HYPERSENSITIVITY IN YOUNG PIGLETS: ITS RELATION TO THE PATHOGENESIS OF *ESCHERICHIA COLI* DISEASE

B. J. SHREEVE AND J. R. THOMLINSON

Department of Veterinary Pathology, University of Liverpool

PLATE XXXIII

SHREEVE AND THOMLINSON (1970a and b) have shown that *Escherichia coli* disease in the young piglet has several features in common with oedema disease of the older pig, and that the lesions bear a close resemblance to those associated with anaphylactic reactions (Thomlinson and Buxton, 1962, 1963; Thomlinson, 1963). The work described in the present paper formed part of an investigation into the pathogenesis of *E. coli* disease in young piglets and its purpose was to determine whether exposure of pregnant sows to an antigenic substance may lead to hypersensitivity in their piglets. Hen-egg albumin was chosen for the immunisation of sows in these experiments, because this was an antigen to which the animals had not previously been exposed.

MATERIALS AND METHODS

Origin of animals. Pregnant Large White sows were obtained from a herd that was known to maintain a good standard of husbandry and health, and were maintained on a commercial sow diet. All piglets were separated from the sow at birth and before they had ingested colostrum. Blood samples were collected, then approximately half the piglets in each litter were returned to the sows for 24 hr; these were the "colostrum-fed" experimental group. The remainder of the piglets were housed separately in cages and were the "colostrum-deprived" group. Experiments on these piglets were carried out as soon as possible after birth.

Collection of blood samples and colostral whey samples. All blood samples from piglets were obtained from the anterior vena cava (Mackenzie, 1961) and a maximum of 4 ml of whole blood was withdrawn at one time. Blood samples from sows were obtained from an auricular vein. Whey was separated from fresh samples of colostrum by the method described by Sharpe (1965). Samples of serum and whey were stored at $-30^\circ$C.

Immunisation of sows. A 5 per cent. solution of hen-egg albumin was prepared in sterile normal saline and centrifuged to remove any undissolved particles; 5 ml of this solution was injected intravenously into an auricular vein in each of five pregnant sows. This injection was repeated with freshly prepared egg albumin solution after 7, 9, 11, 13, 15, 17, 19 and 26 days, the final injection being given 10 days before the expected farrowing date.

Intradermal tests in piglets. Preliminary experiments showed that as much as 50 mg hen-egg albumin dissolved in 0.1 ml normal saline could be injected intradermally into normal piglets whose dams had not been immunised, without provoking any reaction. Doses of 10 mg, 5 mg and 1 mg dissolved in 0.1 ml normal saline were chosen for use in the experiments. A 26-SWG hypodermic needle was used and the injections were made 5 cm below the dorsal border of the neck and 2.5 cm behind the base of the ear. Egg albumin was

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injected on the left side and 0.1 ml sterile normal saline was injected on the right side as a control. In addition, similar tests were carried out on a total of 28 piglets from the litters of three normal sows that had not been immunised.

*Intravenous challenge of piglets.* Immediately after the intradermal tests had been completed, piglets were challenged by injecting 500 mg egg albumin dissolved in 5 ml sterile normal saline into the anterior vena cava. In some experiments, pontamine sky blue 6BX was added to the challenge injection at a final concentration of 1.5 per cent. Piglets from sows SF8 and TJ10 were challenged by the intradermal route only, and those from sow A by the intravenous route only.

*Serological methods.* Antibodies to hen-egg albumin were detected by means of the tanned red blood cell technique described by Boyden (1951).

*Histological methods.* Piglets were killed by stunning and exsanguination 2 hr after intravenous challenge. Immediately after the piglets were killed, portions of skin from the intradermal test sites, lung, heart, liver, spleen, kidney, stomach, intestine and mesenteric lymph-nodes were fixed in 4 per cent. formaldehyde in saline and embedded in paraffin wax. Sections 5 μm thick were stained with haematoxylin and eosin.

*Bacteriological examinations.* Cultures from intestinal contents, liver, spleen, lung, kidney, and heart blood were made on 5 per cent. sheep blood agar and on MacConkey agar, and were incubated at 37°C for 24 hr. Representative colonies resembling *E. coli* were tested by slide agglutination against "OK" antisera.

