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Anaphylaxis in Pigs and its Relationship to the Pathogenesis of Oedema Disease and Gastro-enteritis Associated with *Escherichia coli*

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Summary. Pigs were subjected to active anaphylactic shock using egg albumin and to reversed passive anaphylaxis using *Escherichia coli* (O138). The symptoms and lesions closely resembled those of oedema disease and haemorrhagic gastro-enteritis. Catarrhal enteritis was also observed. There was a relationship between the character of the lesions which were produced and the severity and duration of the anaphylactic symptoms. Further evidence confirmed earlier observations that clinically normal pigs may develop a hypersensitivity to those serotypes of *E. coli* which are associated with these conditions. The results are discussed in relation to the pathogenesis of these diseases, and it is considered that oedema disease and haemorrhagic gastro-enteritis develop from an anaphylactic type of hypersensitivity to *E. coli* rather than from a direct toxaemia arising from the sudden absorption of increased quantities of bacterial polysaccharide.

INTRODUCTION

It is widely held that *E. coli* infection is a common cause of gastro-enteritis in piglets of less than a week old and it has been shown that oedema disease and various forms of gastro-enteritis in older pigs are associated with the multiplication of certain *E. coli* serotypes in the stomach and intestines (Sojka, Erskine and Lloyd, 1957; Roberts and Vallely, 1959; Thomlinson and Buxton, 1962). These serotypes, which are commonly haemolytic, are present in the intestines of healthy pigs (Campbell, 1959; Miura, Sato, Ito, Miyamae, Mitamura and Sakazaki, 1961) and multiply rapidly in association with dietetic and environmental changes (Buxton and Thomlinson, 1961).

Gregory (1955) and Erskine, Sojka and Lloyd (1957) reproduced oedema disease in pigs by inoculating culture filtrates and extracts of these organisms. More recent work has shown that pigs are hypersensitive to the common *E. coli* serotypes associated with oedema disease and haemorrhagic gastro-enteritis; and it has been postulated that these diseases occur as the result of an anaphylactic reaction rather than a direct toxic effect following the rapid absorption of *E. coli* polysaccharide from the intestines (Buxton and Thomlinson, 1961). It has also been shown (Thomlinson and Buxton, 1962) that lesions similar to those of naturally occurring oedema disease and haemorrhagic gastro-enteritis in pigs develop in guinea-pigs in association with protracted passive anaphylactic shock. Furthermore, the experimental introduction of *E. coli* polysaccharide into the stomach results in rapid absorption and anaphylactic shock in sensitized guinea-pigs.

The purpose of the present experiments was to determine the conditions under which anaphylactic shock could be induced in pigs and to study the symptoms and lesions

produced. Crystalline hen-egg albumin was chosen as the antigen for the initial experiments since this substance is non-toxic. It was considered that the active method would be the more practicable means of sensitizing pigs and the techniques were based on the results of preliminary experiments which had been carried out in guinea-pigs. The occurrence of anaphylactic reactions in pigs to *E. coli* was demonstrated by reversed passive anaphylaxis. By this method the reactions were clearly shown to be the result of anaphylaxis as distinct from a direct toxicity of the extracts.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Large White pigs, 6–8 weeks old, were obtained from herds known to maintain good standards of husbandry and health. Commercial sow and weaner meals, similar to those which the pigs had been receiving, were fed as a wet mash. Control and experimental pigs were derived from the same group of animals.

PRODUCTION OF ACTIVE ANAPHYLAXIS WITH EGG ALBUMIN

The pigs weighed approximately 20 kg. The dose of egg albumin was calculated from the same ratio of dose for body weight used for preliminary experiments with guinea-pigs. A 10 per cent solution of egg albumin in saline was centrifuged to remove any undissolved particles. Initially, a dose of 2 g. was given subcutaneously. This was reduced to 1 g. when given intravenously. After varying intervals, a challenge dose of 0·5–1 g. of egg albumin was given intravenously. A further dose of 0·5–1 g. of egg albumin was given subcutaneously 30 minutes later to prolong the reaction.

PREPARATION OF *E. coli* ANTIGEN

Cultures of *E. coli* (O138) were grown on nutrient agar in Roux flasks incubated at 37° for 48 hours. The growth in each flask was harvested in 10 ml. saline and boiled for 2½ hours. The suspensions were pooled and formalin added to a final concentration of 0·25 per cent. Undiluted suspension was used for antiserum production. For reversed passive anaphylaxis, the suspension was diluted 1/10 in saline.

PREPARATION OF *E. coli* ANTISERUM IN PIGS

Antiserum for eliciting reversed passive anaphylaxis was prepared in pigs of approximately 40 kg. weight. Two ml. of *E. coli* (O138) antigen was injected intravenously at intervals of 2–4 days for 25 days. Serum was collected 10 days after the last injection and stored at −25°. The pooled antisera had a bacterial agglutinin titre of 1/10,240.

