PHYSIOLOGIC AND PATHOLOGIC CHANGES IN CALVES GIVEN ESCHERICHIA COLI ENDOTOXIN OR PASTEURELLA MULTOCIDA

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ABSTRACT

PHYSIOLOGIC AND PATHOLOGIC CHANGES IN CALVES GIVEN *ESCHERICHIA COLI* ENDOTOXIN OR *PASTEURELLA MULTOCIDA*

By

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Ten healthy Holstein bull calves, ranging in age from 8 to 36 days, were used in this experiment. Three animals were inoculated subcutaneously with 4.75×10^5 viable *Pasteurella multocida*, strain 656, organisms, and a fourth animal received half of the above dose. Another four animals were injected intravenously with *Escherichia coli* endotoxin (026:B6) at the rate of 0.03-0.088 mg./lb. body weight. The remaining two animals were the control animals.

Clinical observations and blood collections were made at regular intervals. The blood was used for bacteriologic examinations and for determinations of routine hemograms. Serum was separated from the blood samples and was used for the following determinations: glucose, serum glutamic-oxaloacetic transaminiase, serum glutamic-pyruvic transaminase, and cholesterol. Sera from the control animals and from the animals inoculated with the *Pasteurella* organisms were used to inoculate 11-day-old chick embryos of the white leghorn strain. Serum (0.1 ml.) was inoculated into the embryonated eggs intravenously.

Following death of the calves, or termination of the experiment, a complete necropsy was conducted. Specimens from various organs were collected for pathologic and bacteriologic examinations.

This study showed that the physical signs, hematologic findings, and glucose and cholesterol levels followed more or less a similar trend whether the calves were given *Pasteurella multocida* organisms or *Escherichia coli* endotoxin. Levels of serum glutamic-oxaloacetic transaminase were characteristically elevated as a result of tissue damage accompanying *Escherichia coli* endotoxin, whereas serum glutamic-pyruvic transaminase levels were more characteristically elevated during *Pasteurella multocida* infection. The lung had the most prominent changes in both *Pasteurella*-infected calves and *Escherichia coli* endotoxemic calves. Macroscopic and microscopic lesions were strikingly similar for the two groups of calves. *Pasteurella multocida* organisms were isolated from blood and tissues of animals inoculated with that organism. The primary effect of endotoxin was on the blood and blood vascular system, but whether this was a direct effect or mediated through other substances was not determined.

Based on the similarities of the physical, chemical, and necropsy findings for the two groups of calves, there was the suggestion that an endotoxin identical to, or nearly identical to, *Escherichia coli* endotoxin, was released into the animal tissues during the course of pasteurellosis (*Pasteurella multocida*). The detection of such an endotoxin in the blood by a bioassay method employing 11-day-old chick embryos was discussed.

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LITERATURE REVIEW

Definition

The present concept (Ribi *et al.*, 1964) is that endotoxin is a form of a lipopolysaccharide complex which could be released on disruption of bacterial cells or could be extracted from intact bacteria. Schalm (1965) reported that the endotoxin is located on or in the cell wall of gram-negative bacteria and is released on autolysis. However, there is much difference of opinion among investigators as to what part of this macromolecule is responsible for the biological activity. Knox and Bain (1960) isolated from *Pasteurella multocida*, type I, a polysaccharide fraction which is a biologically active endotoxin. Ribi *et al.* (1961) claimed that the biological activity is not related to the lipid fraction but to the large polysaccharide molecule. Osborne (1965), Schalm (1965), and Alaupovic *et al.* (1968) related the toxin to a lipopolysaccharide-protein complex rather than to polysaccharide alone.

Species Susceptibility to Endotoxin

Berczi *et al.* (1966), testing the effect of *Escherichia coli* endotoxin on 11 species of animals, found that there is a certain correlation of endotoxin sensitivity with the phylogenetic maturity of the species. Fish and frogs displayed an extreme resistance to endotoxin while the mammalian species displayed a wide range of sensitivity to endotoxin; for instance, calves were extremely sensitive, whereas rats and mice were rather resistant. Cruchley *et al.* (1967) observed that endotoxins

extracted from different sources have similar biological responses. These include toxicity, pyrogenicity, the ability to induce nonspecific resistance to infection, diarrhea, tumor necrosis and a local and general Shwartzman reaction.