RESULTS

**Intradermal challenge**

*Piglets from immunised sows*

Table I shows the results of intradermal tests carried out on colostrum-deprived and colostrum-fed piglets. Of the colostrum-deprived piglets observed at 30-min. intervals, four developed skin reactions 60 min. after the tests were carried out. These reactions reached their maximum size at 90 min. In the remainder, skin reactions developed at 90 min., except in the case of one piglet in which a reaction developed at 120 min. These reactions were oedematous with erythema towards the centre. Histologically, clusters of neutrophils, which are mainly perivascular in distribution, are present (fig. 1). Haemorrhage is also present in the lesion.

In colostrum-fed piglets, which were observed at 30-min. intervals, skin reactions with marked erythema and oedema developed at 60 min. and reached their maximum size at 90 min. except in one piglet whose reaction did not enlarge after 60 min. In another, some regression had occurred at 120 min. Histologically, perivascular accumulations of neutrophils are more dense than in lesions from colostrum-deprived piglets (fig. 2). In addition, clusters of neutrophils are scattered throughout the lesion, lymphocytes and eosinophils are present, and haemorrhage is marked.

No skin reactions or histological changes were observed in either group of piglets at the sites of injection with normal saline.

*Piglets from normal sows*

A total of seven colostrum-deprived piglets from the litters of three sows and five colostrum-fed piglets from two of these sows were tested intradermally
with 1-mg doses of egg albumin. A further ten colostrum-deprived and six colostrum-fed piglets from the same litters were tested intradermally with 5-mg doses. No skin reactions were observed in any of these piglets. Histologically, slight oedema and a few scattered neutrophils are observed at the

**Table I**

_Dermal reactions in neonatal piglets from immunised sows after intradermal injection of 0.1 ml egg albumin solution_

<table>
<thead>
<tr>
<th>Sow</th>
<th>Piglet</th>
<th>Egg albumin injected (mg)</th>
<th>Dermal reaction at time after challenge (min.)</th>
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<tr>
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<tr>
<td>6556</td>
<td>Colostrum-deprived</td>
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<td>-</td>
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<td></td>
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<td>2</td>
<td>-</td>
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<td></td>
<td>A2</td>
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<td>6</td>
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<tr>
<td></td>
<td></td>
<td>10</td>
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</tr>
</tbody>
</table>

- = Negative; + = 4–7 mm; ++ = 8–11 mm; +++ = 12–15 mm; ++++ = 16–22 mm.

test sites (fig. 3). In the colostrum-fed piglets, some of the neutrophils are perivascular in distribution.

**Intravenous challenge**

_Piglets from immunised sows_

Table II shows the severity of symptoms that developed in colostrum-deprived and colostrum-fed piglets from immunised sows after they were
challenged with an intravenous injection of 500 mg egg albumin. In all the colostrum-deprived piglets, the onset of symptoms was gradual. The first signs were slight trembling and incoordination, which became progressively more severe until, after 2 hr, all the piglets were unable to stand and lay trembling with occasional convulsive movements.

**Table II**

*Severity of symptoms in neonatal piglets from immunised sows after intravenous injection of 500 mg egg albumin in 5 ml sterile normal saline*

<table>
<thead>
<tr>
<th>Sow</th>
<th>Piglet</th>
<th>Severity of symptoms at time after challenge (min.)</th>
</tr>
</thead>
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</tr>
<tr>
<td>6556</td>
<td>Colostrum-deprived 1</td>
<td>+</td>
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<tr>
<td></td>
<td>Colostrum-deprived 2</td>
<td>+</td>
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<tr>
<td></td>
<td>Colostrum-deprived 3</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Colostrum-deprived 4</td>
<td>-</td>
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<tr>
<td>6449</td>
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<td>+</td>
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<td></td>
<td>Colostrum-deprived 6</td>
<td>+</td>
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<tr>
<td></td>
<td>Colostrum-deprived 8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Colostrum-deprived 9</td>
<td>-</td>
</tr>
<tr>
<td>6556</td>
<td>Colostrum-fed 5</td>
<td>+++</td>
</tr>
<tr>
<td></td>
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<td>+++</td>
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<tr>
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<td>Colostrum-fed 7</td>
<td>+++</td>
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<td>+++</td>
</tr>
<tr>
<td>6449</td>
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<td>+++</td>
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<tr>
<td></td>
<td>Colostrum-fed 4</td>
<td>+++</td>
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<tr>
<td>A</td>
<td>Colostrum-fed 6</td>
<td>+++</td>
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<tr>
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<tr>
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<td>Colostrum-fed 8</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Colostrum-fed 10</td>
<td>+++</td>
</tr>
</tbody>
</table>

- = Negative; + = incoordination, trembling, hyperpnoea (mild); ++ = vomiting, diarrhoea (moderate); +++ = collapse (severe).