SEROLOGICAL TESTS

Haemagglutination and antiglobulin haemagglutination tests were done by the methods described previously (Buxton and Thomlinson, 1961).

PRODUCTION OF REVERSED PASSIVE ANAPHYLAXIS WITH *E. coli*

Reversed passive anaphylaxis was induced by intravenous injection of *E. coli* (O138) antigen, followed 30 minutes later by intravenous injection of *E. coli* (O138) pig antiserum. Preliminary experiments had shown that, in the majority of pigs of approximately 40 kg. body weight, no symptoms developed when 10 ml. of a 1/10 dilution of *E. coli*

antigen was injected intravenously. To prepare pigs for reversed passive anaphylaxis, doses varying from 3·8 to 7·5 ml. of the 1/10 dilution of antigen were used. Two control pigs were inoculated with antigen alone; two received antiserum only.

BACTERIOLOGICAL METHODS

Rectal swabs were examined for haemolytic strains of *E. coli* as soon as the pigs arrived on the premises and on every 2nd day until the experiments were completed. The technique has been described previously (Buxton and Thomlinson, 1961). At post-mortem examination, cultures were made on 5 per cent sheep blood agar plates from the stomach, from the small intestine at intervals of about 4 feet, and from the caecum and colon. After overnight incubation, these plates were examined for haemolytic and non-haemolytic strains of *E. coli*. Representative colonies were tested for acid and gas production in MacConkey's broth and examined serologically.

HISTOLOGICAL METHODS

Specimens were fixed in 10 per cent formol saline. Sections were stained with haematoxylin and eosin.

RESULTS

ACTIVE ANAPHYLAXIS TO EGG ALBUMIN

The results of the experiments in which pigs were sensitized by a single subcutaneous dose of egg albumin are shown in Table 1. One animal (No. 1) received a further dose

TABLE 1

SYMPTOMS AND LESIONS IN PIGS AS A RESULT OF ACTIVE ANAPHYLACTIC SHOCK WITH EGG ALBUMIN

Pig No.	*Sensitization*			*Challenge dose of albumin (g.)*		*Symptoms*			*Macroscopic lesions*
	Dose (g.)	*Route*	*Interval before challenge (days)*	*i.v.*	*s.c.*	*Time for development*	*Duration (hours)*	*Severity*	
1*	2	s.c.	8	0·5	0·5	1 minute	2	Moderate	Oedema up to 5 mm. thick in stomach.
2	2	s.c.	12	1	1	5 minutes	3	Moderate	Oedema up to 15 mm. thick in stomach.
3	2	s.c.	12	1	1	Gradually over 1 hour	6	Mild	Catarrhal enteritis. No oedema.
4	2	s.c.	12	1	1	Gradually over 1 hour	6	Mild	Catarrhal enteritis. No oedema.
5	2	s.c.	12	1	1	1 minute	6	Moderate	Slight oedema in stomach.
6	1	i.v.	6	1	1	1 minute	3	Moderate	Oedema up to 5 mm. thick in stomach.
7	1	i.v.	6	1	1	1 minute	4	Moderate	Oedema up to 5 mm. thick in stomach. Haemorrhage near pylorus.

* This animal was given a further challenge dose of 5 g. of egg albumin intravenously after recovery at 2 hours.
s.c.=subcutaneous injection; i.v.=intravenous injection.

of egg albumin after it had recovered from the two challenge doses. Mild symptoms developed slowly and continued for a further 2 hours.

In an experiment not shown in Table 1, two pigs used for antiserum production were given repeated intravenous injections of 1 g. of egg albumin at intervals of 2 days. After injection on the 4th and 6th days, they developed mild anaphylactic shock. On the 8th day, severe symptoms occurred immediately after injection and the pigs collapsed with acute symptoms of respiratory distress. Thereafter, the dose was reduced to 0·1 g. for the remainder of a course of injections lasting 14 days, and on each occasion moderately severe symptoms recurred. After an interval of 24 days the pigs were given a further intravenous injection of 1 g. of egg albumin and showed no ill effects.

The effect of repeated intravenous injection of egg albumin is shown in Table 2. On the 10th day, pig No. 8 was given an additional dose of 1 g. subcutaneously 10 minutes after the intravenous injection. The animal showed severe symptoms for 2 hours and was then killed. The other two pigs (Nos. 9 and 10) were given similar extra doses of egg albumin subcutaneously on the 14th day and killed 2 hours later.

