THE RESPONSE OF YOUNG PIGS TO ESCHERICHIA COLI ENDOTOXIN

by

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INTRODUCTION

The mechanism of disease production by Gram negative bacteria has been the subject of many research projects in past years and still the pathogenesis of disease produced by these organisms is not clearly understood. Endotoxins are produced by these bacteria and their effect is thought to be either a direct physiological response termed endotoxic shock, or an anaphylactic reaction, or a combination of these. Endotoxic shock is not thought to be immunologic in character whereas anaphylaxis would involve an interaction of endotoxin with homologous antibodies present in the affected animal.

Swine are known to be especially susceptible to endotoxin-producing Gram negative organisms. There have been a number of studies in which pigs have been given filtrates of Escherichia coli cells that would be expected to contain endotoxin but in addition would contain many other cellular materials. Therefore, it was believed that it would be desirable to prepare a purified Escherichia coli endotoxin and characterize its effect on pigs. The results obtained from this experiment could be used to provide additional information about the mechanism of the endotoxin reaction in pigs and could be compared with reports in the literature of the effects of endotoxin on other species of animals. In

addition, the results could possibly be related to edema disease of swine which is a common field problem of which the pathogenesis is not fully understood.

LITERATURE REVIEW

Endotoxins

Endotoxins are the toxic principles of the cell wall of many pathogenic and nonpathogenic bacteria (Westphal and Luderitz, 1961), and Braude (1964) reported that endotoxins are produced by all Gram negative bacteria, even noninfectious soil organisms. While the endotoxin produced by Escherichia coli and other Gram negative bacteria has been purified and used in many experiments, its biological activity continues to puzzle investigators (Stetson, 1961).

Endotoxin was first isolated by Boivin and Mesrobeanu (1935) by extraction with trichloroacetic acid. Noll and Braude (1961a, 1961b) modified this procedure to extract endotoxin from Escherichia coli. Westphal and Luderitz (1954) extracted endotoxin with phenol, and the method has been modified by Tauber and Russell (1961). A third general method of purifying endotoxin which utilizes aqueous ether has been presented by Ribi et al. (1959).

The cell wall of Escherichia coli organisms, according to Weidel et al. (1960), is made up of an external thick amorphous lipoprotein layer, an internal mucopolypeptide layer principally responsible for maintaining size and shape of the cell, and the middle lipopolysaccharide layer containing the endotoxic O antigen. Endotoxins comprise about one fourth of the dry weight of the bacterial cell wall or about

8 to 12 per cent of the dry weight of the cell (Fukushi, 1964). Endotoxins, as normally isolated, are very large molecules containing lipids, polysaccharides, and protein or peptide-like substances (Ribi et al., 1961a, 1961b). However, endotoxins may be aggregations of hapten into micells and gells (Milner et al., 1963). Endotoxin is quite stable, remaining toxic after exposure to 60 C and storage at room temperatures (Merchant and Packer, 1961). Endotoxins are also resistant to tryptic digestion but labile to mild acid hydrolysis (Burrows, 1951).

Many warm-blooded animals are susceptible to the action of bacterial endotoxins including, in addition to man, the mouse, rat, rabbit, sheep, goat, horse, dog, pig, and a number of birds, while in general the cold-blooded animals are not affected (Burrows, 1951). The rabbit, according to Burrows (1951), is perhaps the most susceptible animal on the basis of body weight. The mouse is quite uniformly susceptible to a medial lethal dose of 0.1 to 0.5 mg. of purified endotoxin (Burrows, 1951).

Endotoxins all produce the same general clinical signs in experimentally exposed animals, regardless of which bacterium furnishes the toxin (Braude, 1964; Burrows, 1951; Thomas, 1958). Tauber and Russell (1961) found that there are marked chemical but not toxicological differences between endotoxins from different bacteria. The culture medium and

the method of extracting the endotoxin also affect the chemical structure of the endotoxin (Fukushi et al., 1964).

The role of different constituents in endotoxin, such as lipids, polysaccharides and proteins, is presently not fully understood. Noll and Braude (1961a) believed that toxicity was associated with the lipid portion of the endotoxin molecule, that its immunogenic potency was associated with the protein, but that serological specificity resulted from the polysaccharide moiety. Ribi et al. (1959, 1961b) showed that lipids isolated from endotoxins lost most of the activity associated with endotoxins and that endotoxins of very low lipid content were as potent as those prepared by other methods. They believed that the polysaccharide portion accounted for most of the toxicity. These observations and other evidence make it seem most likely that the entire molecule is essential for the action of endotoxin in producing disease (Braude, 1964).

The mode of action of endotoxin in animals is still not clear, but various investigators have proposed either a direct physiological effect or an anaphylactic reaction as being responsible for the toxicity (Braude, 1964; Stetson, 1955). Stetson (1961) compared the effects of endotoxin and tuberculin in rabbits and found that these substances produced similar responses. He concluded from these results that the reaction to endotoxin was allergic in character. Braude (1964) repeated these tests and found several small but significant

differences between the effects of endotoxin and of tuberculin. Weil and Spink (1957) compared endotoxic shock and anaphylactic shock in dogs and found that in both forms of shock there was an explosive release of histamine into the blood. They concluded that endotoxic shock is closely related to but a different form of anaphylactoid shock. Westphal and Luderitz (1961) suggested that two unrelated mechanisms may occur in endotoxic shock, a primary physiological toxicity and an immunological reaction manifested only in animals presensitized to endotoxins by prior exposure to Gram negative bacteria. Evidence for this was provided by the fact that animals maintained free of ordinary bacterial pathogens were found to be much more resistant to endotoxin than normally reared animals (Schaedler et al., 1962).

Antibodies to endotoxin are found in almost all normal animals and this is probably a result of symbiotic bacteria containing endotoxin that live in the intestinal tract of mammals (Braude, 1964). Following the injection of endotoxin into animals having antibodies to endotoxin, both the antibodies and complement disappeared from the blood (Braude, 1964). When antibody-containing serum was injected into rabbits, it did not lower their resistance to large doses of endotoxin, but "vaccinating" doses of endotoxin did increase the rabbits' resistance (Braude, 1964). Sweeney et al. (1960) found that antibodies did not give protection to toxic doses

of portions of Escherichia coli cells. Soltys (1963) proposed that antibodies are not produced for the highly toxic portion of the endotoxin molecule and therefore, do not fully neutralize endotoxin.

The toxic manifestations of lethal doses of endotoxin in experimental animals have been described by Thomas (1954) and follow a quite definite pattern. There was usually a latent period, often lasting one hour, during which animals appeared well. They then became less active, were disinclined to feed, and showed increasing generalized weakness. During the next few hours they became ataxic and finally were unable to stand. Fever appeared after 3 or 4 hours in some animals and hypothermia often developed in the terminal stages. Respiratory distress indicated by rapid, labored breathing was a conspicuous clinical sign. Some species developed retching and all species showed a profuse fluid diarrhea. In the agonal stage, the animals became completely immobilized and unresponsive to stimuli, and there often was generalized convulsive seizures. Death occurred most often between 4 and 24 hours following injection of the endotoxin.

Escherichia coli endotoxin injected into dogs caused excitement, gagging, apathy, a fall in blood pressure, and finally coma (Weil and Spink, 1957). Dogs and cats given Escherichia coli endotoxin by Hinshaw et al. (1957) showed respiratory distress as indicated by rapid, labored breathing. Postmortem examination of these dogs showed foci of edema,

congestion and hemorrhage in the lungs and an increase in weight of the lungs. Hinshaw et al. (1957) found that endotoxin caused constriction of pulmonary venules and/or small veins resulting in a rise in pressure in the pulmonary artery without a rise in left atrial pressure. The sudden fall in blood pressure in animals given endotoxin was explained by Gilbert (1960) by the fact that there was a sharp decrease in cardiac output due to a decreased venous return. This decrease in venous return resulted from a redistribution of total blood volume which involved a pooling of blood in the liver, lung, and intestines. Another early response to the injection of endotoxin often was a rise in body temperature. Braude (1964) reported that this probably results in a slowing of the circulation of blood through the skin, which reduces the normal rate of radiation of body heat from the surface.

The administration of Escherichia coli endotoxin to dogs resulted in a pronounced response of the gastro-intestinal tract including vomition and evacuation of the bowels (Weil and Spink, 1957). Turner and Berry (1962) showed that vomition is stimulated by endotoxin in species of animals capable of vomiting and that gastric emptying is inhibited in all species by suppression of gastric motility. The initial effect of endotoxin on the intestine was a pooling of blood associated with a rise in portal vein pressure and a significant increase in venous diameter (Hinshaw and Nelson, 1962). Elevated capillary hydrostatic pressure may result in intestinal edema

according to Meyer and Visscher (1962).

Experimental evidence indicated that the effect of endotoxin on the kidney was primarily vascular and not nephrotoxic (Gillenwater et al., 1963). Dogs given Escherichia coli endotoxin showed an average of a 25 per cent decrease in weight of kidneys which was independent of blood pressure changes (Hinshaw and Bradley, 1957). They observed that the capsular surface became wrinkled and the kidney became smaller in size. Renal dysfunctions observed by Hinshaw et al. (1959) included anuria, oliguria, hematuria, and uremia resulting from the effects of systemic hypotension and consequent renal ischemia.

