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ANAPHYLACTIC SHOCK IN THE ALBINO RAT

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It has been known for a long time that rats are difficult to sensitize to foreign proteins (Longcope, 1922). Probably the first workers to give a comprehensive description of anaphylactic shock in the rat were Parker & Parker (1924), who recorded symptoms of dyspnoea, abdominal cramps, progressive hypothermia and collapse. At autopsy there were congestion and haemorrhage in several tissues, particularly in the small intestine where desquamation of the epithelium was also noted. In recent years, anaphylaxis in the rat has been reported by Halpern, Liacopoulus & del Castillo (1955) and by Mota (1957). The Dale–Schultz reaction, so characteristic of sensitized guinea-pig tissues, has been noted in the rat by Parker & Parker (1924), Kellaway (1930), Suden (1934), and Campbell & Nicoll (1940).

The difficulty in sensitizing rats has led to the use of accessory means of aggravating the phenomenon. Such methods have included simple changes in the diet (Seegal & Khorazo, 1929), the operation of suprarenalectomy (Flashman, 1926; Wyman, 1929; Dews & Code, 1953) or of hypophysectomy (Molomut, 1939), the use of Freund's adjuvant (Lipton, Stone & Freund, 1956) or the use of *Haemophilus pertussis* vaccine (Malkiel & Hargis, 1952*b*).

From a review of the literature on the production of anaphylactic shock in the rat, the following points emerge:

(1) The time of incubation following sensitization is a factor of primary importance. Shock is only produced after an incubation period of at least 10 days; on the other hand, it is greatly reduced if the challenge is given after 21 days or more (Hochwald & Rackemann, 1946).

(2) For the regular production of the Dale–Schultz reaction, passive sensitization with heterologous serum appears to be superior to active sensitization (Kellaway, 1930).

(3) Severe or fatal shock is rare though the symptoms and lesions produced usually follow the pattern described by Parker & Parker (1924).

(4) The highest number of fatal reactions occur when *H. pertussis* vaccine is used as an accessory agent for sensitization (Malkiel & Hargis, 1952*b*).

The object of the present work was to obtain more detailed information about the role played by *H. pertussis* vaccine in aiding sensitization of the rat to foreign protein. It is known that in mice treatment with *H. pertussis* vaccine renders the animal more sensitive to histamine (Parfentjev & Goodline, 1948; Malkiel & Hargis, 1952*a*), and since these experiments have been completed a similar action in mice has been found for 5-hydroxytryptamine (5-HT) (Kind, 1957; Kallos & Kallos-Deffner, 1957). No experiments of this kind with 5-HT have so far been performed in rats, and the present results show that in this species treatment with *H. pertussis* vaccine increases the sensitivity to 5-HT as well as to histamine. The time courses of the hypersensitivity to the two amines differ although there is some overlap, and this period of overlap coincides with the condition in which severe anaphylactic shock can be produced.

MATERIALS AND METHODS

Animals. Female albino rats (125–175 g) obtained from the Agricultural Research Council's Field Station at Compton were used in all experiments. For passive sensitization, injections of plasma from sensitized rats were made into female guinea-pigs (400–600 g). The diets consisted of cubes, for rats (No. 41, Association London Flour Millers Ltd.) and for guinea-pigs (No. 18B). Drinking water was allowed *ad lib.*

Antigens. (1) Horse serum (Burroughs Wellcome, Ltd., London); (2) Fresh egg white, mixed with an equal volume of N-saline and strained; (3) Ovalbumin (4% (w/v)) in N-saline; (4) Hog mucin (1·5% (w/v)) suspended in N-saline.

Whooping cough vaccine. Haemophilus pertussis vaccine, phase I, 20,000 × 10^6 organisms/ml., grown in Cohen and Wheeler's liquid medium.

Sensitization. 1 ml. of one of the antigens, with or without 1 ml. of *H. pertussis* vaccine, intraperitoneally.

Challenge. 1 ml. of the corresponding antigen intravenously under light ether anaesthesia 12–18 days after sensitization, unless otherwise stated in the text.

Assessment of shock

The various signs and symptoms of shock were recorded on an arbitrary scale, as follows:

1. Not responding to painful stimuli, score of 1 mark;
2. Hair ruffled, 1 mark;
3. Moderate drop in body temperature, 2 marks;
4. Severe drop in body temperature, 3 marks;
5. Dead in 4 hr, 5 marks;
6. Dead in 24 hr, 3 marks.

On this scale of marking, the maximal possible score for any rat is 10 (e.g. the sum of scores for signs 1, 2, 4 and 5).

Injections of fresh egg white into normal rats produce peripheral oedema, and the fall in blood pressure produced by anaphylactic shock masks this oedema formation. Absence of oedema is therefore a positive sign of shock and presence of oedema a negative sign. The assessment values used for egg-white anaphylaxis were as follows:

1. Not responding to painful stimuli, score of 1 mark;
2. Hair ruffled, 1 mark;
3. Moderate drop in body temperature, 1 mark;

4. Severe drop in body temperature, 2 marks;
5. Absence of oedema, 1 mark;
6. Presence of oedema, minus 2 marks;
7. Dead in 4 hr, 5 marks;
8. Dead in 24 hr, 3 marks.