TABLE 2

SYMPTOMS IN PIGS AFTER REPEATED INTRAVENOUS INJECTION WITH 1 g. OF EGG ALBUMIN

Time of injection (days after first dose)	*Pig No.*	*Symptoms of anaphylaxis*		
		Time for development (minutes)	*Duration (hours)*	*Severity*
3	8	5	2	Mild
	9	5	2	Moderate
	10	5	2	Mild
6	8	2	2	Severe
	9	2	2	Moderate
	10	2	2	Moderate
10	8*	1	Killed after 2 hours	Severe
	9	1	4	Severe
	10	1	4	Severe
14	9*	1	Killed after 2 hours	Severe
	10*	1	Killed after 2 hours	Severe

* 1 g. of egg albumin subcutaneously 10 minutes after the intravenous dose.

SYMPTOMS OF ANAPHYLAXIS

The first signs of severe anaphylactic shock were rapid circling movements accompanied by incoordination, staggering, coughing and intense vascular congestion of the skin, especially of the ears, nose and around the eyes. The conjunctivae also were intensely congested. A convulsion followed immediately, and the pig collapsed with acute symptoms of respiratory distress. Respiration ceased for as long as 30 seconds, cyanosis developed and it appeared that the animal would not survive. Laboured respiration recommenced

slowly. After a few minutes the animal was breathing at twice the normal rate. Coughing and yawning occurred at intervals and this was followed in 5 minutes by retching, vomiting and defaecation. Repeated vomiting and straining with protrusion of the rectum continued at intervals. Usually at this stage there was a period of restlessness. The pig stood still or moved about unsteadily and at intervals vigorously rubbed its nose and occasionally other parts of its head or shoulders against the wall or floor, suggesting severe local irritation of the skin. The body surface became cold. Congestion in the skin was localized mainly around the head, the extremities, and the lower parts of the body.

Severe muscular tremors developed after 20–40 minutes and usually continued for 30 minutes. The tremors were most noticeable in the head but affected the whole body and resembled those observed in natural oedema disease (Timoney, 1950; Thomlinson and Buxton, 1962). Some swelling of the eyelids was observed at this time. The respiratory symptoms and cyanosis persisted after the tremors had ceased. The pig rose to its feet and moved unsteadily if stimulated, but would lie down again almost immediately. Animals that had shown severe symptoms recovered rapidly. Two pigs (Nos. 9 and 10) (Table 2) which were allowed to survive after having shown severe symptoms for 4 hours following an injection on the 10th day, recovered after 30 minutes and accepted a small amount of food at the end of that time.

When the symptoms were moderate, congestion of the skin and cyanosis were usually marked but the respiratory symptoms were less acute. Defaecation occurred, but none of the animals vomited. Muscular tremors were milder and occurred for shorter periods.

When symptoms were mild, little or no congestion of the skin occurred. Heavy and rapid breathing was accompanied by coughing and yawning. The animals showed stiffness and incoordination, and preferred to lie down. Recovery was more gradual in animals which had shown mild or moderate symptoms and none of these animals accepted food until the following day.

Three unsensitized control pigs were given 1 g. of egg albumin intravenously, followed by a further 1 g. subcutaneously 30 minutes later. No symptoms developed and the animals were killed after 2 hours.

POST-MORTEM LESIONS (ACTIVE ANAPHYLAXIS)

Lesions varied with the severity and duration of symptoms. Pigs developing mild anaphylaxis showed no evidence of oedema.

Lesions resembling naturally occurring oedema disease (Shanks, 1938; Timoney, 1950) developed in five animals that had shown moderate symptoms. All these animals showed oedema of the face, eyelids and abdominal wall. There was a marked excess of serous fluid in the pericardial and peritoneal cavities. The stomachs of three of these animals showed oedema of the submucosa which extended throughout the cardiac and fundic zones. The wall of the intestine was thickened and showed occasional patches of congestion in the mucosa; in some cases there was obvious oedema. Oedema occurred in the mesentery, particularly between the coils of the colon. The mesenteric veins and lymphatics were engorged, and the accompanying lymph nodes were oedematous. There was often oedema of the walls of the gall bladder and major bile ducts.

In severely affected animals there was both oedema and haemorrhage in the stomach (Fig. 1). Haemorrhages were confined to the fundic zone in one animal, and in another