The intravenous administration of endotoxin quickly results in hematologic changes in experimental animals. Experiments with radioactive tracers have demonstrated that the endotoxin almost instantly enters white blood cells and acts to drive them out of circulation (Braude et al., 1955a). Within a few minutes after injection of endotoxin, 60 per cent of the white blood cells disappeared from the blood stream (Braude, 1964; Weil and Spink, 1957; Davis and Smibert, 1963). Braude (1964) showed that the first to go are granulocytes and that during the next few hours the lymphocytes gradually declined in number. After the leukocytes disappear from the blood, they have been observed in the tissues of the lung and other organs (Smith et al., 1957; Braude, 1964). Injection of Escherichia coli endotoxin into animals caused a rapid decrease

in levels of circulating platelets (Roy et al., 1962). Herring and Herion (1963) showed that endotoxin is almost completely removed from the blood within ten minutes. A few hours after the injection of the endotoxin, granulocytes were observed to suddenly flood into the blood stream, increasing the number of white blood cells to an abnormally high level (Davis and Smibert, 1963; Braude, 1964). The outpouring of white cells probably represented a release of reserves from the centers of blood-cell formation and storage in the bone marrow (Braude, 1964). Carozza and Hills (1962) showed that endotoxins cause an increase in the blood coagulation time of rabbits independent of the hypotensive action of endotoxins. Davis and Smibert (1963) reported that after injection of endotoxin, a decrease in the blood glucose level and a decrease in the serum concentration of arginine, aspartate, glutamate, methionine, alanine, serine, proline, tyrosine, phenylalanine, leucine, isoleucine, and lysine occurred. The serum concentration of ammonia, ornithine, histidine, glycine, cystine, and urea increased.

The administration of endotoxin to experimental animals resulted in histamine release into the bloodstream (Hinshaw et al., 1958, 1961). Schayer (1960) showed that administration of endotoxin caused an increase in the activity of histidine decarboxylase which changes histidine to histamine. Comparative studies of the haemodynamic actions of histamine and endotoxin showed a definite similarity of action in a number

of vascular parameters such as hemoconcentration and intravascular fluid loss (Hinshaw et al., 1958, 1961a, 1961b). These findings provided evidence that the early release of histamine accounts for at least part of the effects in endotoxin shock (Hinshaw et al., 1962).

Endotoxins are also responsible for producing the Swartzman reaction. Swartzman (1937) found that if a somewhat less than fatal dose of endotoxin is injected intravenously into a rabbit and then followed with another intravenous dose 12 to 24 hours later, the second dose results in massive destruction of the kidneys. In this reaction fibrin appeared to collect in the glomeruli of the kidneys and resulted in an obstruction of circulation leading to necrosis of the kidney (Braude, 1964). This syndrome has been termed a generalized Swartzman reaction. Swartzman (1937) found that if the first "preparing" injection of endotoxin is made subcutaneously and followed by a second endotoxin injection given intravenously the result is a severe necrosis of the skin at the site of the first injection. In this localized Swartzman reaction, white blood cells, platelets, and fibrin caused interference with blood circulation resulting in death of the affected tissues (Thomas, 1954).

The final effect of toxic levels of endotoxin in an animal is death. Necropsy examination of animals dying from endotoxin may show a great variance in character and severity of gross lesions. Edema of the lungs, edema of the gall bladder, and enteritis are common necropsy lesions caused by endotoxin

(Weil and Spink, 1957). The administration of trophic hormones of the hypophysis prior to endotoxin resulted in an increased amount of hemorrhage in affected tissues of animals (Westphal and Luderitz, 1961). Other factors, such as the intestinal flora (Schaedler et al., 1962), intraintestinal pools of endotoxin (Wiznitzer et al., 1960) and the presence of neutralizing antibodies, may also affect the character of lesions observed.

Edema Disease

The effect of endotoxin in pigs is of particular interest because of the disease syndrome known as edema disease. Shank (1938) made the first report of edema disease on cases that he observed in northern Ireland. A short while later Hudson (1938) reported cases of the disease from England. Few cases of this disease were observed during the first years following these reports, and it was not until 1945 that edema disease began to assume considerable importance as an economic problem (Timoney, 1950). Hutchings and Doyle, as reported by Timoney (1950), were the first to report the disease in the United States. At about the same time, Schofield, as reported by Timoney (1950), in Canada and McIntosh (1950) in South Africa reported the condition. With cases of the disease being reported by Cameron-Stephen (1963) in Australia, the disease is now known to be present in every major swine producing country of the world.

The cause of edema disease has been a matter for speculation for over 25 years. Timoney (1950) was the first to suggest that the disease must be a specific toxemia originating in the intestine. He demonstrated that it was possible to experimentally reproduce the clinical signs and lesions of edema disease in pigs by using the supernatant fluid from centrifuged intestinal contents of affected animals. This observation was confirmed by Gregory (1960b) providing evidence that a specific enterotoxemia originating in the intestine is responsible for the development of the disease. Schofield and Davis (1955) and Gregory (1955) were the first to point out the predominance of hemolytic strains of Escherichia coli in the intestinal contents of affected animals. Sojka et al. (1957) demonstrated with serologic tests that three serotypes of beta-hemolytic Escherichia coli were associated with most cases of the disease. These same authors then used a freeze-thaw method to produce an extract of the Escherichia coli serotypes involved in edema disease that, when injected intravenously into pigs, produced a syndrome indistinguishable from edema disease. Since that time other workers have conducted similar experiments, and they believe that the active principle is endotoxin (Davis and Smibert, 1963). A newer concept expressed by Thomlinson and Buxton (1963) is that interaction of endotoxin with specific antibodies results in anaphylactic shock causing the typical syndrome.

The incidence of edema disease in swine has been reported to be related to the practice of self-feeding high protein rations and the use of antibiotics as growth stimulants in rations (Glantz, 1962; Timoney, 1950). A change in management, such as weaning, together with feeding of rich concentrate rations has been observed to predispose to an outbreak of edema disease because these factors provide the ideal conditions for rapid growth of Escherichia coli in intestinal contents (Bennett, 1964). Glantz (1962) showed that serotypes of Escherichia coli involved in cases of edema disease are quite resistant to antibiotics that are commonly used as growth stimulants in swine rations. He observed that antibiotics may inhibit the growth of other bacteria in the intestine, and, as a result, the edema disease-producing serotypes of Escherichia coli are encouraged to grow.

Clinical signs of edema disease were described by Lamont (1953). He observed that clinical signs usually indicate a sudden onset, in fact often the first indication of the presence of the disease was finding several pigs dead. A staggering gait was one of the most frequently observed clinical signs and was very characteristic of the disease. The final stages were often characterized by partial or complete paralysis and stupor. Edema of the face and eyelids was also observed in many cases. Diarrhea was occasionally present prior to more severe clinical signs. Severe dyspnea was often observed. The body temperature was usually normal. Only the

best pigs contracted the disease, and the runts were not affected. The fatality rate according to Gelenczie (1959) is often 50 to 70 per cent of the affected pigs.

Bennett (1964) reported that necropsy of pigs dying from edema disease may show a great variance in the severity and character of gross lesions. A complete absence of gross lesions was found in some cases of the disease. However, evidence of edema was present in many cases and was found in subcutaneous tissues, lungs, perirenal tissues, gall bladder, stomach submucosa, intestinal submucosa and colonic mesentery. Another form of the disease described by Harding (1960), Tutt and Gale (1957) and Bennett (1964) has been called mulberry heart disease. In cases of this type, diffuse hemorrhages are present on the heart and petechial hemorrhages on the kidneys.

METHODS OF PROCEDURE

Endotoxin Production

The endotoxin used in this experiment was prepared, with slight modifications, by the method of Tauber and Russell (1961). The method is a modification of the procedure used by Westphal (1954). A culture of Escherichia coli serogroup 0138, which was isolated by Dr. Paul C. Bennett from the intestine of a pig with a clinical case of edema disease, was utilized in this project. The stock culture was maintained on an agar slant at 5 C. The following medium was used for culturing the organism: (grams per liter) $MgSO_4 \cdot H_2O$, 0.2; KH_2PO_4, 1.5; Na_2HPO_4, 6.0; protein hydrolysate[1], 10.0; and glucose, 5.0. A 50 per cent glucose solution was autoclaved for 15 minutes at 121 C and added in proper concentration to the basic medium which had previously been autoclaved 30 minutes at 121 C. Approximately 5 ml. of a 6 to 8 hour culture of the organism was inoculated into each liter of medium. A total of 20 batches of medium, each consisting of 15 liters of media divided into 3 six-liter Erlenmeyer flasks and incubated in a water bath at 37 C, were used for growth of the bacterial cells (Figure 1). Air was bubbled through the medium and growth of the organism was vigorous. After incubation, blood agar plates were streaked to check for contaminants. The

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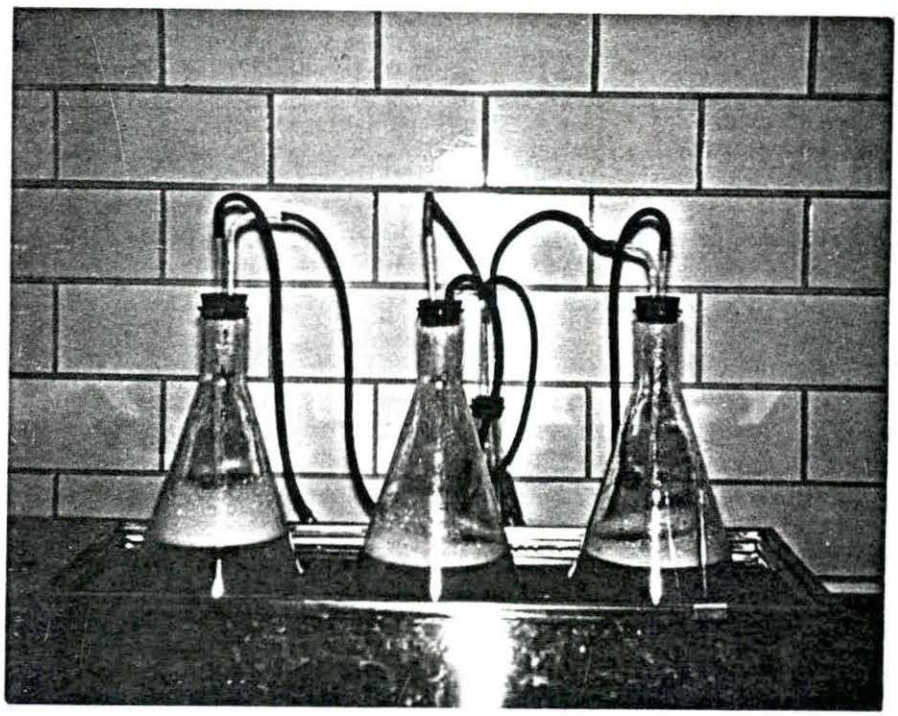
[1]Amigen, Mead Johnson and Company, Evansville, Indiana.