Again, on this scale of marking, the maximum possible for any rat is 10 (e.g. the sum of scores for signs 1, 2, 4, 5 and 7).

Results have been recorded as the mean shock values expressed as percentages of the maximal possible scores of groups of four or more rats. On this system of assessment, shock values over 20% denote the presence of anaphylactic shock.

Extraction of rat tissues and assay procedures for histamine and 5-hydroxytryptamine

The tissues were either extracted with trichloroacetic acid and assayed on the isolated guinea-pig ileum for histamine, or extracted with acetone and assayed on the isolated rat uterus for 5-HT. The methods have been described in detail elsewhere (Parratt & West, 1957). All values of histamine and 5-HT in this paper refer to the base.

Perfusion of tissues of rats

Rats were anaesthetized with intraperitoneal injections of urethane (1·5 g/kg), and either the hind quarters or the alimentary canal with liver were perfused. The hind quarters were perfused through the abdominal aorta, using oxygenated Locke's solution, and the venous effluent was collected from the vena cava (Feldberg & Mongar, 1954). The alimentary canal and liver were perfused through the thoracic aorta as it pierces the diaphragm. The abdominal aorta and the inferior vena cava were clamped above the level of the renal vessels, the lumbrical and ovarian vessels being ligated. The perfusate was collected from the inferior vena cava above its junction with the hepatic vein. The venous effluent was tested for histamine on the atropinized guinea-pig ileum and for 5-HT on the atropinized rat uterus. Evans blue dye was injected intra-arterially at the conclusion of each experiment to verify the areas which were perfused.

Histaminase activity of rat tissues

Histaminase activity was estimated in homogenates of the intestine, since that tissue is the chief source of this enzyme in the rat. The method was that used by Wicksell (1949).

RESULTS

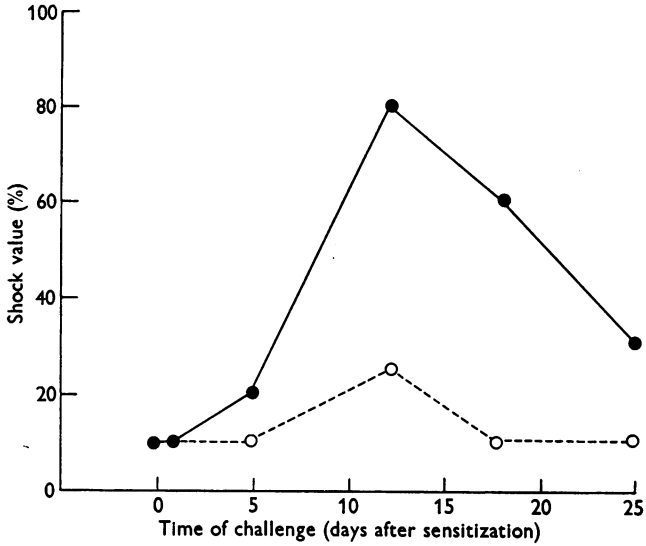
Anaphylactic shock in the rat with various antigens

Horse serum. Groups of rats were sensitized by a single injection of horse serum and challenged within 5 min or 1, 5, 12, 18 or 25 days after sensitization. The shock values obtained have been plotted in Text-fig. 1. Anaphylactic shock occurred only when the challenge was given 12 days after sensitization, and even then it was of a mild character (shock value 25%) and no deaths were recorded.

When *H. pertussis* vaccine was added to the sensitizing dose of horse serum and groups of rats were similarly challenged, intense shock was produced 12 days after sensitization (Text-fig. 1). In 45 out of 66 animals (i.e. 68%) the shock was fatal. Occasionally fatal anaphylactic shock was also seen when the challenging dose was given up to 18 days after sensitization.

The symptoms of the anaphylactic shock are as follows. Immediately after injection, respiration becomes hurried and severe diaphragmatic movements

occur. Frothy exudate appears from the nose. Quickly the animal assumes a peculiar 'kangaroo-like' attitude, standing upon its hind paws with its body hunched. The hair is ruffled, there is loss of reaction to painful stimuli, and the body temperature and blood pressure fall. Following this stage, some animals show signs of recovery, both the body temperature and blood pressure gradually returning to normal levels. Recovery is then complete in 24 hr. Other animals exhibit progressive shock which is characterized by severe prostration, weakness, cyanosis, exophthalmos and convulsions. Death usually occurs within 4 hr, but is sometimes delayed.