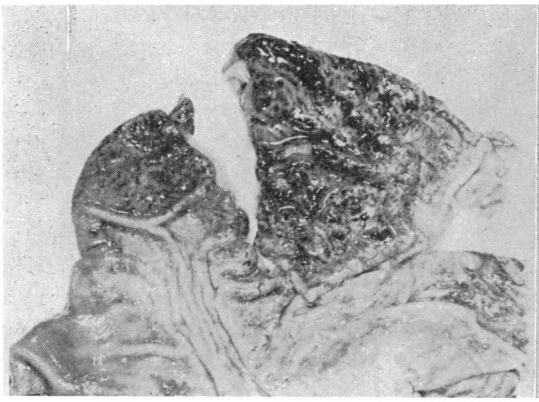

FIG. 1

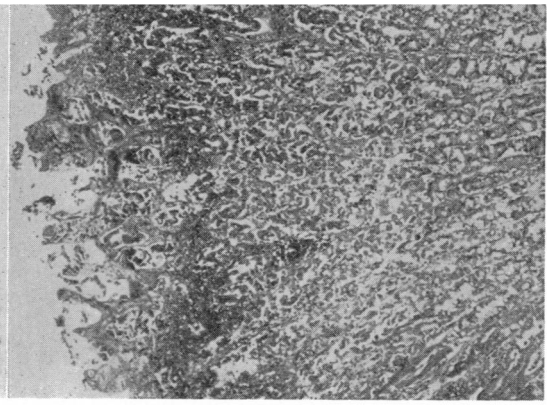

FIG. 2

FIG. 1. Anaphylaxis to egg albumin. Haemorrhage and oedema in the stomach.

FIG. 2. Anaphylaxis to egg albumin. Haemorrhage and necrosis in the gastric mucosa. Haematoxylin and eosin. ×42 approx.

occupied the greater part of the cardiac and fundic zones. In the two pigs that developed catarrhal enteritis the mesenteric lymph nodes were enlarged and haemorrhagic.

CONTROLS (POST-MORTEM)

There were no lesions in these animals.

HISTOLOGY (ACTIVE ANAPHYLAXIS)

Stomach

Haemorrhagic lesions showed desquamation and necrosis of the epithelium (Fig. 2). The endothelial cells lining blood vessels, particularly arteries, were swollen. There were thrombi in some of the veins. The mucosa was infiltrated with lymphocytes, plasma cells and eosinophils. Many plasma cells had a swollen cytoplasm and contained Russell bodies. These cells were also present in the oedema fluid especially near the large blood vessels (Fig. 3).

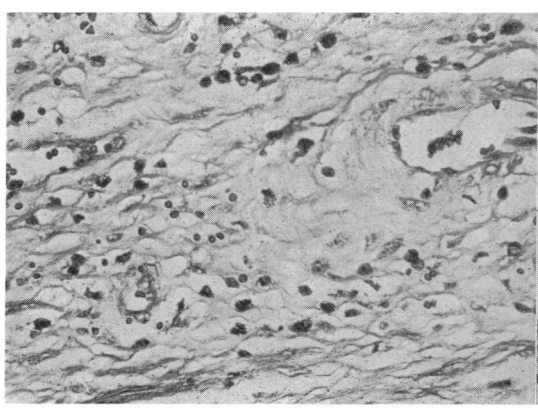

FIG. 3

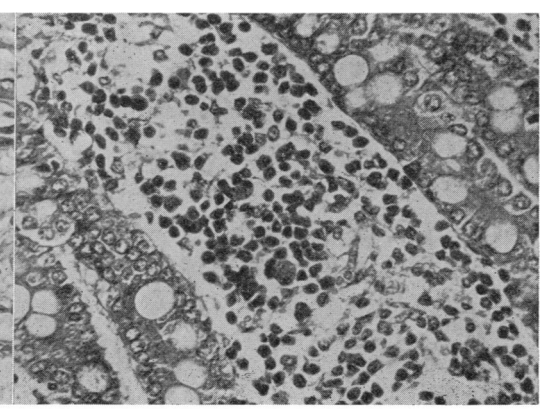

FIG. 4

FIG. 3. Anaphylaxis to egg albumin. Cellular infiltration in the oedema fluid. Haematoxylin and eosin. ×290 approx.

FIG. 4. Anaphylaxis to egg albumin. Cellular infiltration in mucosa of small intestine. Haematoxylin and eosin. ×290 approx.

In the absence of gross haemorrhage the cellular infiltrations were similar but less dense.

Intestine

Cellular infiltrations similar to those found in the stomach were present in the mucosa (Fig. 4). The lymphoid follicles were hyperplastic. Plasma cells were particularly numerous in catarrhal enteritis.

Mesenteric Lymph Nodes

There was hyperplasia of the germinal centres. Mitotic figures were seen in some cells; others showed pyknotic nuclei. There were numerous plasma cells in the medullary cords.

Lungs

The lesions were similar to those found in guinea-pigs (Thomlinson and Buxton, 1962). There was emphysema, congestion, broncho-constriction and peribronchial oedema (Fig. 5).