Figure 1. Photograph of apparatus used for batch culture of Escherichia coli.



bacterial cells were killed by the addition of formalin solution to a final concentration of 0.3 per cent. The bacterial cells settled out quite readily on standing in the refrigerator at 5 C and final separation of the cells from the media was accomplished by centrifugation at 1000 x g. for 10 minutes. The cells were washed twice in a 0.85 per cent sodium chloride solution and stored at -20 C in approximately 100 gram aliquots. The yield was approximately 2 grams of cells per liter of medium.

The extraction of the endotoxin from the bacterial cells also followed the method described by Tauber and Russell (1961). About 50 gram amounts of cells were suspended in 45 ml. of distilled water, poured into a Waring blender and stirred for two minutes. A mixture of 110 grams of phenol and 67 ml. of distilled water was poured into the blender and the contents were stirred for eight minutes. The mixture was cooled to 20 C and centrifuged at 4 C for ten minutes at 3000 x g. The clear upper phase was siphoned off and the lower (phenol) phase containing the proteins was extracted with 30 ml. of distilled water and centrifuged again. Another 50 gram portion of bacterial cells was similarly treated. The upper phase from each preparation which contained the endotoxin and nucleoproteins were combined. The combined supernatants were placed in a cellophane tube and dialyzed for 48 hours against several changes of distilled water.

The next step in the preparation of the endotoxin was purification. One mg. of dry sodium chloride was added to each ml. of dialyzed supernatant followed by the addition of two volumes of cold acetone. The endotoxin was precipitated together with nucleic acids and a small quantity of other materials. The precipitate was removed by 3000 x g. centrifugation for ten minutes and dissolved in 50 ml. of distilled water. Cold acetone (0.2 volume) was added to the solution containing the endotoxin and the mixture was centrifuged for one hour in a Servall type RC 2 centrifuge at 31,000 x g. The supernatant was discarded. The precipitate was dissolved in 15 ml. of distilled water and lyophilized. The yield of endotoxin was approximately 2 mg. per gram of bacterial cells.

The endotoxin was prepared for use in this experiment by dissolving it at a concentration of 5 mg. per ml. in 0.85 per cent saline solution.

Antiserum Production

Antiserum to Escherichia coli was prepared in 3-month-old pigs. A bacterin was prepared by culturing the same organism used in preparing the endotoxin (Escherichia coli serogroup 0138) in the same medium as used for preparation of the endotoxin. The cells were killed by heating at 65 C for 30 minutes in a water bath. The cells were then separated by centrifugation and washed 3 times in normal saline solution. The cells were suspended to a concentration equivalent to that of the

number 7 tube of the McFarland nephelometer scale. Hyper-immunization of the pigs was accomplished by administration of two series of injections of the prepared antigen. The pigs were first given four injections beginning at 1 ml. and increasing to 2 ml. amounts of the antigen at intervals of two days. Thirty days later the pigs were given an additional three injections of 2 ml. amounts of the antigen at two day intervals. Seven days after the final injection of bacterin the pigs were bled out and the serum separated. The serum was heated at 56 C for 30 minutes and filtered through a Selas 02 filter. The antiserum was stored at -20 C. The titer of this antiserum was determined to be 1 to 83,886,080 by the method described on page 24.

Experimental Animals

Animals used in this experiment consisted of mice, naturally reared pigs, and colostrum-deprived pigs. White Swiss 20 gram mice were obtained from a commercial source[1]. The naturally reared pigs were the offspring of four crossbred sows purchased locally and moved to our facilities prior to farrowing. The pigs were utilized at three weeks of age when their weights varied from 5.75 to 13.5 lbs. Colostrum-deprived pigs were taken by Cesarean section and placed immediately in rearing boxes in an isolation unit. Their diet consisted of

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[1]Midwest Animal Colony, Corning, Iowa.

a commercially prepared canned milk[1] and a commercial baby pig prestarter ration[2]. These pigs were used at four weeks of age when they weighed from 6.5 to 11.4 lbs.

Treatment of Animals

One litter of pigs was utilized at a time and they were randomized, weighed, and the dosage of endotoxin was calculated for each. The pigs were placed in a recumbent position, and the predetermined amount of endotoxin was injected into the anterior vena cava.

The pigs were bled from the anterior vena cava using 20 ga. 1 1/2 in. needles and 5 cc. syringes prior to injection of endotoxin and at intervals of 15 minutes, 1 hour, 4 hours, and 8 hours after injection of endotoxin. Blood samples were allowed to clot at 4 C and the serum removed by centrifugation. An aliquot of serum was removed for complement titration and the remaining serum stored at -20 C. Heparinized blood samples were also collected at the same time intervals, and the plasma was separated from the cells by centrifugation. Plasma samples were also stored at -20 C.

Histological Techniques

Tissues for histological examination were collected as soon after death as possible. The tissues were fixed in 10

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[1]SPF-Lac, Borden Co., New York, New York.

[2]"4 x 4" Pre-Weaner, Walnut Grove Products Co., Atlantic, Iowa.

per cent phosphate buffered formalin solution, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin. The sections were cut 8 microns in thickness and stained with Delafield's hematoxylin and ethyl eosin.

Serological Techniques

Serums were tested for the presence of anti Escherichia coli endotoxin antibodies by the indirect hemagglutination method of Neter (1952) as modified by Gilbert and Braude (1962). Swine erythrocytes were collected in Alsever's solution and washed four times in 0.85 per cent sodium chloride solution. Ten mg. of endotoxin were dissolved in 1.0 ml. of normal saline solution and 4.5 ml. of 0.25N NaOH was added. This mixture was incubated for three hours at 37 C. The solution was then neutralized with 0.25N HCl. The NaOH treated endotoxin was mixed with 1.0 ml. of packed red blood cells and incubated for 30 minutes at 37 C. Excess endotoxin that had not adhered to the red blood cells was removed by three washings with saline solution. The sensitized red blood cells were suspended in 0.85 per cent sodium chloride solution at a concentration of 2 per cent.

The swine blood serum samples to be tested were heated at 56 C for 30 minutes and serial two-fold dilutions were made in 0.85 per cent sodium chloride solution containing one per cent swine serum which contained no detectable antibodies for Escherichia coli endotoxin. One drop of the sensitized red

blood cells was added to the diluted serum samples. The mixture was shaken, incubated at 37 C for one hour, and refrigerated overnight at 4 C before reading the test. A positive test was characterized by a matting of the red blood cells in the bottom of the tube and a negative test by the formation of a button of red blood cells.

Serum lysozyme was determined by a modification of the method of Smolelis and Hartsell (1949). Lysis of a suspension of Micrococcus lysodeikticus organisms was measured with a spectrophotometer[1] at a wave length of 540 mu. Ultraviolet killed dried Micrococcus lysodeikticus organisms[2] were suspended in M/15 phosphate buffer solution at pH 6.2 to yield an optical density of 0.34 when diluted with an equal volume of buffer solution. All reagents were warmed to 37 C and 0.25 ml. of serum to be assayed and 1.75 ml. of buffer solution were placed in a cuvette. The substrate suspension (2 ml.) was added and the optical density of the resultant mixture was measured and recorded. Following incubation at room temperature for one hour, the cuvette was shaken to uniformly suspend unlysed organisms and the optical density was again determined. Percentage of lysis of the substrate was calculated from the optical density readings. Lysozyme activity was determined from a standard curve prepared by measuring the lytic activity

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[1]Spectronic 20, Bausch and Lomb, Rochester, New York.

[2]Bacto-Lysozyme Substrate, Difco Laboratories, Detroit, Michigan.

of known amounts of purified lysozyme[1] on the substrate.

Titration of serum for hemolytic complement activity was performed by a modification of the procedure of Mayer (Kabat and Mayer, 1961). The modification consisted of reducing the total reaction volume to 1.5 ml. by scaling down the quantity of each of the reagents proportionately. Dilutions of serum to be titrated for complement activity were made in pH 7.5 isotonic veronal buffer in a total volume of 0.5 ml. One ml. of a suspension of optimally sensitized sheep red blood cells (10^8 cells per ml.) was added. The mixture was incubated for 60 minutes in a water bath at 37 C with frequent agitation. Tubes in which the degree of hemolysis was estimated to fall between 20 and 80 per cent were centrifuged to sediment unlysed cells. Optical density of the clear supernatant fluids was determined in a spectrophotometer at a wave length of 540 mu. The fraction of cells lysed was determined and the 50 per cent unit calculated for the respective serum by application of the von Krogh equation.

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[1]Bacto-Lysozyme, Difco Laboratories, Detroit, Michigan.

RESULTS

Toxicity of Endotoxin

The toxicity of the prepared Escherichia coli endotoxin was first determined in mice. Doses of 0.05, 0.1, 0.15, 0.2, and 0.3 mg. were tested and 5 mice were given the endotoxin intravenously at each dosage level. Deaths occurred between 7 and 34 hours after injection. However, most deaths occurred around 10 hours. No correlation between dosage rate and time of death was observed. The results of these tests are presented in Table 1. The LD_{50}, as determined by the method of Reed and Muench (1938), of the endotoxin for mice was determined to be 0.125 mg. of endotoxin per mouse.

Table 1. The influence of dosage levels of Escherichia coli endotoxin upon death rate in 20 gram white swiss mice

Dosage level	Number of mice	
	Died	Lived
0.05 mg.	1	4
0.1 mg.	2	3
0.15 mg.	3	2
0.2 mg.	4	1
0.3 mg.	5	0

The toxicity of the endotoxin was next determined by injection of 4 dosage levels into 18 three-week-old colostrum-fed pigs. Pigs receiving a lethal dose of endotoxin died as early as one hour after injection and as late as 32 hours. The results of these tests are presented in Table 2.