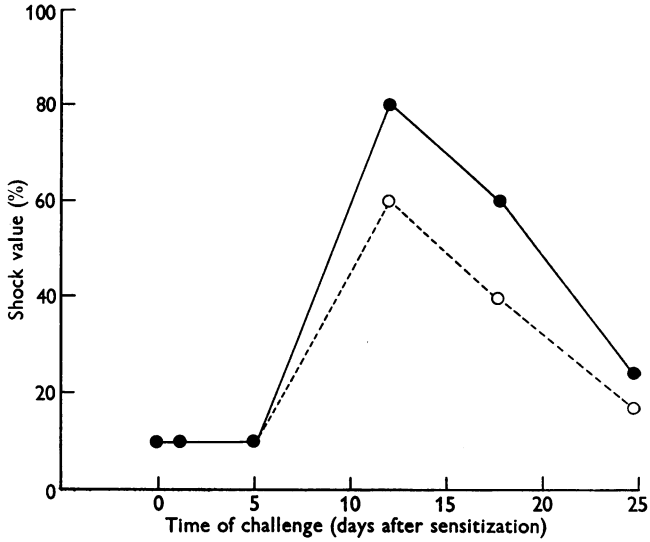


Text-fig. 1. Mean shock values (expressed as percentages of the maximum) of groups of rats when challenged with horse serum at varying times after sensitization by horse serum (○ - - - ○) or by horse serum and *H. pertussis* vaccine (●——●).

In rats killed 15 min after challenge, there is slight to moderate congestion of the abdominal viscera, particularly in the small intestine. Microscopically there are petechial haemorrhages in the submucosa, eosinophil infiltration in the mucosa and submucosa, and oedema of the columnar cells lining the villi (Pl. 1, fig. 1). In rats killed 1 hr after challenge, congestion of the viscera is more pronounced and ecchymotic patches appear in the walls of the small intestine. Microscopically, haemorrhages in the submucosa are extensive and the oedema extends to the villi, where the epithelial cells at the top show desquamation (Pl. 1, fig. 2). Rats dying from anaphylactic shock in 2–4 hr show severe congestion of the whole alimentary canal and peritoneum, blood and mucus being found in the lumen of the gut (Pl. 2). The liver and spleen are also congested. A constant microscopic feature at this stage is almost complete

desquamation and disintegration of the mucosa of the small intestine (Pl. 1, fig. 3). Occasionally petechial haemorrhages are found in the lungs.

Fresh egg white. Rats were sensitized by a single injection of fresh egg white, and challenged immediately or on 1, 5, 12, 18 or 25 days after sensitization. The shock values have been plotted in Text-fig. 2. Moderately severe shock was noted only when the challenge was given 12 or 18 days after sensitization. When *H. pertussis* vaccine was added to the sensitizing dose of egg white and rats were similarly challenged, the shock values were raised (Text-fig. 2).



Text-fig. 2. Mean shock values (expressed as percentages of the maximum) of groups of rats when challenged with egg white at varying times after sensitization by egg white (○ - - - ○) or by egg white and *H. pertussis* vaccine (●——●).

Maximal figures were again obtained 12–18 days after sensitization. The symptoms and pathological changes were similar to those recorded in the previous section using horse serum and *H. pertussis* vaccine.

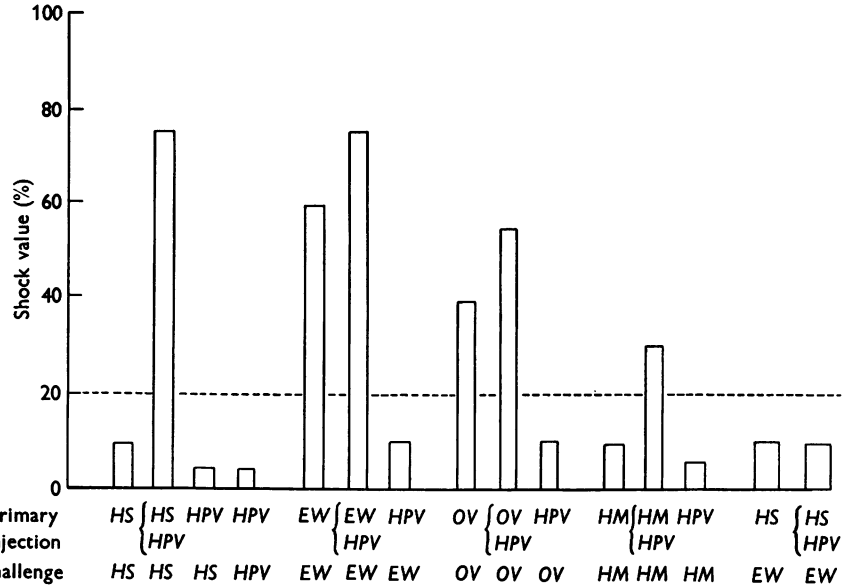
Ovalbumin. As with egg white, the shock value after ovalbumin sensitization and challenge was increased by treatment with *H. pertussis* vaccine, from 40 to 55% at 12 days after sensitization.

Hog mucin. This was found to be a poor antigen in the rat, and treatment with *H. pertussis* vaccine only resulted in a maximal shock value of 30% (Text-fig. 3).

In rats pre-treated with only *H. pertussis* vaccine 12 days previously, the primary intravenous injection of any one of the foreign proteins used or of *H. pertussis* vaccine itself in the dose employed for aiding sensitization failed to produce a shock-like condition. Likewise the injection of a non-specific

protein into rats sensitized with a specific protein and *H. pertussis* vaccine failed to produce significant shock (Text-fig. 3).