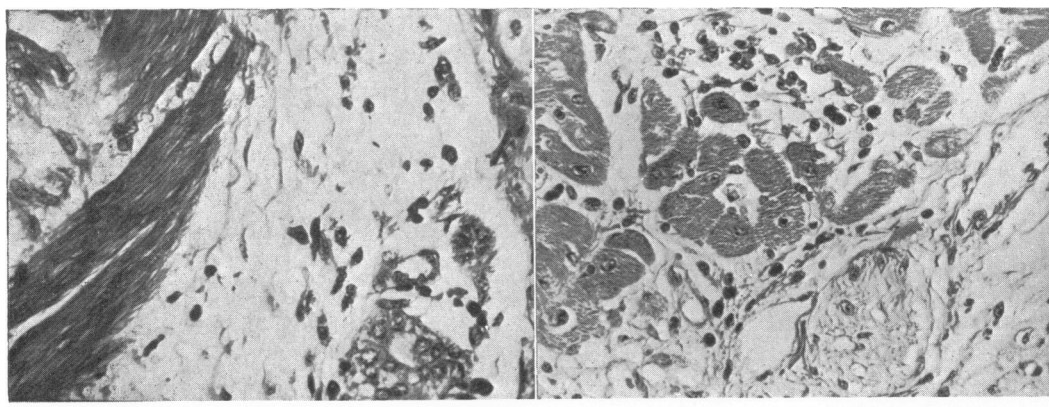

FIG. 5 FIG. 6

FIG. 5. Anaphylaxis to egg albumin. Peribronchial oedema. Haematoxylin and eosin. ×290 approx.

FIG. 6. Anaphylaxis to egg albumin. Oedema, cellular infiltrations and degenerative changes in myocardium. Haematoxylin and eosin. ×290 approx.

Liver

The connective tissue of the portal tracts was oedematous. The oedematous lesions were infiltrated with lymphocytes, plasma cells and eosinophils. Many of the Kupffer cells and hepatic parenchymal cells were swollen.

Heart

In the majority of pigs there were subendocardial haemorrhages. There was oedema and haemorrhage of the interstitial tissue of the myocardium; this was most marked near the large blood vessels. These areas were infiltrated with lymphocytes, plasma cells and eosinophils (Fig. 6). In some areas there was degenerative change in cardiac muscle cells.

In those pigs which were used for antiserum production and had received repeated

doses of egg albumin over a long period, hyperplasia of the spleen and lymph nodes was well marked. Plasma cells and eosinophils were particularly numerous in the gastro-intestinal mucosa and in the portal tracts of the liver. Fibroblasts had proliferated in these areas and there was an increase in perilobular fibrous tissue in the liver.

REVERSED PASSIVE ANAPHYLAXIS TO *E. coli*

The results of these experiments are shown in Table 3. The doses of antigen and antiserum for pig No. 14, and for the control animals, were based on the ratio of dose to body weight used in pig No. 13.

TABLE 3

SYMPTOMS AND LESIONS IN PIGS AS A RESULT OF REVERSED PASSIVE ANAPHYLACTIC SHOCK WITH *Escherichia coli* (O138)

Pig No.	*Weight (kg.)*	*Doses for sensitization and passive shock*		*Symptoms*		*Macroscopic lesions*
		Antigen 1/10 (ml.)	*Antiserum (ml.)*	*Time for development*	*Severity*	
11	48·0	5·0	40*	Gradually over 5 minutes	Moderate	Slight oedema in stomach wall. Congestion of small intestine. Oedema of gall bladder.
12	46·0	5·0	60	Gradually over 40 minutes	Moderate	Slight oedema in stomach wall. Thickened areas in mucosa of small intestine. Submucous oedema in colon. Oedema of gall bladder.
13	49·5	7·5	60	1 minute	Severe	Oedema and small haemorrhagic lesions in stomach. Intense congestion in small and large intestine. Oedema of gall bladder.
14	25·3	3·8	31	1 minute	Severe	Oedema in stomach wall. Intense congestion of small and large intestine. Marked oedema of gall bladder.
Control 1	25·0	—	31	—	—	—
Control 2	22·7	—	28	—	—	—
Control 3	27·5	4·2	—	—	—	—
Control 4	29·3	4·4	—	10 minutes	Mild	Slight oedema in stomach. Oedema of gall bladder.

* This animal received a further 20 ml. of antiserum intravenously 15 minutes after the shocking dose of antiserum.

SYMPTOMS OF REVERSED PASSIVE ANAPHYLAXIS

The symptoms were similar to those observed in pigs challenged with egg albumin, except that a more prolonged reaction occurred and there were no signs of recovery

after 4 hours when the animals were killed. In the severely affected pigs, generalized muscular tremors occurred 15 minutes after the animals had received the antiserum. These bouts of trembling continued for periods of from 15 to 30 minutes and recurred at intervals of about 30 minutes. Trembling was accompanied by occasional kicking movements of the hind limbs. Retching, defaecation, coughing and yawning occurred at intervals and often preceded the attacks of trembling. Milder symptoms included slight trembling followed by more severe attacks 30–60 minutes later. The control pig (Control No. 4) developed symptoms of dyspnoea and general dullness after 10 minutes, followed by muscular tremors. The animal recovered after 3 hours.