Table 2. The influence of dosage levels of Escherichia coli endotoxin upon death rate of colostrum-fed pigs

Dosage level	Number of pigs	
	Died	Lived
1 mg./kg.	2	3
2 mg./kg.	3	3
4 mg./kg.	4	1
6 mg./kg.	2	0

No correlation between dosage rate and time of death was observed. The LD_{50}, as determined by the method of Reed and Muench (1938), of the endotoxin for three-week-old colostrum-fed pigs was found to be 1.8 mg. of endotoxin per kg. of body weight.

Toxicity of the endotoxin was also determined for colostrum-deprived pigs. Six colostrum-deprived pigs that had been given antiserum 16 hours previously and 9 pigs without previous treatment were injected intravenously with 2 mg. of endotoxin per kg. of body weight. These results are presented in Table

3. Three additional pigs that were treated with antiserum were given 3 mg. of endotoxin per kg. of body weight; 2 of these 3 pigs succumbed to the endotoxin.

Table 3. Comparison of death rates of pigs given 2 mg. per kg. of body weight of Escherichia coli endotoxin

Treatment of pigs	Number of pigs	
	Died	Lived
Colostrum-fed, no antiserum	3	3
Colostrum-deprived, no antiserum	4	5
Colostrum-deprived, antiserum	0	6

Clinical Signs

Mice given toxic levels of Escherichia coli endotoxin showed only minor effects during the first two to three hours following injection. Thereafter, they became progressively more reluctant to move and refused to eat or drink. Depression, a rough haircoat, and diarrhea were apparent in many of the mice prior to death.

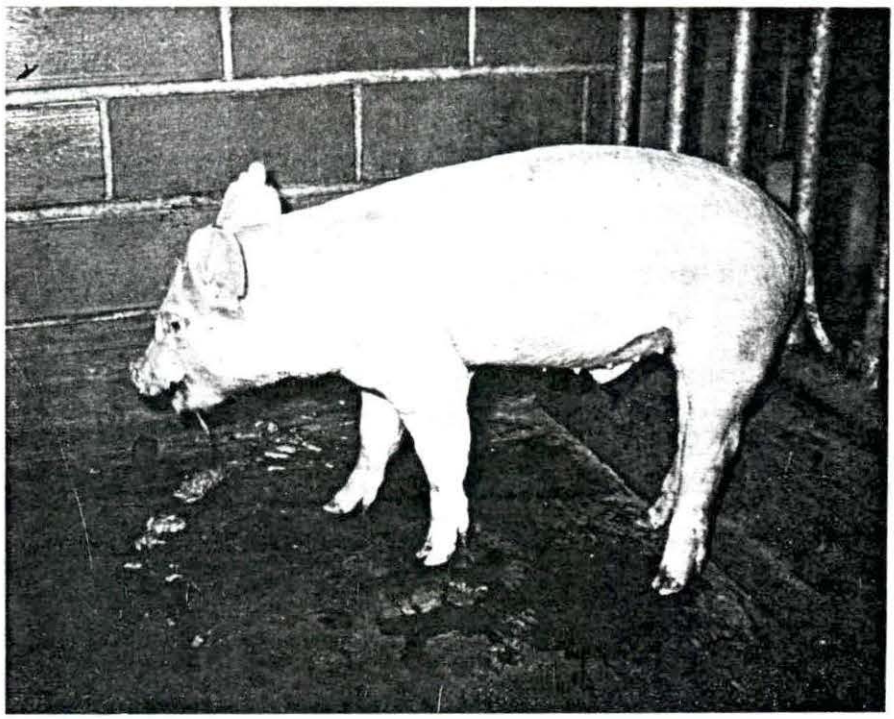
Clinical signs of toxicity caused by endotoxin in colostrum-fed three-week-old pigs followed a definite pattern for the first 3 to 4 hours irrespective of dosage. Following this period, animals receiving a lethal dose developed more severe signs. The first sign of toxicity, observed 10 to 20 minutes after administration of endotoxin, was frequent clamping of

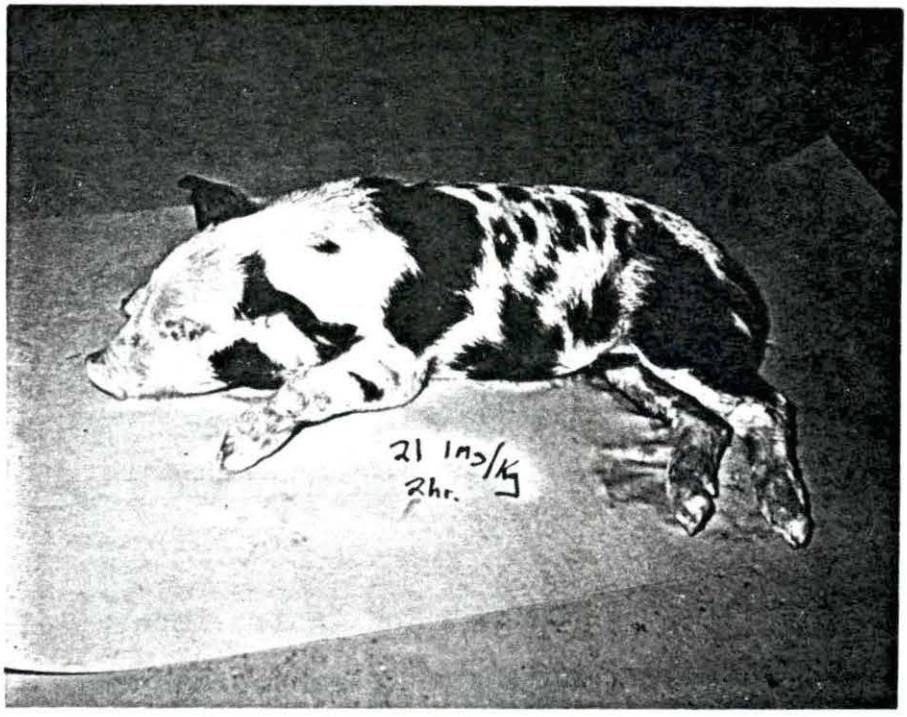
jaws accompanied by salivation and vomition of food and mucus (Figure 2). Intermittent vomition continued for as long as 30 minutes. The pigs developed various degrees of incoordination and within an hour many were recumbent and unable to rise (Figure 3). At about one hour the respiratory rate increased markedly and breathing became labored. In about half the pigs, large amounts of soft feces were voided. At approximately 3 hours, many of the pigs that eventually died appeared to make a partial recovery, but they soon passed into the typical final phase of the syndrome showing more severe clinical signs. Respiration became shallow and irregular, and the extremities became cold. A paddling motion of the legs was observed in some cases prior to death. The signs of illness appeared to abate after about 24 hours in those pigs that survived.

The clinical signs in colostrum-deprived pigs given endotoxin were almost identical to those observed in colostrum-fed pigs. However, in those pigs given antiserum intravenously 16 hours prior to administration of endotoxin, a violent convulsion occurred within 10 to 15 seconds and continued for about 60 seconds. Tnis convulsion was characterized by complete cessation of respiration for the first 30 seconds of the period and a bluish coloration of the skin over the snout and ears. Following this reaction, the pigs appeared to return to normal until vomition and other characteristic signs developed at about 20 minutes. However, clinical signs were less severe

Figure 2. Photograph of a pig vomiting following the administration of Escherichia coli endotoxin.

Figure 3. Photograph of a pig recumbent and unable to rise 2 hours following the administration of Escherichia coli endotoxin.




21 mg/kg
2hr.

in these pigs than in those not given antiserum prior to endotoxin.

Gross Pathologic Lesions

The principal gross lesions in pigs dying from the effects of Escherichia coli endotoxin are summarized in Table 4.

Table 4. Occurrence of principal gross lesions in pigs given an intravenous injection of Escherichia coli endotoxin

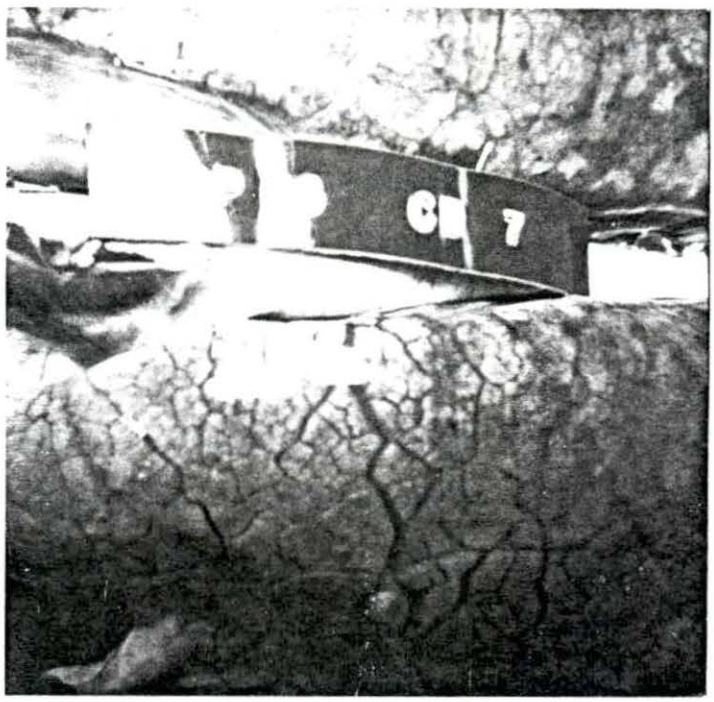
Lesion	Number of pigs showing lesions			
	Severe	Moderate	Mild	Absent
Edema of lungs	3	4	6	3
Hyperemia of stomach wall	1	2	7	6
Edema of gall bladder	0	2	9	5
Catarrhal enteritis	3	1	3	9

Interlobular edema of the lungs was the most common necropsy finding (Figure 4). This manifestation was most severe in those pigs dying within the first 6 hours after injection. Hemorrhage was present on the marginal surface of the lungs in 4 of the 16 pigs, and excessive thoracic fluid was found in 2 pigs.