It is possible that the challenging dose of antigen exerts part of its action by functioning as a stress agent. The effect of a non-specific stress was therefore tested 12 days after a primary injection of *H. pertussis* vaccine. Rats were anaesthetized with ether and the abdomen and lower extremities immersed in hot water at 60° C for 10 sec. After drying the animals they were allowed to recover. Severe oedema of the hind paws developed at the same rate as in heated uninjected rats and 4 hr after immersion there was no congestion in the jejunum.



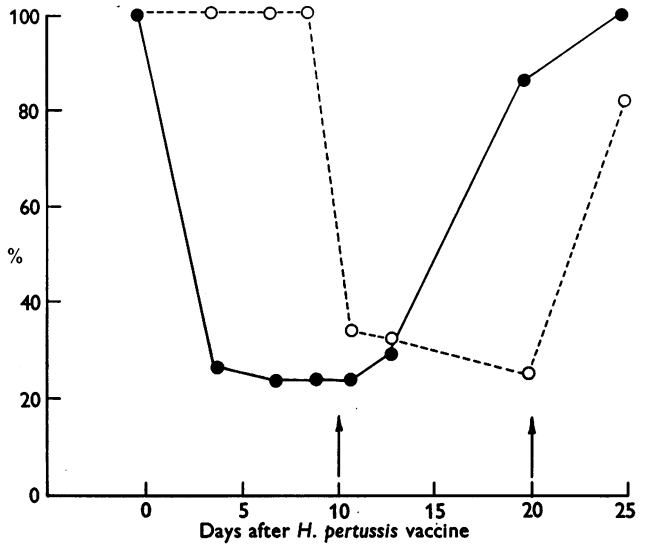
Text-fig. 3. Comparison of shock values (expressed as percentages of the maximum) of groups of rats on intravenous challenge 12 days after primary injections of various antigens, or *H. pertussis* vaccine (*HPV*), or both. Antigens used were horse serum (*HS*), egg white (*EW*), ovalbumen (*OV*), and hog mucin (*HM*). Shock values over 20% denote the presence of anaphylactic shock.

The mode of action of Haemophilus pertussis vaccine

Primary toxicity of the vaccine. Although the dose of the vaccine used for aiding sensitization did not produce any noticeable reaction in the rats, four times this dose (i.e. $80{,}000 \times 10^6$ organisms) killed 10 out of a total of 42 rats (i.e. 24%). Morbid changes were similar to those noted in anaphylaxis, congestion of the gastrointestinal tract being prominent. The remaining 32 rats showed mild degrees of shock, with recovery in 24 hr.

Toxicity of histamine and 5-HT. As in mice, treatment with *H. pertussis* vaccine rendered the rats more sensitive to histamine and 5-HT. Four days

after such treatment, the animals were nearly five times more sensitive to histamine than were the untreated control animals. Lethal doses of histamine in such treated rats produced death in less than 2 hr, with intestinal lesions which were often similar to those found in animals undergoing active anaphylaxis 12 days after sensitization with horse serum and *H. pertussis* vaccine. This increased sensitivity to histamine persisted for about 10 days and then slowly returned to levels found in untreated control rats (Text-fig. 4).



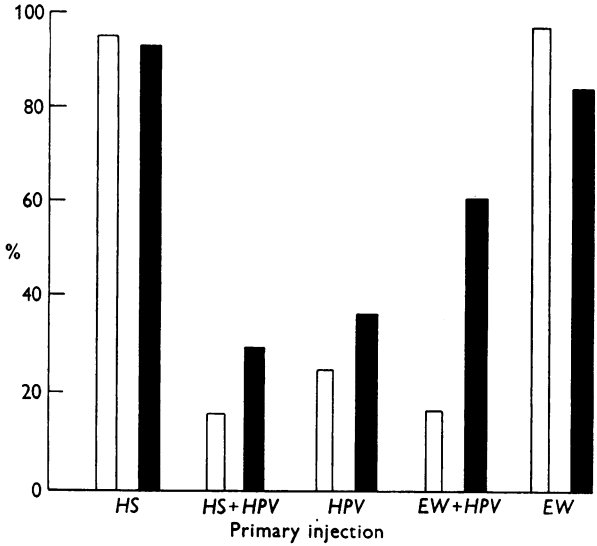
Text-fig. 4. Comparison of the time period of the hypersensitivity of rats to histamine (●——●) and to 5-HT (○ - - - ○) after treatment with *H. pertussis* vaccine with that of anaphylactic sensitivity to foreign protein (as indicated between the two arrows). Ordinates: LD_{50} values in the treated rats expressed as percentages of the values obtained in control rats (histamine, 600 mg/kg intraperitoneally; 5-HT, 30 mg/kg intravenously). Abscissae: time in days after treatment with *H. pertussis* vaccine.

The sensitivity of the rats to intravenous doses of 5-HT following treatment with *H. pertussis* vaccine was unaltered for about 10 days, then for the next 10 days it increased three fold, and lastly it gradually returned to levels found in the control rats (Text-fig. 4). Death usually occurred within 15 min of intravenous administration, congestion of the lungs and abdominal viscera being present.