In the colostrum-fed piglets, severe shock developed immediately. The first sign was violent excitement followed by a period of apnoea that lasted for several seconds. Breathing recommenced and was laboured at first, then hyperpnoea developed. Within 5 min. after challenge, all the piglets were trembling and lying on their sides with their eyes closed. They were very incoordinated when forced to stand. Straining occurred and yellow faeces, porridge-like in consistency, were voided. The faeces became more fluid until, after 30 min., diarrhoea was observed in all except one piglet. These symptoms continued for 60 min., when a gradual recovery occurred. After 120 min. all the piglets were able to stand but most showed some incoordination. Three colostrum-deprived and two colostrum-fed piglets, which received intravenous injections of normal saline as controls, remained normal.
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Piglets from normal sows

No clinical response was observed in seven colostrum-deprived piglets from the litters of three normal sows or in six colostrum-fed piglets from two of these sows when they were given an intravenous injection of 500 mg egg albumin.

Serological findings

In all cases, the serum and colostral whey antibody titres of the immunised sows were 2560 when the sows farrowed. No antibodies were detected in sera from the colostrum-deprived piglets. The serum antibody titres of the colostrum-fed piglets from these sows were all within the range 640–1280 immediately before challenge and 320–640 after intravenous challenge. No antibodies were detected in sera from the normal sows or their piglets.

Gross lesions

Piglets from immunised sows

Colostrum-deprived piglets. All these piglets had an excess of clear fluid in the pleural and pericardial cavities. Petechial haemorrhages and a few ecchymoses were present in the lungs of four of the eight piglets, and in three of these some scattered petechiae were observed on the epicardium. In the majority of these piglets, the lungs, livers, spleens and corticomedullary junctions of the kidneys were congested. No gross abnormalities were observed in the stomachs or intestines except that oedema was present in the mesentery of the spiral colon and in the mesenteric lymph-nodes. Oedema affecting the wall of the gall bladder occurred in three piglets and the ureters were oedematous in two. Subcutaneous oedema up to 1 cm thick, affecting the ventral abdominal wall and inguinal region, was observed in all the piglets. In piglets that received pontamine blue with the challenge dose, the oedema fluid in all these areas was stained blue. In control piglets given an injection of normal saline and pontamine blue, the only lesion was slight oedema of the ventral abdominal wall. The oedema fluid in these piglets was not stained.

Colostrum-fed piglets. The gross lesions in these piglets were similar to those in the colostrum-deprived group, except that petechial haemorrhages and ecchymoses were more numerous in the lungs and epicardium, and occurred in the majority of piglets. Also, petechial haemorrhages were numerous in the mucosa of the gastric fundus and duodenum, and the mucosa of the small intestine was hyperaemic. The mesenteric blood vessels and the lymphatic vessels were engorged. In some cases, petechial haemorrhages were present in the mesenteric lymph-nodes and in others the nodes were haemorrhagic. Subcutaneous oedema was observed in only four of the nine piglets. Oedema affecting the gall-bladder wall and the portal area of the liver was observed in three piglets and much haemorrhage occurred in association with this lesion. Marked subserous oedema was present in the region of the lesser curvature of the stomach in two piglets. In piglets that received pontamine blue at the
time of challenge, the oedema fluid was stained blue. No lesions were observed
in control piglets given an injection of normal saline.

**Piglets from normal sows**

No lesions were observed in colostrum-deprived and colostrum-fed piglets
from normal sows after challenge with egg albumin.