Edema of the gall bladder and gastro-hepatic ligament was also a characteristic lesion as it was observed in 11 of the 16 pigs (Figure 5). The livers appeared normal except for a

Figure 4. Photograph showing intralobular edema of the lung of a pig caused by administration of Escherichia coli endotoxin.

Figure 5. Photograph showing edema of the gall bladder and gastro-hepatic ligament in a pig following injection of Escherichia coli endotoxin.

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very pale appearance in the two pigs surviving for 36 hours after injection.

Petechial hemorrhages were present on the surface of the kidneys of two pigs, and hyperemia and hemorrhage were evident in the medulla of the kidneys of two of the pigs (Figure 6).

The stomachs of all pigs contained food even though all pigs vomited after injection of the endotoxin. An area of hemorrhage on the mucosa of the stomach was observed in 10 of the 16 pigs. The principal site of this lesion was in the fundic area at the greater curvature of the stomach. Three of the pigs that showed the intense reddening of the gastric mucosa also showed edema of the stomach wall in the affected area (Figure 7).

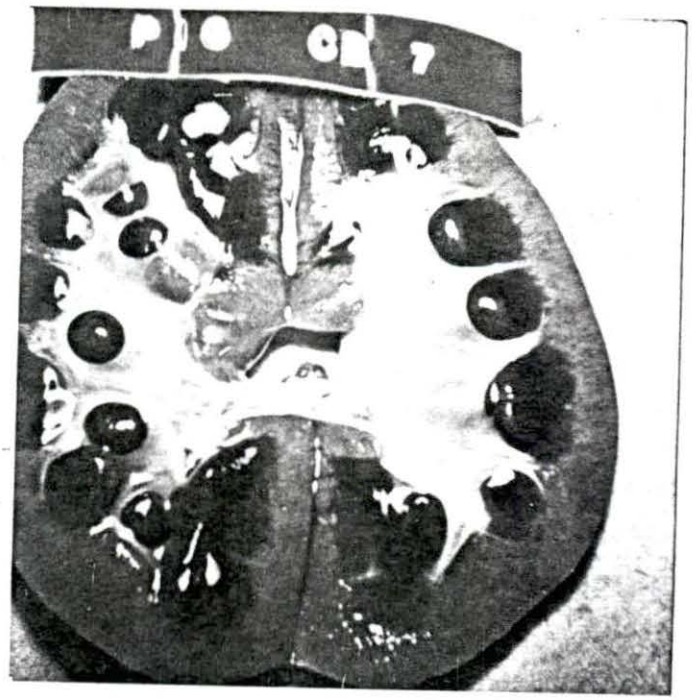
Catarrhal enteritis was a quite consistent finding in the colostrum-fed pigs, but not in the colostrum-deprived pigs. The serosal surface of the intestines was hyperemic in one pig (Figure 5). The mesenteric lymph nodes were edematous and hemorrhagic in most of the pigs.

Microscopic Pathologic Lesions

The principal microscopic lesions observed in pigs receiving a lethal dose of Escherichia coli endotoxin are summarized in Table 5. The lesions observed were mainly those of edema, severe congestion and some hemorrhage.

Figure 6. Photograph showing hemorrhage in medulla of porcine kidney following administration of Escherichia coli endotoxin.

Figure 7. Photograph showing edema of submucosa of the stomach of a pig caused by injection of Escherichia coli endotoxin.



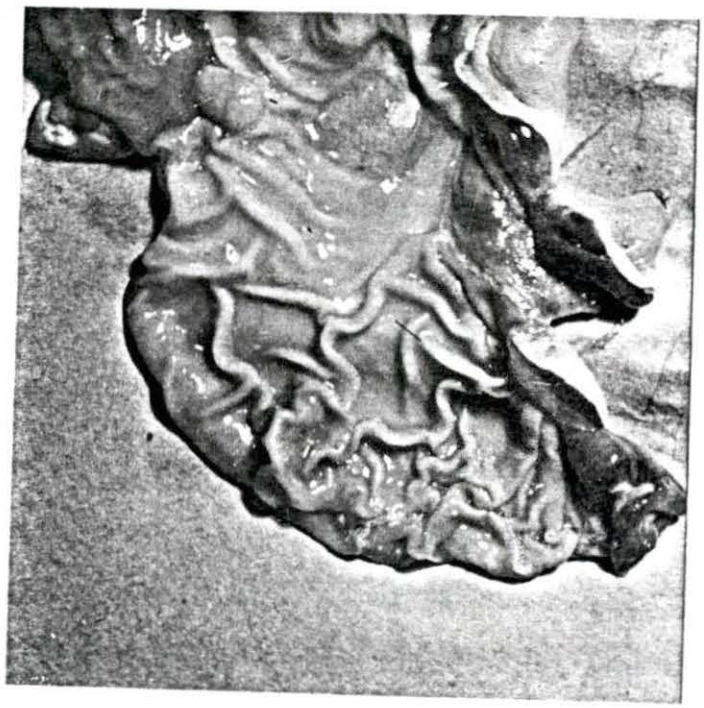


Table 5. Occurrence of principal microscopic lesions in pigs given an intravenous injection of Escherichia coli endotoxin

Lesion	Number of pigs showing lesions			
	Severe	Moderate	Mild	Absent
Congestion and hemorrhage of lungs	0	3	12	1
Congestion and hemorrhage of liver	0	0	11	5
Congestion and hemorrhage of kidneys	1	3	9	3
Congestion and hemorrhage of stomach wall	1	1	8	6
Eosinophils in mesenteric lymph nodes	1	0	15	0

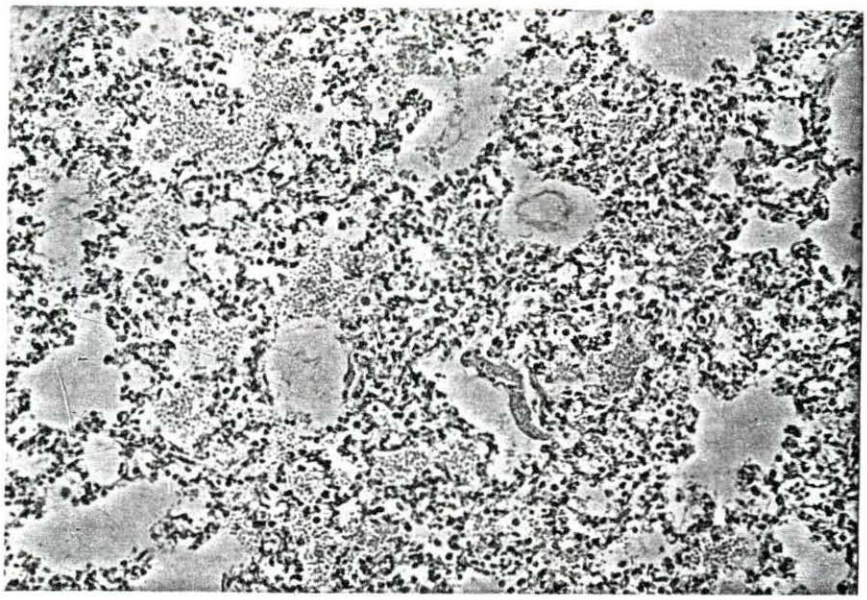
Microscopic examination of sections of the lungs showed congestion and in some cases hemorrhage into the alveolar spaces in 15 of the 16 pigs (Figure 8). In addition, lesions indicating the presence of edema were observed in the lungs of most of the pigs.

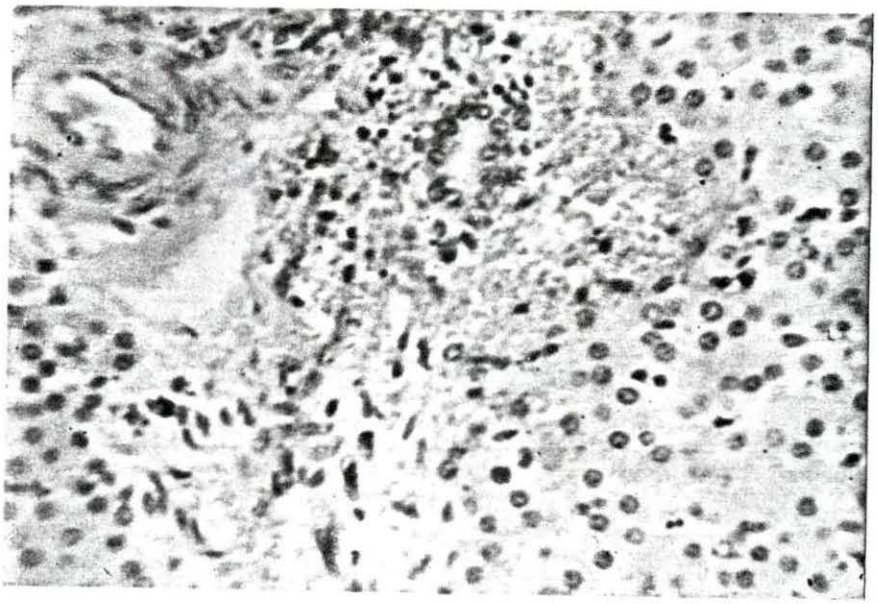
The liver showed congestion in 11 of the pigs (Figure 9). Severe congestion with some diapedesis of red blood cells was present in the medulla of the kidneys in 13 of the pigs.

Hemorrhage and congestion were observed in the gastric mucosa and submucosa of 10 of the pigs, and edema of the gastric mucosa was observed in 3 of these (Figure 10). The mesenteric lymph nodes showed severe congestion and hemorrhage in 5

Figure 8. Photomicrograph of a section of lung showing hemorrhage into alveolar spaces following administration of Escherichia coli endotoxin. Hematoxylin and eosin stain. X 100.