Thus following treatment with *H. pertussis* vaccine there is an early phase (up to 10 days) when the rats are more sensitive to histamine, an intermediate phase (10–18 days) when they are more sensitive to both histamine and 5-HT, followed by a late phase of a few days when the rats are more sensitive to 5-HT but not to histamine. When the challenge is given during the intermediate phase, full anaphylactic shock develops, whereas given in the early

or late phase (or beyond 25 days) the shock produced is insignificant, though the incubation period between the sensitizing dose and the challenging dose of the antigen is kept constant.

The sensitization of the animals by *H. pertussis* vaccine with horse serum, or by *H. pertussis* vaccine with egg white, also resulted in an increased sensitivity to histamine and 5-HT on the 12th day, whereas sensitization with horse serum alone or with egg white alone either failed to render the rats more sensitive than the untreated control rats or produced only a slight sensitization (Text-fig. 5).



Text-fig. 5. Comparison of the hypersensitivity of rats to histamine, □, and to 5-HT, ■, 12 days after the primary injection of antigen, or *H. pertussis* vaccine (*HPV*), or both. Antigens used were horse serum (*HS*) and egg white (*EW*). LD_{50} values in the treated rats expressed as percentages of the values obtained in control rats.

Action of histamine liberators. Compound 48/80, a releaser of both histamine and 5-HT, and polymyxin B, a compound which preferentially releases histamine (Parratt & West, 1957), were given intraperitoneally and deaths were recorded over 24 hr. In untreated rats, the LD_{50} for compound 48/80 is 2 mg/kg and for polymyxin B is 5 mg/kg. In rats treated with *H. pertussis* vaccine 12 days previously, both substances were found to be twice as toxic as in untreated rats.

Amine content of the tissues. The histamine and 5-HT contents of certain tissues were assayed in control rats, in rats 12 days after sensitization with horse serum and *H. pertussis* vaccine, and in rats in which anaphylactic shock had been produced. The results are shown in Table 1. The values were about the same in all three groups of rats except in the jejunum of the shocked rats,

where there was an apparent lowering of both the histamine and 5-HT levels which could be attributed to oedema of the tissue.

Urinary histamine. The histamine content of the urine of rats pre-treated with *H. pertussis* vaccine 12 days previously was compared with that of uninjected control animals. Urine was collected over periods of 5 hr and assayed directly on the isolated guinea-pig ileum. Rats pre-treated with *H. pertussis* vaccine excreted on the average 3·32 μg histamine in this period whereas the controls excreted 3·45 μg.

TABLE 1. Comparison of the histamine and 5-HT contents (μg/g) of tissues of control rats, rats sensitized 12 days previously with horse serum and *H. pertussis* vaccine, and similarly sensitized rats after challenge with horse serum. All values are the means of four or more rats

Tissue	Histamine			5-HT		
	Control	Sensitized	Shocked	Control	Sensitized	Shocked
Spleen	1·2	1·0	0·9	4·0	4·2	4·1
Lungs	10·0	12·0	10·0	2·0	2·2	2·0
Jejunum	22·2	20·0	15·0	4·6	3·8	3·3
Subcutaneous connective tissue	35·5	37·0	38·0	1·0	0·9	0·8

To determine whether the renal mechanism for excretion of histamine of rats pre-treated with *H. pertussis* vaccine is as efficient as that of control rats, intraperitoneal doses of histamine (2 mg/kg) were given to groups of animals and the urine collected and assayed as previously described. Rats pre-treated with *H. pertussis* vaccine excreted on the average 25·5 μg histamine in 5 hr, whereas the controls excreted 22·3 μg.

Release of histamine and 5-HT by antigen from perfused sensitized tissues. Rats were sensitized by a single injection of horse serum or egg white, with or without *H. pertussis* vaccine, and their hind quarters or alimentary canal with liver were perfused 12 days later. After the injection of antigen, the perfusate was collected every 15 min for 1 hr and assayed for histamine. At this time, the release of histamine had returned to basal levels. There was wide variation in the histamine release from experiment to experiment, but pre-treatment with *H. pertussis* vaccine did not significantly alter the histamine release. It was of the same order as that from rats in which sensitization had not been aided by *H. pertussis* vaccine and in which either horse serum or egg white was the antigen.

In most experiments 5-HT was also assayed in the perfusate, but in no case was the release greater than the basal level before antigen. Normal rat plasma added to the perfusion fluid before the injection of antigen also did not increase the release of either amine.

Typical intestinal haemorrhagic patches sometimes developed in the isolated perfused sensitized intestines on addition of antigen to the perfusion fluid. The intensity of this reaction was not as severe as in the intact animal, since the blood from the tissues had been partly washed out by the perfusing fluid.

Such haemorrhagic patches were never seen in perfused intestines of normal rats or of rats pre-treated with *H. pertussis* vaccine alone.

Tissue histaminase. There was no significant alteration in the histaminase activity of intestinal homogenates of rats pre-treated with *H. pertussis* vaccine 12 days previously when compared with that of similar extracts taken from untreated control animals.