**Histological findings**

**Piglets from immunised sows**

*Lungs.* Pulmonary emphysema is extensive in all the colostrum-fed piglets.
Discrete areas of alveolar congestion are present, and there are macrophages
in the alveoli. Oedema and haemorrhage are present in the interlobular septa.
In colostrum-deprived piglets, there are discrete foci of haemorrhage. Pul-
monary emphysema occurs only in a few cases and is less extensive.

*Heart.* Foci of haemorrhage in the myocardium are more frequent in the
colostrum-fed piglets.

*Liver.* Lymphatics are engorged in the portal spaces and some infiltration
of the surrounding connective tissue with polymorphs, lymphocytes and macro-
phages has occurred.

*Stomach.* In colostrum-fed piglets, some focal shedding of glandular
epithelium has occurred, and the affected areas of mucous membrane are infil-
trated with polymorphs, lymphocytes and eosinophils. Subserous oedema is
present in the region of the lesser curvature, and the lamina propria and sub-
mucosa of the fundic zone are oedematous. In the colostrum-deprived piglets,
the only significant lesion is oedema of the fundic zone.

*Intestine.* No significant lesions are present in the small intestine. In the
colostrum-deprived piglets, lymphocytes and red blood cells are numerous
in the oedematous areas of the mesocolon.

*Mesenteric lymph-nodes.* The subcapsular lymphatics are dilated. In
colostrum-deprived piglets, eosinophils are numerous in the cortex, but in the
colostrum-fed group an increase of polymorphs and macrophages has also
occurred.

No lesions are present in control piglets given an injection of normal saline.

**Bacteriological findings**

No potentially pathogenic serotypes of *E. coli* were isolated from any of
the piglets.

**DISCUSSION**

The results of these experiments show that sows may confer hypersensi-
tivity on their young, and that this may occur by means other than transfer
of antibody in the colostrum. Hen-egg albumin, when injected intradermally,
provoked no macroscopic skin reaction and very little histological response in neonatal colostrum-deprived or colostrum-fed piglets from normal sows. In contrast, oedematous and erythematous skin reactions with a marked histological response developed in colostrum-deprived piglets from immunised sows. In colostrum-fed piglets from these sows, skin reactions developed marginally earlier and showed a more marked cellular response. This difference between these two groups of piglets was shown more clearly after intravenous challenge, when the symptoms shown by the colostrum-fed group resembled classical acute anaphylactic shock.

Chase (1947) described erythema and oedema in immediate hypersensitivity reactions and considered that the antibodies concerned were similar to those responsible for anaphylactic hypersensitivity. Similar reactions have been reported in actively and passively sensitised guinea-pigs (Dienes and Mallory, 1932). Infiltration with polymorphonuclear leucocytes is a feature of immediate hypersensitivity reactions (Gell and Hinde, 1954) and occurs also in the early stages of Arthus reactions, which develop in the presence of circulating antibodies (Opie, 1924; Culbertson and Kent, 1935; Fischel and Kabat, 1947). No circulating antibodies were detected in the colostrum-deprived piglets, and it would thus appear that the skin reactions that occurred in this group of animals were of the immediate hypersensitivity type.

The acute reaction to intravenous challenge observed in colostrum-fed piglets contrasts with the slow and prolonged reaction in the colostrum-deprived group. The degree of hypersensitivity shown by the two groups of piglets would depend not only on the dose of antibody acquired but also on the duration of the latent period for fixation of antibody. When the dose of antibody is large, as was the case in the colostrum-fed piglets, the latent period for maximal sensitisation will be short (Benacerraf and Kabat, 1949). It is probable that sensitisation in the colostrum-deprived piglets occurred as a result of prolonged exposure to small amounts of antibody. The relatively slow anaphylactic reaction that occurred in these animals is consistent with a lower level of sensitisation, and the clinical signs were reminiscent of those that occur in pigs after the administration of E. coli endotoxin (Thomlinson and Buxton, 1963).

Under normal circumstances, absorption of E. coli antibodies from colostrum by the piglet is limited to 24 hr after birth (Speer et al., 1959). It is probable, therefore, that the titre of acquired antibodies will have reached its maximum 24-48 hr after birth. By this time, the natural E. coli flora of the intestine will have become established (Smith, 1965). If, at that time, one serotype of E. coli became dominant, a relatively large amount of endotoxin of that particular serological affinity would be absorbed and exert a challenge effect. Passive sensitisation from the colostrum could therefore explain the relatively high incidence of E. coli disease in piglets 2-4 days old. A higher titre of colostral antibody relative to the challenge dose of endotoxin would have a protective effect.