Figure 9. Photomicrograph of a section of liver showing hemorrhage surrounding a bile duct following administration of Escherichia coli endotoxin. Hematoxylin and eosin stain. X 410.





pigs. Infiltration of the mesenteric lymph nodes with eosinophils was observed in all 16 of the pigs (Figure 11). Eosinophils were also observed in the intestinal submucosa and the thymus of several of the pigs.

Serologic Findings

Serologic tests to determine serum levels of antibody to Escherichia coli endotoxin did not reveal the presence of antibodies in the serum of any of the colostrum-fed pigs before or after injection of endotoxin. Three of the colostrum-deprived pigs showed antibody titers of 1:160 prior to injection of endotoxin, but no antibodies were detectable 15 minutes after injection.

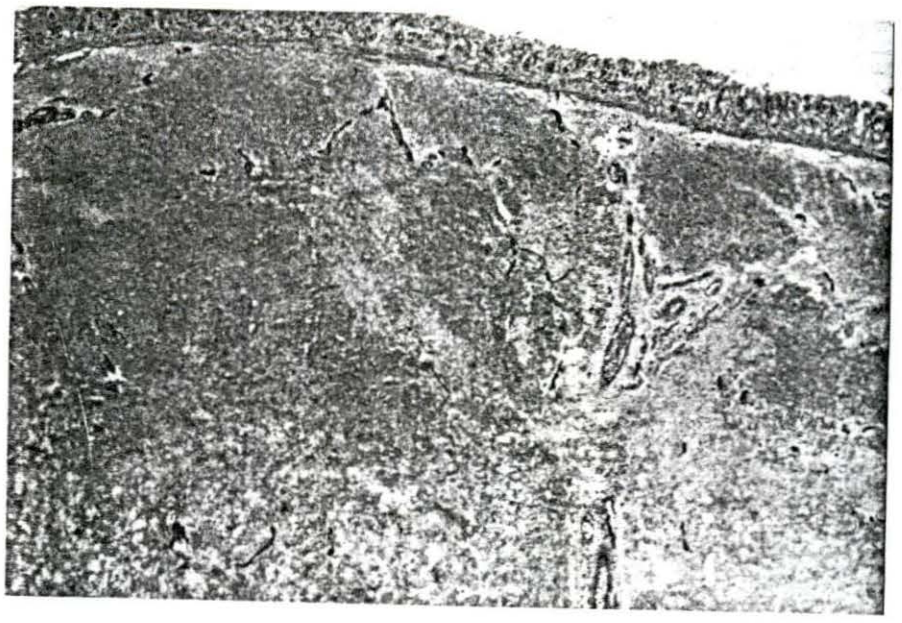
The serums of colostrum-deprived pigs given antiserum prior to administration of endotoxin possessed an average antibody titer of 1:290,000. Fifteen minutes following administration of endotoxin the antibody titer was reduced to 1:34,000, and after 8 hours to 1:8,000 (Table 6). Figure 12 shows these results in graphic form.

Pigs which survived the administration of Escherichia coli endotoxin responded with the production of antibodies. The average antibody titer of 6 pigs at 1 week after injection of endotoxin was 1:250 (Table 7).

Serum lysozyme levels showed a marked increase following the administration of Escherichia coli endotoxin to colostrum-fed pigs (Table 8). A similar increase in serum lysozyme

Figure 10. Photomicrograph showing congestion and hemorrhage in the submucosa of a pig stomach following administration of Escherichia coli endotoxin. Hematoxylin and eosin stain. X 100.

Figure 11. Photomicrograph showing infiltration of eosinophils into the mesenteric lymph nodes of a pig following administration of Escherichia coli endotoxin. Hematoxylin and eosin stain. X 410.



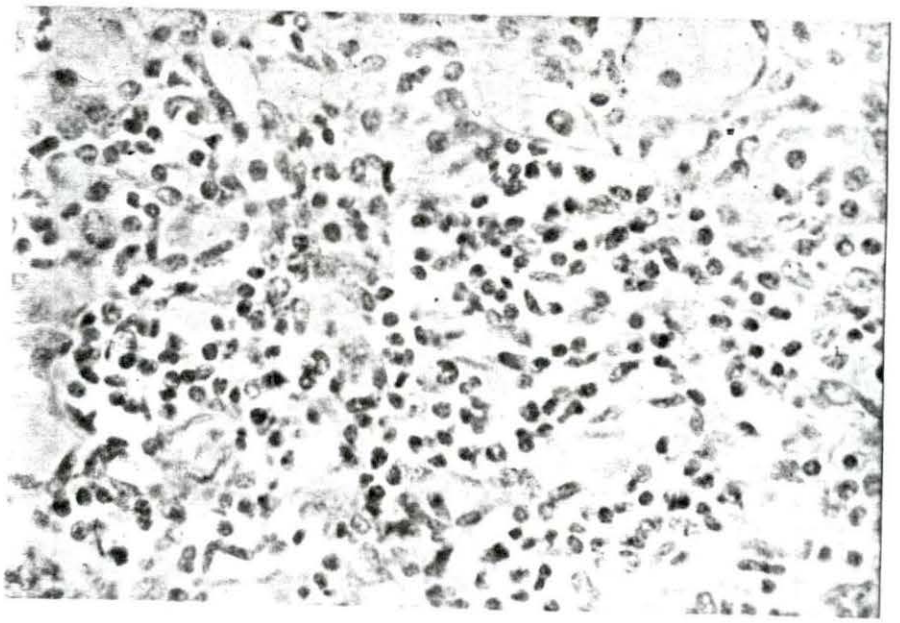


Table 6. Antibody titers for *Escherichia coli* endotoxin of serums from colostrum-deprived pigs given antiserum prior to administration of endotoxin

Pig no.	Dosage of endotoxin	Death	Time after administration of endotoxin				
			Preinoc.	15 min.	1 hr.	4 hr.	8 hr.
CD G	2 mg./kg.	No	327,680	10,240	10,240	2,560	2,560
CD O	2 mg./kg.	No	655,360	81,920	81,920	40,960	20,480
CD 1	2 mg./kg.	No	327,680	81,920	40,960	20,480	20,480
CD 3	2 mg./kg.	No	327,680	40,960	20,480	10,240	5,120
CD 6	2 mg./kg.	No	163,840	20,480	10,240	10,240	10,240
CD 9	2 mg./kg.	No	163,840	5,120	5,120	5,120	640
CD 21	3 mg./kg.	Yes	163,840	20,480	10,240	5,120	2,560
CD 25	3 mg./kg.	Yes	163,840	40,960	20,480	10,240	10,240
CD 27	3 mg./kg.	No	327,680	5,120	2,560	2,560	1,280
Average titer			291,270	34,133	22,471	11,936	8,180

Figure 12. Graph showing average serum antibody titers in pigs following administration of Escherichia coli endotoxin.

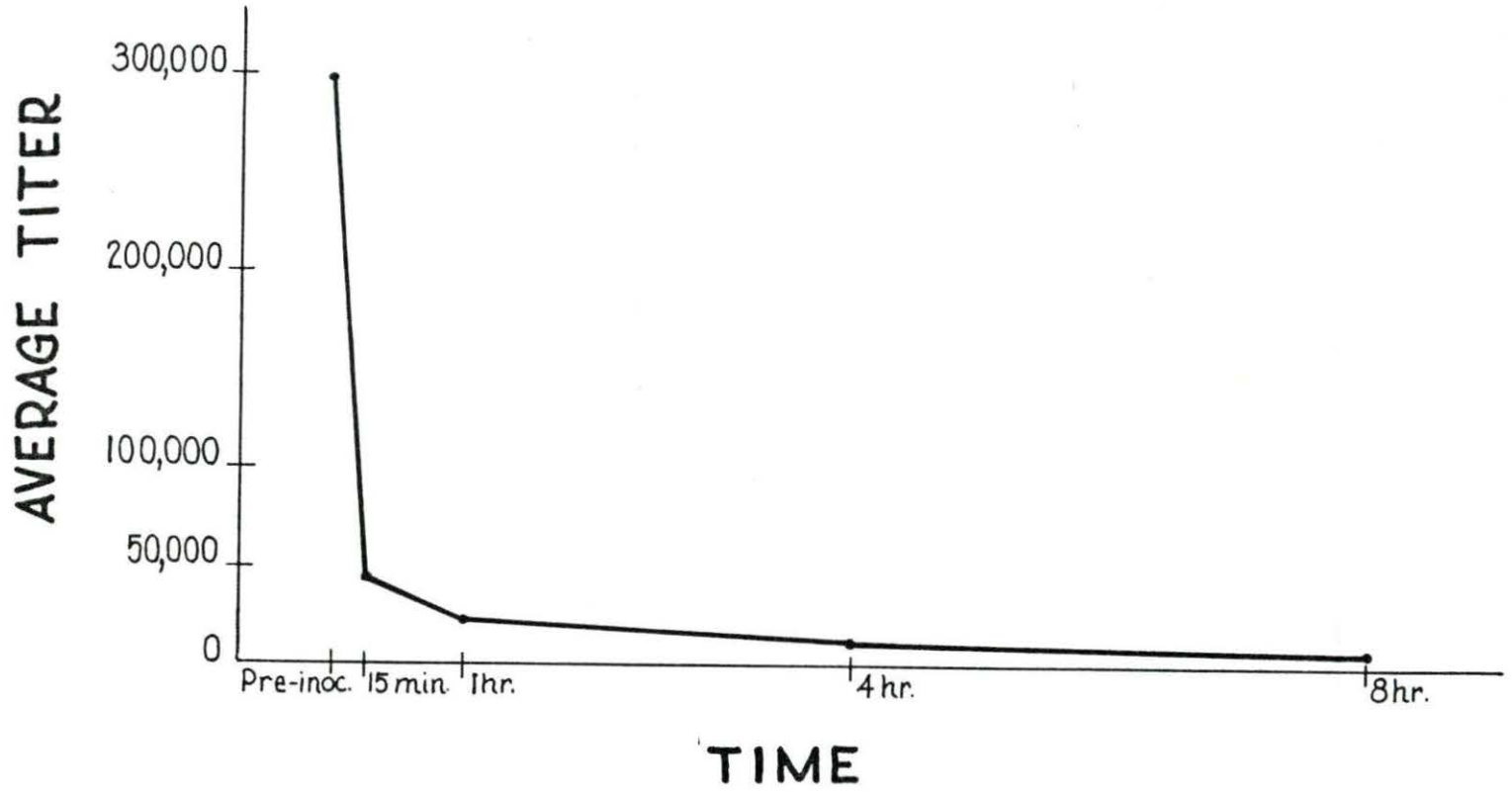

AVERAGE TITER
300,000
200,000
100,000
50,000
0
Pre-inoc.
15 min.
1 hr.
4 hr.
8 hr.
TIME