Dale–Schultz reaction. The response of the uterus or colon of the sensitized rat to antigen was tested by suspending strips of tissue in an isolated organ bath containing magnesium-free de Jalon fluid at 30° C. As early as 6 days after sensitization, a small contraction was sometimes noted; between 10 and 15 days, there was always a contraction; after more than 20 days, both preparations no longer responded to the antigen. Pre-treatment of the rat with *H. pertussis* vaccine did not modify the Dale–Schultz reaction and desensitization occurred normally on addition of the second dose of antigen.

TABLE 2. Precipitin reaction of rat plasma 9 days after sensitization with horse serum or with horse serum and *H. pertussis* vaccine. Precipitate shown on a relative scale from + + to 0

Sensitizing treatment	Concentration of antigen added to plasma (%)	Plasma dilution 1/5	1/50	1/500	1/1000
Horse serum	50	+ +	+	±	0
	10	+	±	0	0
Horse serum + *H. pertussis* vaccine	50	+ +	+	±	0
	10	+	±	0	0

Precipitin reaction. Rats were sensitized by a single injection of horse serum with or without *H. pertussis* vaccine 9 days previously. Plasma from such animals was collected, serially diluted with N-saline and incubated at 37° C during the night with diluted horse serum. Plasma from untreated rats was similarly diluted and incubated with diluted horse serum. A positive reaction was judged when a clear distinction existed between the amount of precipitate formed in the tube containing treated rat plasma and that formed in the tube containing a similar dilution of untreated rat plasma. Results shown in Table 2 indicate that *H. pertussis* vaccine does not influence the production of precipitating antibodies in the rat.

Passive transfer of antibodies. Rats were sensitized by a single injection of horse serum with or without *H. pertussis* vaccine 9 days previously. Plasma from such animals was injected intraperitoneally into guinea-pigs, which were challenged 24 hr later by an intravenous injection of horse serum (1 ml.). Respiratory distress and moderate shock was observed in all animals; *H. pertussis* vaccine failed to modify the response. The lungs of guinea-pigs killed 30 min later presented slight to moderate emphysema and the pleural mast cells were mostly disrupted. When passive transfer of antibodies was carried out 5 or 15 days after sensitization, the resulting shock on challenge was definitely

milder, and at 22 days after sensitization the plasma failed to confer passive sensitivity to horse serum.

Plasma from rats sensitized with only horse serum 9 days previously was also injected into normal rats and into rats which had received *H. pertussis* vaccine 12 days previously. The rats were challenged 24 hr later with intravenous doses of horse serum. An average shock value of 45% was obtained in the experiments in which the rats had received *H. pertussis* vaccine previously, compared with an average shock value of 10% in normal rats. Such a result suggests that *H. pertussis* vaccine magnifies the effects of the antigen-antibody reaction, and in fact some recipient rats died in 24 hr, with petechial haemorrhages in the intestine.

In another experiment, a rabbit received intraperitoneal injections of horse serum (each of 1 ml.) every day for 5 days. Ten days later the rabbit was bled and its serum tested for precipitin. A positive reaction was noted in a dilution of 1 in 800. 2 ml. of the undiluted serum was then injected intraperitoneally into each of six control rats and six rats which had received *H. pertussis* vaccine 12 days previously. Both groups of animals were challenged 24 hr later with intravenous doses of horse serum and the shock assessed. The shock value in the rats which received *H. pertussis* vaccine was 45% and in the control group it was 10%.

These results indicate therefore that pre-treatment of recipient rats with *H. pertussis* vaccine facilitates the passive transfer of both homologous and heterologous antibodies, but no such enhancement occurs if donor rats are similarly treated instead of the recipients.

DISCUSSION

The present results show first that the anaphylactic shock in the albino rat produced by horse serum is relatively mild, but that a more severe shock can be elicited when egg white is used as an antigen, and secondly that treatment with *H. pertussis* vaccine always aggravated the shock produced by either antigen, thus confirming and extending the results obtained by Malkiel & Hargis (1952*b*).

Anaphylaxis in the rat is characterized by a progressive circulatory collapse, the underlying mechanism of which is apparent when the abdominal viscera are examined. The earliest lesions occur in the small intestine and consist of oedema, haemorrhage and eosinophil infiltration in the submucosa. The oedema rapidly spreads to the villi and in severe cases separates the mucosa from the submucosa. Desquamation of the mucosa is followed by internal haemorrhage leading to progressive fatal collapse. The target organ is probably the small intestine itself, since similar lesions are occasionally found in isolated sensitized intestine on perfusion with antigen. It is possible that changes occurring elsewhere in the body play a subsidiary role in the development of the lesions.

A summary of the chief events in the time course of anaphylaxis in the rat either in the presence or absence of *H. pertussis* vaccine is as follows. During the period of 5 to 10 days following sensitization, precipitins appear in the blood and can be transferred passively to guinea-pigs. In this period, no active anaphylaxis can be produced though a minimal Dale–Schultz reaction is sometimes obtained. For the next 10 days active anaphylactic shock can be produced and the Dale–Schultz reaction is maximal. Beyond 20 days these responses rapidly diminish to pre-sensitization levels.