POST-MORTEM LESIONS (REVERSED PASSIVE ANAPHYLAXIS)

The two animals that developed the most severe symptoms (Table 3) showed the most marked lesions in the stomach and intestines. In the stomachs of both animals the oedematous lesion was situated in the submucosa of the cardiac zone and was about 4 mm. thick. The greater part of the duodenum and jejunum, and the whole of the large intestine was severely congested. In other areas of the intestine the Peyer's patches were intensely haemorrhagic (Fig. 7). All the animals showed oedema of the gall bladder and a

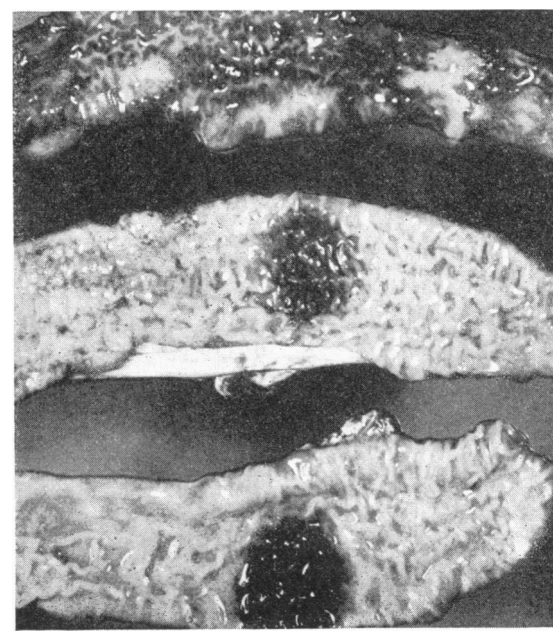

FIG. 7. Reversed passive anaphylaxis to *Escherichia coli*. Haemorrhagic lesions in the intestines.

marked excess of serous fluid in the peritoneal and pericardial cavities. The mesenteric lymph nodes were enlarged, congested and oedematous.

HISTOLOGY (REVERSED PASSIVE ANAPHYLAXIS)

The histological lesions (Figs. 8–11) were identical with those already described in pigs suffering from active anaphylaxis to egg albumin.

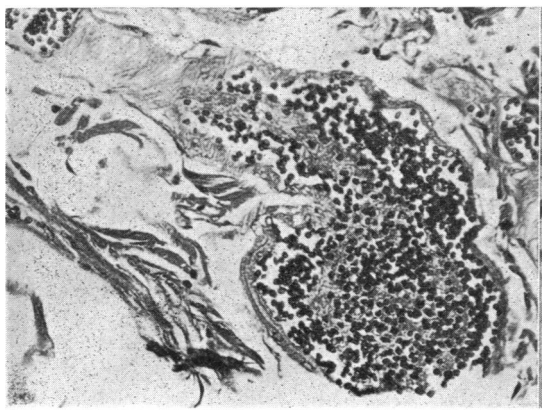

FIG. 8

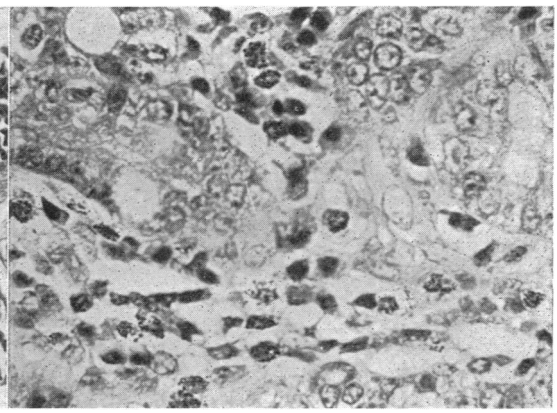

FIG. 9

FIG. 8. Reversed passive anaphylaxis to *Escherichia coli*. Thrombus in wall of small intestine. Haematoxylin and eosin. × 130 approx.

FIG. 9. Reversed passive anaphylaxis to *Escherichia coli*. Cellular infiltration in mucosa of small intestine. Haematoxylin and eosin. × 525 approx.