Table 7. Active antibody response in pigs following administration of Escherichia coli endotoxin

Pig no.	Dosage of endotoxin	Time after injection of endotoxin			
		Preinoc.	1 wk.	2 wks.	4 wks.
4	2 mg./kg.	0	640	x[a]	x
22	2 mg./kg.	0	320	x	x
23	4 mg./kg.	0	160	x	x
50	1 mg./kg.	0	160	160	160
51	1 mg./kg.	0	80	80	80
52	1 mg./kg.	0	160	80	40

[a]Not tested.

Table 8. Average levels of lysozyme in micrograms per ml. of serum in colostrum-fed pigs given Escherichia coli endotoxin

Dosage of endotoxin	No. of pigs	Time after injection of endotoxin		
		Preinoc.	15 min.	1 hr.
1 mg./kg.	6	0.19	0.39	0.59
2 mg./kg.	7	0.23	0.58	0.59
4 mg./kg.	6	0.48	0.86	0.77

levels was also observed in colostrum-deprived pigs in the case of those given only endotoxin as well as those pretreated with antiserum and then given endotoxin (Table 9).

Table 9. Average levels of lysozyme in micrograms per ml. of serum in pigs given Escherichia coli endotoxin

Treatment of pigs	No. of pigs	Time after injection of endotoxin		
		Preinoc.	15 min.	1 hr.
Colostrum-fed, no antiserum	19	0.29	0.59	0.63
Colostrum-deprived, no antiserum	11	0.26	0.67	0.59
Colostrum-deprived, antiserum	8	0.26	0.60	0.52

Hemolytic complement activity in blood serum of colostrum-fed pigs given Escherichia coli endotoxin was slightly reduced 15 minutes after administration of endotoxin and continued to decline slowly during the following 4 hours (Table 10). Serum complement levels also decreased slightly in colostrum-deprived pigs given the endotoxin. However, colostrum-deprived pigs pretreated with antiserum 16 hours before administration of Escherichia coli endotoxin showed a very sharp drop in serum complement levels. Three pigs given antiserum intravenously one hour after administration of endotoxin showed a sudden

drop to undetectable levels of serum complement 15 minutes after the antiserum was given, but an average of 5.5 C^1H_{50} units was present in the serum of these pigs 3 hours thereafter (Table 11).

Table 10. Average hemolytic complement activity[a] in blood serum of colostrum-fed pigs given Escherichia coli endotoxin

Dosage of endotoxin	No. of pigs	Time after injection of endotoxin				
		Preinoc.	15 min.	1 hr.	4 hrs.	8 hrs.
1 mg./kg.	6	47.4 (6)[b]	44.1 (6)	38.7 (6)	36.1 (5)	40.0 (4)
2 mg./kg.	7	59.8 (7)	51.8 (7)	46.7 (5)	39.6 (2)	52.2 (1)
4 mg./kg.	8	59.7 (8)	56.2 (8)	47.1 (6)	36.7 (2)	54.5 (1)

[a]Hemolytic complement activity expressed in C^1H_{50} units.

[b]Number of samples represented given in parentheses.

Table 11. Average hemolytic complement activity[a] in blood serum in pigs treated different ways and given Escherichia coli endotoxin

Treatment of pigs	No. of pigs	Time after injection of endotoxin				
		Preinoc.	15 min.	1 hr.	4 hrs.	8 hrs.
Colostrum-fed, no antiserum	7	59.8 (7)[b]	51.8 (7)	46.7 (5)	39.6 (2)	52.2 (1)
Colostrum-deprived, no antiserum	8	55.8 (8)	47.3 (8)	45.6 (8)	45.8 (8)	45.6 (7)
Colostrum-deprived, antiserum	8	42.8 (8)	3.0 (8)	12.9 (8)	20.2 (8)	22.3 (8)
Colostrum-deprived, antiserum (one hour after endotoxin)	3	36.8 (3)	32.0 (3)	29.2 (3)	5.5 (3)	10.6 (3)

[a]Hemolytic complement activity expressed in C^1H_{50} units.

[b]Number of samples represented given in parentheses.

DISCUSSION

The Escherichia coli endotoxin produced in this study was found to be quite toxic for mice and young pigs. The LD_{50} of the endotoxin for mice (.125 mg.) is similar to the lethal dose of 0.1 to 0.5 mg. per mouse described by Burrows (1951) and less than the 0.25 mg. to 0.4 mg. per mouse reported by Tauber and Russell (1961). This variation in toxicity could result from differences in the culture medium used (Fukushi et al., 1964), in methods of preparation of the endotoxin (Fukushi et al., 1964), in the type of organism used in production of the endotoxin (Tauber and Russell, 1961), and in susceptibility of the mice. Schaedler et al. (1962) reported that mice raised in an unsanitary environment or fed diets containing antibiotics were more susceptible to endotoxin than those raised in a more ideal environment.

Purified Escherichia coli endotoxin has been studied in several species of animals, but its use was not found to have been reported in pigs. If the LD_{50} of endotoxin for mice is calculated on a kg. basis, the resulting LD_{50} of 6.25 mg. per kg. of body weight may be compared with the LD_{50} of 1.8 mg. per kg. of body weight in pigs. These results might only indicate a species difference, but could possibly result from a decreased tolerance to endotoxin with an increase in size of the animal. It was observed during the course of this experiment that the larger animals within a litter appeared to be

more severely affected by a given dosage level of endotoxin.

Clinical signs resembling quite closely those reported in the literature for other species of animals follwed the intravenous administration of the prepared Escherichia coli endotoxin into pigs. Typical signs of gagging, vomition, diarrhea, and respiratory distress observed in this experiment are also characteristic of the effect of endotoxin when administered to dogs and cats (Hinshaw et al., 1957; Weil and Spink, 1957). Jones and Smith (1964) prepared extracts of Escherichia coli bacterial cells using either alternate freezing and thawing or ultrasonic disintegration, and this material, when injected into pigs, caused clinical signs similar to those obtained in the present experiment. However, the pigs used by Jones and Smith (1964) were 7-12 weeks old and in some cases they showed violent convulsions immediately after injection of the Escherichia coli extract. The pigs appeared to recover in 2-3 minutes and the reaction would appear to be similar to the one produced in the present experiment by administration of antiserum prior to the endotoxin.

The most characteristic postmortem lesions observed in the pigs dying from endotoxin administration were edema of the lungs, edema of the gall bladder, and catarrhal enteritis; all of these lesions are reported by Weil and Spink (1957) in other species of animals given endotoxin. The gross lesions

of edema of the stomach wall, congestion and hemorrhage of the stomach mucosa, and congestion and hemorrhage of the kidneys, produced by extracts of Escherichia coli bacterial cells administered to pigs by Jones and Smith (1964), were also similar to those lesions produced in the present experiment. In addition, the lesions of edema of the lungs, edema of the stomach wall, congestion and hemorrhage of the kidneys and others produced in this experiment by intravenous injection of Escherichia coli endotoxin are typical lesions observed in naturally occurring cases of edema disease in swine.

The administration of endotoxin quickly results in hematologic changes in experimental animals. Braude (1964) reported that endotoxin administered intravenously was almost immediately picked up by leukocytes. Following the administration of endotoxin, the white cells were observed to quickly disappear from the blood stream (Coulter, 1965). This direct effect of endotoxin on leukocytes is very likely related to the increased lysozyme content of blood serum observed following administration of endotoxin in this experiment. This suggests that many leukocytes are lysed within the blood stream for it is known that leukocytes contain large amounts of lysozyme which may be released by destruction of the white blood cells (Myrvik and Weiser, 1955).

There is evidence that not all of the white blood cells disappearing from the blood stream are lysed. The fate of other leukocytes may be explained by the experiments of

Rubenstein et al. (1962) who demonstrated with an indirect fluorescent antibody technique that leukocytes bearing endotoxin were present in the walls of blood vessels. In addition, fluorescent aggregates of endotoxin, which they thought represented disrupted leukocytes, were also present in the walls of these blood vessels. The diapedesis of leukocytes may be the mechanism of transport of endotoxin into extravascular spaces. Here the endotoxin may cause direct cellular changes similar to those observed by Karnovsky et al. (1964) who studied the effects of endotoxin on leukocytes with an in vitro system. They demonstrated by electron microscopy that the most conspicuous part of the effect of endotoxins on leukocytes is a surface active property causing increased vesicle formation within these cells typical of changes in white blood cells having undergone phagocytic activity. This particular activity of endotoxin may be exerted in a similar manner on other cells of the body such as those of blood vessel walls. This effect may result in changes that allow hemorrhage and edema to occur in surrounding tissues. These changes were observed in this experiment by microscopic examination of lungs, stomach wall, liver, and kidneys.

A possible role for specific antibody in the toxicity of endotoxin was also studied in this experiment. Serologic tests showed that the pigs used in this experiment were free of antibodies to Escherichia coli endotoxin except for very low titers in 3 animals. Further evidence that antibodies were

not involved was presented by the finding that there was no marked drop in serum complement levels following administration of the endotoxin. These results indicate that the effects of endotoxin observed in this experiment involved a direct physiologic response.