H. pertussis vaccine does not appear to influence the essential characteristics of the anaphylactic reaction in the rat. It does not render the primary injection of foreign protein toxic to the animals; it does not affect the specificity of the response on challenge; and it does not alter the duration of the anaphylactic sensitivity. The intensity of the systemic anaphylactic reaction is increased but otherwise it is not modified. The potentiating action is also manifest in anaphylaxis induced by passive transfer of both homologous and heterologous antibodies, though the anaphylactic response of isolated smooth muscle is little changed.

The potentiating action of *H. pertussis* vaccine on the anaphylactic reaction in the rat could be the result, among other factors, of either an increased formation of antibodies, or an increase in the amount of amines released, or an increase in the sensitivity of the tissues to the released amines. The present results give no evidence for increased formation of antibodies or for increased release of amines. *H. pertussis* vaccine does not increase the precipitin titre, nor does it increase the power of the plasma to sensitize guinea-pigs passively. The amine content of the tissues is not raised by treatment with *H. pertussis* vaccine and antigen, and the release of amines from perfused sensitized tissues by antigen is unchanged. But there is an increase in the sensitivity of the tissues to the released amines. Treatment of rats with *H. pertussis* vaccine renders them hypersensitive to both histamine and 5-HT, although the time courses of these two phenomena are different. There is some overlap, however, and it is only during this period of hypersensitivity to both amines that rats undergo severe anaphylactic shock when challenged with antigen. Therefore at least one factor responsible for the potentiating action of *H. pertussis* vaccine on the anaphylactic reaction is the hypersensitivity of the tissues to histamine and 5-HT. It is doubtful, however, if this is the full explanation as the tissues at this period of time may also be hypersensitive to other substances.

SUMMARY

1. Anaphylactic shock in the rat is characterized by progressive circulatory collapse.

2. The target organ is the small intestine, where oedema and haemorrhage in the submucosa and desquamation of the mucous membrane occur.

3. Anaphylactic shock in the rat is aggravated by pre-treatment with *H. pertussis* vaccine.

4. Treatment with *H. pertussis* vaccine does not alter the content of histamine and 5-HT in the tissues, nor does it alter the urinary excretion of histamine.

5. Treatment with *H. pertussis* vaccine does not alter the histaminase content of the intestinal wall which is the chief source of this enzyme in the rat.

6. Treatment with *H. pertussis* vaccine does not increase the release of histamine and 5-HT either from perfused tissues on addition of antigen, or from the tissues of an animal undergoing anaphylactic shock.

7. Treatment with *H. pertussis* vaccine does not increase the amount of precipitin formed in response to the injection of antigen.

8. The only observed effect of *H. pertussis* vaccine is to increase the sensitivity of the animals to histamine and 5-HT, but it is not clear whether this phenomenon alone is responsible for the increased sensitivity to anaphylactic shock.

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REFERENCES

CAMPBELL, D. H. & NICOLL, P. A. (1940). Studies on *in vitro* anaphylaxis and release of an active non-histamine material from sensitized guinea-pig lungs. *J. Immunol.* **39**, 103–112.

DEWS, P. B. & CODE, C. F. (1953). Anaphylactic reactions and concentrations of antibody in rats and rabbits: effects of adrenalectomy and of administration of cortisone. *J. Immunol.* **70**, 199–206.

FELDBERG, W. & MONGAR, J. L. (1954). Comparison of histamine release by compound 48/80 and octylamine in perfused tissues. *Brit. J. Pharmacol.* **9**, 197–201.

FLASHMAN, D. H. (1926). The effect of suprarenalectomy on active anaphylactic shock in the white rat. *J. infect. Dis.* **38**, 461–468.

HALPERN, B. N., LIACOPOULUS, P. & DEL CASTILLO, C. P. (1955). L'anaphylaxie experimentale chez le rat albino. *C.R. Soc. Biol., Paris*, **149**, 314–319.

HOCHWALD, A. & RACKEMANN, F. M. (1946). Studies on the physiology of anaphylactic shock. *J. Immunol.* **53**, 191–199.

KALLOS, P. & KALLOS-DEFFNER, L. (1957). Effect of inoculation with *H. pertussis* vaccine on susceptibility of albino mice to 5-hydroxytryptamine (serotonin). *Int. Arch. Allergy, N.Y.*, **11**, 327–345.

KELLAWAY, C. H. (1930). The anaphylactic reaction of the isolated uterus of the rat. *Brit J. exp. Path.* **11**, 72–80.

KIND, L. S. (1957). Sensitivity of pertussis-inoculated mice to serotonin. *Proc. Soc. exp. Biol., N.Y.*, **95**, 200–201.

LIPTON, M. M., STONE, S. H. & FREUND, J. (1956). Systemic and local anaphylaxis in the albino rat. *J. Immunol.* **77**, 453–461.

LONGCOPE, W. T. (1922). Insusceptibility to sensitization and anaphylactic shock. *J. exp. Med.* **36**, 627–643.