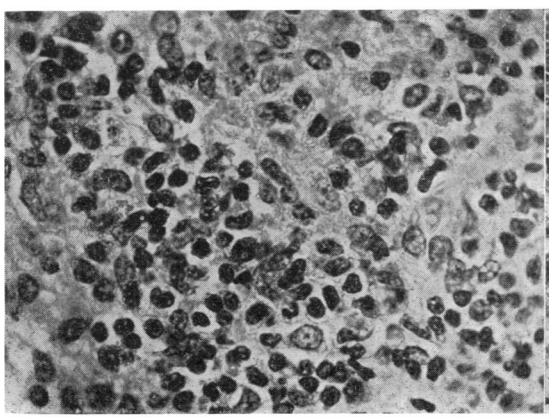

FIG. 10

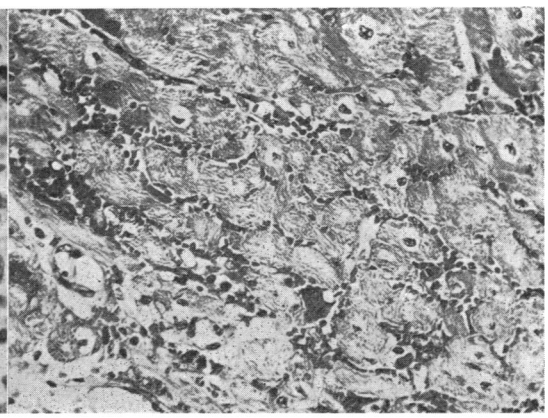

FIG. 11

FIG. 10. Reversed passive anaphylaxis to *Escherichia coli*. Plasma cell proliferation in lymph node. Haematoxylin and eosin. × 525 approx.

FIG. 11. Reversed passive anaphylaxis to *Escherichia coli*. Haemorrhage, oedema and cellular infiltration in myocardium. Haematoxylin and eosin. × 130 approx.

LESIONS IN CONTROL PIGS

Control pigs Nos. 1–3 (Table 3) showed no lesions. Pig No. 4 showed slight oedema of the stomach and oedema of the gall bladder. The mesenteric lymph nodes were enlarged and oedematous. There was an area of congestion in the caecum. Histologically, there were large numbers of plasma cells and eosinophils in the intestinal mucosa.

HUMORAL ANTIBODIES

Haemagglutination and antiglobulin haemagglutination tests were done on pig sera before the experiments were started. No haemagglutinating antibodies were detected but antiglobulin haemagglutinating antibodies to *E. coli* (O138) were present in all samples (Table 4). It was perhaps significant that the highest antibody titre occurred in the serum from the control pig (Control No. 4) which developed mild signs of anaphylaxis 10 minutes after being inoculated with antigen.

TABLE 4

ANTIGLOBULIN HAEMAGGLUTINATION TESTS FOR *Escherichia coli* (O138) ANTIBODIES IN SERA FROM PIGS BEFORE THEY WERE SUBJECTED TO REVERSED PASSIVE ANAPHYLAXIS WITH *Echerichia coli* (O138)

Experimental Pig No.	*Titre*	*Control Pig. No.*	*Titre*
11	1/160	1	1/640
12	1/640	2	1/160
13	1/1280	3	1/1280
14	1/2560	4	1/10240

BACTERIOLOGY

Bacteriological tests were made to see if any abnormal multiplication of *E. coli* occurred in the gastro-intestinal tract either before or during the experiments. A few colonies of haemolytic *E. coli* were isolated from rectal swabs from two pigs used for the experiments on reversed passive anaphylaxis. These organisms were found in one sample, 7 days before the experiments were carried out, and were identified as *E. coli* (O141). All the other *E. coli* strains isolated from rectal swabs were non-haemolytic and failed to agglutinate with antisera to the common serotypes associated with oedema disease and gastro-enteritis (Sojka, Lloyd and Sweeney, 1960).

Cultures of *E. coli* obtained at post-mortem examination were usually non-haemolytic and failed to agglutinate with any available antisera. In one animal (Pig No. 2, Table 1) half the colonies obtained from the caecum were haemolytic. In another (Pig No. 6, Table 3) two-thirds of the colonies from the ileum were of this type. In three other animals (No. 7, Table 1, Nos. 8 and 10, Table 3) a few haemolytic colonies were obtained from the ileum. All the haemolytic strains were *E. coli* (O8).

None of these organisms were present in unusually large numbers. These results confirm the observations of Campbell (1959), Miura *et al.* (1961) and Buxton and Thomlinson (1961) that these organisms are present in small numbers in clinically normal pigs and show that no significant multiplication occurred during the experiments.

DISCUSSION

The experiments described in this paper have shown that pigs are readily susceptible to anaphylactic shock from either an egg albumin or *E. coli* antigen–antibody system, and that the resulting reactions resemble those of naturally occurring oedema disease and haemorrhagic gastro-enteritis. In actively sensitized pigs, the severity of the symptoms and lesions elicited by a standard challenge dose of egg albumin varied with the route of

sensitization and the time which elapsed before challenge. Hypersensitivity developed more rapidly when the sensitizing antigen was given intravenously, and repeated doses of antigen were necessary to produce severe shock in 10–14 days. When pigs were subjected to reversed passive anaphylaxis from an *E. coli* antigen–antibody system, typical symptoms of bowel oedema commenced immediately after the antibody had been injected.