The pigs pretreated with antibody 16 hours prior to administration of the endotoxin did show evidence that endotoxin reacted with the antibody. Serologic tests showed clearly by the drop in average antibody titer from 1:290,000 at preinoculation to 1:34,000 at 15 minutes following intravenous administration of the endotoxin, and by the sharp drop in serum complement levels, that an antibody-antigen reaction occurred. This sudden drop in antibody titer and serum complement level was accompanied by an anaphylactic type reaction in the pigs. Although this acute reaction developed, it did not appear to contribute to the toxic activity of the endotoxin.

The acute reaction observed by Jones and Smith (1964) in some of the 7-12 week-old-pigs to which they gave extracts of Escherichia coli cells appeared to be apparently similar to the anaphylactic type reaction produced in the present experiment. In the experiment reported by Jones and Smith (1964) the pigs had apparently acquired antibodies to Escherichia coli from the Escherichia coli organisms normally present in the intestine. The acute reaction in their experiment also

did not appear to contribute to the toxicity of the Escherichia coli cell extracts.

Thomlinson and Buxton (1963) reported that the toxic effects of Escherichia coli for pigs was due to an anaphylactic reaction. They prepared an antigen composed of a suspension of Escherichia coli cells and used this to prepare an antiserum in pigs. The antigen was then given to 4 pigs and 30 minutes later these pigs were injected with antiserum to produce a reversed passive anaphylaxis. The pigs showed clinical signs of trembling, retching, defecation, coughing, and yawning. The pigs were sacrificed 4 hours later and the gross lesions observed included edema of the gall bladder, some edema and hemorrhage of the stomach wall, and congestion of the intestines. The authors concluded that the pathogenesis of edema disease in swine is associated with an anaphylactic type of hypersensitivity to Escherichia coli rather than a direct toxemia from the bacterial polysaccharide.

The results reported by Thomlinson and Buxton (1963) contrast to some extent with the findings of the present experiment using a purified Escherichia coli endotoxin. However, the differences may be due to the use of whole Escherichia coli cells which would contain a relatively low content of endotoxin, the fact that whole Escherichia coli cells would contain many other protein components that could enter into antigen-antibody reactions, the use of a reversed passive anaphylaxis test which may give different results than a

direct anaphylactic test system, and the fact that they sacrificed the pigs 4 hours following administration of the Escherichia coli cells. However, some similarities are present in the results of the two experiments. One of their 2 control pigs given only the Escherichia coli cell suspension and no antiserum showed clinical signs and necropsy lesions somewhat similar to those observed in this experiment. In addition, they attributed the necropsy findings of edema of the gall bladder, edema and hemorrhage of the stomach wall, and congestion of the intestine to the anaphylactic reaction. However, these necropsy findings were very similar to the lesions observed in the present experiment where no anaphylactic reaction occurred. In the 3 pigs in the present experiment given antiserum after endotoxin administration, there were no apparent differences in clinical signs or postmortem lesions as compared to pigs given endotoxin alone. This might indicate that perhaps the lesions observed by Thomlinson and Buxton (1963) were actually a direct result of the endotoxin.

The antiserum appeared to provide protection against the endotoxin in pigs in the present experiment given levels of endotoxin just above the LD_{50} level of endotoxin for pigs. However, when this dosage was increased, protection was no longer provided by the antiserum. These results are similar to the observations made by Braude (1964) in rabbits that antibody does not give protection against large doses of endotoxin but, in most instances, does increase resistance to

endotoxin. Soltys (1963) proposed that antibody is not produced for the highly toxic portion of the endotoxin molecule and that this might explain the limited reduction in toxicity of endotoxin resulting from homologous antibody.

The role of histamine and other similar materials must also be considered in a discussion of the pathogenesis of endotoxin. Hinshaw et al. (1962) showed that in mature dogs histamine, 48/80 (a histamine-releasing agent), and endotoxin produce similar vascular effects such as a decrease in venous return, pooling of blood and a rapid decrease in systemic arterial pressure. However, Hinshaw et al. (1962) found that pups differed from mature dogs in that, instead of the characteristic rapid fall, there is a gradual decline in systemic arterial pressure. This effect of endotoxin in young animals resembled the action of endotoxin in mature dogs that were given an injection of a histamine-releasing agent a few minutes prior to the endotoxin (Hinshaw et al., 1962). Reddin and Spink (1964) showed that the maximal rise in blood histamine after injection of endotoxin in the adult was 0.5 μg. per cent as compared to a maximal rise of 0.05 μg. per cent in puppies. The puppies were found to be 4 times as susceptible to endotoxin as adult animals. One of the most significant differences between young and adult animals is a variation in antibody titer for Escherichia coli. Perhaps endotoxin itself does not cause a release of histamine, but rather an endotoxin-antibody reaction causes the release of

this and similar substances. The decreased susceptibility to endotoxin observed in mature dogs as compared to pups may be due to homologous antibodies present in higher concentrations in mature animals. This antibody could result in neutralization of the endotoxin as observed in rabbits by Braude (1964) and in pigs in the present experiment.

The final effect of endotoxin on an experimental animal is death. The method by which endotoxin causes death is not clearly understood. Coulter (1965) showed that iron deficient pigs were much more susceptible to endotoxin than pigs given iron parenterally. Iron deficient pigs have low hemoglobin levels and, therefore, anoxia appears to be involved in the pathogenesis of endotoxin. Pigs given endotoxin in the present experiment showed clinical signs of extreme respiratory distress which may also be due to anoxia. The cyanotic appearance of the skin on the ears, nose, and legs prior to death of the pigs also indicated anoxia. The surface active property observed in experiments with leukocytes by Karnovsky et al. (1964) might also cause similar changes in erythrocytes making them less capable of transporting oxygen. In addition, endotoxin may interfere with the transfer of oxygen from the alveoli in the lungs to the red blood cells. Part of this effect may be physical as edema of the lungs results in a mechanical obstruction to oxygen transfer. The anoxia, when severe, finally leads to complete circulatory collapse and death.

The syndrome produced in this study appears to be similar in many respects to that observed in naturally occurring cases of edema disease in swine. The cause of death in the pigs in this experiment was concluded to be a direct physiological effect of the endotoxin. This suggests that field cases of edema disease in swine are also the result of a similar response to endotoxin. The role of antibody in field cases of edema disease may be to prolong the course of the disease allowing more marked lesions of edema to develop as observed in some cases. In field cases of edema disease, pigs may develop an endotoxemia when certain serotypes of Escherichia coli multiply in large numbers in the intestines because of ideal conditions for growth such as feeding large amounts of high protein concentrate feeds. However, this in itself is not enough to cause edema disease as lysis of the bacterial cells must occur and the endotoxin must be absorbed from the intestine. Outbreaks of edema disease are commonly associated with dietary and management changes which may result in temporary physiologic changes in the intestine, perhaps enzymatic in nature, that may cause disruption of the Escherichia coli cells and allow absorption of endotoxin.

SUMMARY

The response of young pigs to Escherichia coli endotoxin, purified by a phenol-water extraction method, was studied by intravenous administration of the endotoxin into colostrum-fed and colostrum-deprived pigs. The endotoxin was injected into 19 three-week-old colostrum-fed pigs weighing between 5.75 and 13.5 lbs. at dosage levels of 1, 2, 4, and 6 mg. per kg. of body weight. The LD_{50} of the endotoxin for pigs was found to be 1.8 mg. per kg. of body weight.

The role of anaphylaxis in endotoxemia was studied in 19 colostrum-deprived pigs. Passively administered high titer anti-Escherichia coli swine serum appeared to prevent deaths from endotoxin at the 2 mg. per kg. of body weight dosage level but did not prevent deaths in pigs given a 3 mg. per kg. dosage level. Antiserum pretreated animals showed an acute anaphylactic reaction beginning 10 to 15 seconds after injection of the endotoxin and lasting for about 60 seconds. This reaction was characterized by complete absence of respiration for about 30 seconds, a drop in serum antibody titer for Escherichia coli from 1:290,000 at preinoculation to 1:34,000 at 15 minutes following administration of the endotoxin, and by a drop in hemolytic complement activity to very low and in many cases undetectable levels 15 minutes after injection of endotoxin.

Clinical signs of toxicity in pigs, caused by Escherichia coli endotoxin administration, involved a characteristic

syndrome. Vomition began between 10 and 20 minutes and lasted up to 30 minutes. Incoordination was apparent in many pigs about one hour after endotoxin administration. Following this, respiration became labored and many pigs voided large amounts of soft feces. Terminal signs observed included shallow and irregular respiration, cold cyanotic extremities, and a paddling motion of the legs. Deaths occurred between one and 32 hours after injection of endotoxin.

Gross pathologic lesions were most pronounced in the lungs and digestive tract. Intralobular edema of the lungs, hyperemia and edema of the stomach wall, edema of the gall bladder, and catarrhal enteritis were commonly observed lesions.

Microscopic pathologic lesions were mainly those of edema, congestion, and hemorrhage. The lungs showed edema and congestion and in some cases hemorrhage into alveolar spaces. Severe congestion was observed in the liver and kidneys of most of the pigs. Hemorrhage and congestion of the gastric mucosa was present in almost all pigs, and some pigs showed edema of the stomach wall.

The destruction of leukocytes by Escherichia coli endotoxin was responsible for a marked effect on lysozyme levels in blood serum in the pigs. The average preinoculation lysozyme level for all pigs was 0.28 micrograms per ml. of serum and this was increased to an average of 0.62 micrograms per ml. of serum 15 minutes after injection of endotoxin.

The results of this experiment indicate that the pathogenesis of endotoxemia in pigs involves a direct physiologic response and not anaphylaxis. In addition, the findings of this study provide evidence that field cases of edema disease in swine are also due to a direct physiologic response to Escherichia coli endotoxin.

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