MALKIEL, S. & HARGIS, B. J. (1952*a*). Anaphylactic shock in the pertussis vaccinated mouse. *Proc. Soc. exp. Biol., N.Y.*, **80**, 122.

MALKIEL, S. & HARGIS, B. J. (1952*b*). Histamine sensitivity and anaphylaxis in the pertussis vaccinated rat. *Proc. Soc. exp. Biol., N.Y.*, **81**, 689–691.

MOLOMUT, N. (1939). The effect of hypophysectomy on immunity and hypersensitivity in rats with a brief description of the operative technique. *J. Immunol.* **37**, 113–131.

MOTA, I. (1957). Action of anaphylactic shock and anaphylotoxin on mast cells and histamine in rats. *Brit. J. Pharmacol.* **12**, 453–456.

PARFENTJEV, I. A. & GOODLINE, M. A. (1948). Histamine shock in mice sensitized with *H. pertussis* vaccine. *J. Pharmacol.* **92**, 411–413.

PARKER, J. T. & PARKER, F. (1924). Anaphylaxis in the white rat. *J. med. Res.* **44**, 263–287.

PARRATT, J. R. & WEST, G. B. (1957). 5-hydroxytryptamine and tissue mast cells. *J. Physiol.* **137**, 169–178.

SEEGAL, B. C. & KHORAZO, D. (1929). Anaphylaxis in the white rat as influenced by diet. *Arch. Path.* (*Lab. Med.*) **7**, 827–834.

SUDEN, C. T. (1934). Reactions of rat uterus excised and *in situ* to histamine and anaphylaxis. *Amer. J. Physiol.* **108**, 416–423.

WICKSELL, F. (1949). A simplified method for estimating the histaminolytic activity of plasma in pregnancy. *Acta physiol. scand.* **17**, 359–369.

WYMAN, L. C. (1929). Studies on suprarenal insufficiency. *Amer. J. Physiol.* **89**, 356–361.

EXPLANATION OF PLATES

PLATE 1

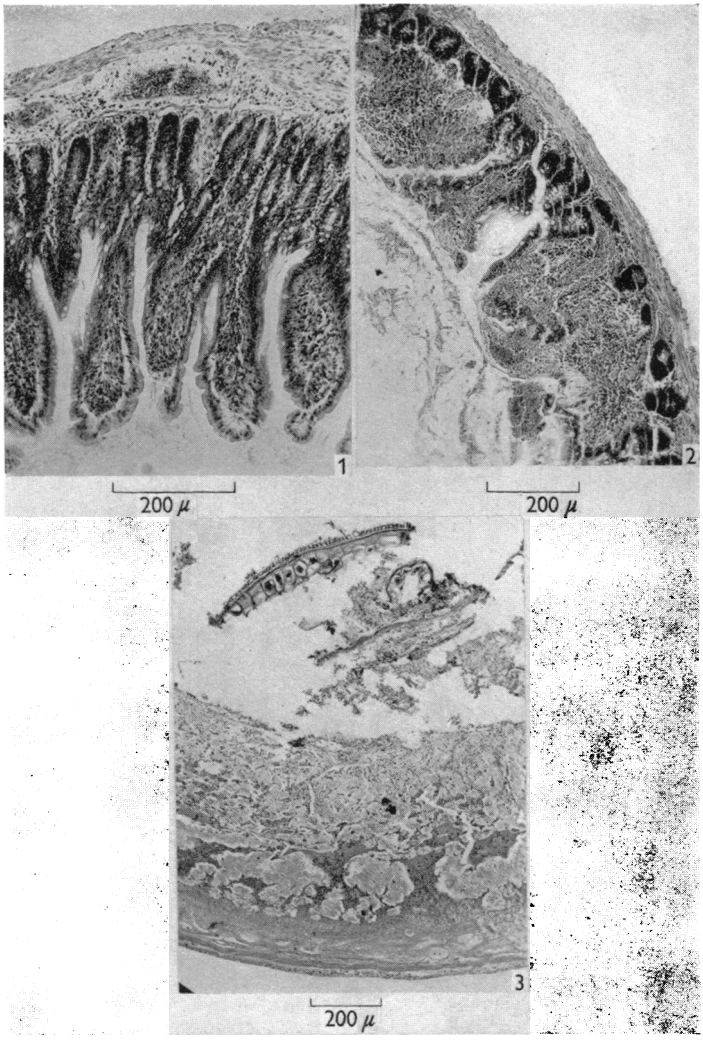
Fig. 1. Rat. Small intestine. Haematoxylin and eosin (explanation in text). 15 min after anaphylaxis.

Fig. 2. 1 hr after anaphylaxis.

Fig. 3. 2 hr after anaphylaxis.

PLATE 2

(*A*) Control rat; 2 hr after horse serum intravenously. (*B*) Sensitized rat (horse serum 12 days previously); 2 hr after horse serum intravenously. There is congestion of the blood vessels of the abdomen. (*C*) Sensitized rat (horse serum and *H. pertussis* vaccine 12 days previously) 2 hr after horse serum intravenously. There is considerable haemorrhage into the lumen of the small intestine.



(*Facing p.* 584)


A
B
C