Characteristic lesions of oedema disease developed in animals that had shown moderately severe symptoms for a relatively short time. Mild symptoms, however, which occurred over a prolonged period resulted in the development of a catarrhal enteritis in the absence of stomach lesions. The severe and protracted symptoms resulting from reversed passive anaphylaxis to *E. coli* gave rise to typical lesions of haemorrhagic gastroenteritis.

Histologically, the various lesions produced as a result of these hypersensitive reactions were characteristic of an anaphylactic reaction, and resembled those previously described in the natural disease (Ohshima and Miura, 1961; Thomlinson and Buxton, 1962). Particularly significant features were damage to the blood vessels with haemorrhage and thrombosis, oedema of the gall bladder, gastro-intestinal tract, lungs and portal tract, and also the nature of the cellular infiltrations. Lymphocytes, plasma cells and eosinophils are the predominant cell types in protracted anaphylactic shock in guinea-pigs, and in naturally occurring oedema disease and haemorrhagic gastroenteritis. These cell types also occurred in the lesions described in this paper from experimentally shocked pigs. In these latter experiments cellular infiltrations, particularly of the portal areas of the liver and the myocardium, were more marked in cases of prolonged anaphylactic reactions and in those animals which had received a number of immunizing doses of antigen. The myocardial lesions were strikingly similar to those described by Harding (1960) in cases of mulberry heart disease, which is associated with multiplication of *E. coli* in the intestines (Thomlinson and Buxton, 1962), and may be an atypical form of oedema disease (Lamont, Luke and Gordon, 1950).

Cellular infiltrations, similar to those occurring in the intestines of diseased pigs, have been described in local hypersensitive reactions occurring in the intestines of other animal species (Kirsner, Elchlepp, Goldgraber, Ablaza and Ford, 1959; Goldgraber and Kirsner, 1959a, b; More and Movat, 1959) and are indicative of cellular antigen–antibody reactions. The massive accumulation of plasma cells in various sites after prolonged anaphylactic shock in pigs, occurs also in the natural disease and is suggestive of repeated stimulation of the reticulo-endothelial system with *E. coli* antigen. The formation of Russell bodies in these cells is characteristic of a secondary antigenic response (White, 1954). Increased numbers of eosinophils are also indicative of an antigen–antibody reaction in the tissues. Litt (1961) concluded from his experiments with various antigen–antibody systems in guinea-pigs that the accumulation of eosinophils is one of the consequences of an immunological reaction.

Further evidence that oedema disease and haemorrhagic gastro-enteritis are manifestations of an anaphylactic type of hypersensitivity is provided by the results of the antiglobulin haemagglutination tests recorded by Buxton and Thomlinson (1961) and also in this paper. Clinically normal pigs develop antibodies against serotypes of *E. coli* commonly associated with these diseases. In our experiments on reversed passive anaphylaxis, one of the control pigs (Control No. 4) showed mild symptoms of anaphylaxis after the injection of antigen. Typical oedematous lesions were observed in this carcase. It may be significant that not only did all the animals in this group have detectable serum antibodies (Table 4) but that the serum from this particular individual had the

highest antibody titre of the group. It seems probable that this pig was more hypersensitive to *E. coli* (O138) than the other members of the group.

The symptoms, lesions, cellular responses and antibody levels of pigs suffering from oedema disease and haemorrhagic gastro-enteritis are all indicative of a hypersensitive anaphylactic type of reaction. Serotypes of *E. coli* associated with these conditions, and probably other antigenically related enteric organisms, may be present in the intestines of healthy pigs. Polysaccharides from these organisms will be constantly absorbed from the intestines and removed by the reticulo-endothelial system (Ravin, Rowley, Jenkins and Fine, 1960). During this process antibodies are produced and can be detected by the antiglobulin haemagglutination test. Any assessment of the immune status of these animals is limited by the techniques used for demonstrating antibodies. Moreover, the results of tests for humoral antibody do not necessarily reflect, either quantitatively or qualitatively, the state of tissue antibodies (Garvey and Campbell, 1959). It is evident, however, that clinically normal pigs may have developed different degrees of hypersensitivity to serotypes of *E. coli* in the intestines. The sudden and rapid multiplication of any one of these serotypes may lead to increased absorption of bacterial polysaccharide by the tissues of these hypersensitive animals. The experimental evidence in this paper suggests that whether the reaction of the host leads to oedema disease, haemorrhagic gastro-enteritis or catarrhal enteritis depends upon the severity and duration of the hypersensitive reaction. This reaction will be determined by the immune status of the individual and by the rate of multiplication of *E. coli* in the intestines and the consequent rate of absorption of bacterial polysaccharide by the tissues.

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