Hypersensitivity in the colostrum-deprived piglets was probably due to transference of small amounts of maternal antibody across the placental
barrier. An alternative mechanism would be transference of antigen to the pig foetus which is itself immunologically competent after the 80th day of gestation (Binns, 1967). Evidence of transplacental transference of antibody in the sow has been provided by the experiments of Segre and Kaeberle (1962) and Myers and Segre (1963). On the other hand, Kim, Bradley and Watson (1966) considered that the normal sow's placenta prevented any transference of antibody to the foetus, but demonstrated that multiple injections of antigens containing *E. coli* endotoxin could lead to transference of antibody, presumably as a result of damage to the placental barrier. In our own investigations in a herd affected with *E. coli* disease (Shreeve and Thomlinson, in press) we found an increase of potentially pathogenic *E. coli* serotypes, accompanied by evidence of endotoxin absorption, in pregnant sows in association with stress occasioned by routine husbandry procedures before parturition. These factors could have led to placental damage and sensitisation of the piglets. In the present work, the final sensitising injection was given 10 days before parturition and it is possible that placental damage could have occurred at this time. On the other hand, it is possible that the failure of some authors to demonstrate antibody in serum from colostrum-deprived piglets may have been due to its fixation in the tissues.

The oedematous lesions at the various sites closely resembled those observed in anaphylaxis in older pigs (Thomlinson and Buxton, 1963) and the greater severity of the pulmonary lesions in the colostrum-fed group was consistent with the higher degree of sensitisation of these piglets. In naturally occurring oedema disease of weaned pigs, oedematous lesions affecting the subserous tissue of the lesser curvature of the stomach, the mesocolon, lungs and gall bladder have all been described in addition to the classical lesion at the greater curvature of the stomach (Timoney, 1950, 1956; Ohshima and Miura, 1961; Thomlinson and Buxton, 1962). Similar lesions have been described in natural cases of *E. coli* disease in young piglets (Shreeve and Thomlinson, 1970a and b), and oedematous lesions affecting the ventral abdominal wall have also been described in association with *E. coli* infection (Thomlinson and Buxton, 1962). Edwards (1961) described a similar lesion in association with a protein deficiency, and the occurrence of slight oedema at this site in some of our colostrum-deprived piglets suggests that such a lesion may arise from this cause. However, it is clear from the results of our experiments with pontamine blue that this lesion may also occur as a result of increased vascular permeability arising from an anaphylactic reaction. It is possible, therefore, that anaphylaxis may play a part in the pathogenesis of *E. coli* disease in young piglets, whether or not they have received colostrum. Colostrum may sensitise or protect—the result depends upon its antibody titre in relation to the challenge dose of endotoxin.

**Summary**

Pregnant sows were immunised with hen-egg albumin and intradermal tests were carried out on their piglets after birth. Colostrum-deprived piglets from immunised sows were hypersensitive to egg albumin, and it is postulated
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PLATE XXXIII

**FIG. 1.**—Dermal reaction in a colostrum-deprived piglet from an immunised sow following intradermal challenge with 1 mg egg albumin. Haematoxylin and eosin (HE). × c. 24.

**FIG. 2.**—Dermal reaction in a colostrum-fed piglet from an immunised sow following intradermal challenge with 1 mg egg albumin. HE. × c. 24.

**FIG. 3.**—Dermal reaction in a colostrum-deprived piglet from a normal sow following intradermal challenge with 1 mg egg albumin. HE. × c. 24.
that these piglets may have been sensitised through the transference of small amounts of antibody across the placental barrier. Antibody present in colostrum also conferred passive sensitisation. The symptoms and lesions that developed in the piglets after intravenous challenge were characteristic of anaphylactic shock, and were compared with those found in naturally occurring E. coli infection.

We wish to acknowledge the financial support of the Pig Industry Development Authority. Our thanks are due to Professor D. L. Hughes for helpful advice. We are grateful to Mrs M. W. Harling for technical assistance and to Mr G. Weston for the photomicrographs.

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