General

Endotoxin in Laboratory Animals

Extensive research has been performed on laboratory animals in contrast to research on large animals. Consequently, much of our present knowledge about endotoxin was derived primarily from the smaller animal species.

Physical signs. A lot of variations exist between these species in clinical manifestations, but the current clinical concepts as given by Osborne (1965) include chill, biphasic fever, respiratory distress, depression, hypotension, cold extremities, anorexia, oliguria and, occasionally, anuria. Zahi and Hutner (1944) and Berry *et al.* (1959) noticed hypothermia in mice following the administration of endotoxin. Berczi *et al.* (1966) reported vomition in the cat 1 to 2 hours after endotoxin was administered as being characteristic. Sheagren *et al.* (1967) mentioned that the Rhesus monkey during endotoxic shock may show hypothermia if left uncovered during the period of shock.

Hemodynamic and other tissue reactions to endotoxin. The presence of disseminated vascular lesions in dogs, cats, rabbits, rats and mice, resulting in congestion, hemorrhage, thrombosis and necrosis in different organs as well as changes in blood, were well documented by many investigators. Bennett and Beeson (1953) found leukopenia in rabbits following the administration of endotoxin and Thomas and Jones (1959)

discovered that endotoxin could cure leukemia in rats. Berenheimer and Schwartz (1964) examined 17 bacterial toxins for their capacity to disrupt rabbit leukocyte lysosomes and liver lysosomes. They found that there was a correlation between the hemolytic property of a toxin and its capacity to disrupt lysosomes. Berry *et al.* (1959) determined blood sugar, liver and muscle glycogen, and total body carbohydrate in mice receiving *Salmonella* endotoxin. They found complete depletion of the carbohydrates. Hinshaw *et al.* (1960 and 1961) reported a rise in plasma histamine in the dog after injection of endotoxin. Cohn and Morse *et al.* (1960) noted that endotoxin stimulates the phagocytic activity of the leukocytes in rabbits. Kuida *et al.* (1961) found little variation in reactions to endotoxins in species such as the cat, rabbit, and monkey. Nykiel and Glaviano (1961) demonstrated in the dog that endotoxin stimulates the medulla of the adrenal gland and hence increases epinephrine in the adrenal venous blood. Palmerio *et al.* (1962) observed increased catecholamines in the blood of the rabbit as well as injury to the myocardium when a bacterial endotoxin was administered. Ushiba *et al.* (1962) found that gram-negative bacilli in the intestine of mice multiplied rapidly following intraperitoneal injection of bacterial endotoxin, while bacteria such as *Lactobacilli*, which normally exist in a larger number than gram-negative bacilli, remained constant in number. Hinshaw and Nelson (1962) noted splanchnic pooling of blood following the injection of an endotoxin. Hemodynamic reactions have been studied in the dog more than in any other species according to Alican (1962), who cited from the work of others the following responses to endotoxin; hemoconcentration, plasma loss, increased plasma hemoglobin, bloody diarrhea, hemorrhagic necrosis of the bowel mucosa, marked lymph flow in the intestine, and adrenal stimulation. Herring *et al.* (1963)

observed that circulating endotoxin in rabbits was distributed between plasma and platelets. Muller and Smith (1963) showed that there was a rise in hematocrit during endotoxic shock in the dog. They also noted an immediate fall in blood pressure, in cardiac output, and in central venous pressure. Petersdorf and Shulman (1964) found that injected bacterial endotoxin in rabbits and dogs was taken by the reticuloendothelial system. Schaedler and Dubos (1964) reported that NCS mice, which were free of ordinary mouse pathogens as well as intestinal *Escherichia coli*, were highly resistant to lethal effects of endotoxin. Osborne (1965) observed that microembolism, thrombosis, pulmonary edema, ischemia, and anoxia of many organs, notably the kidneys and lungs, were among the most common findings in bacterial endotoxic shock. Schalm (1965) gave the following sequence of events after administration of endotoxin: arteriolar constriction, fever, hypoglycemia, and leukopenia followed by leukosis. Berczi *et al.* (1966) reported extensive edema and hemorrhage in the cat after administration of endotoxin. Bounous *et al.* (1966) observed that the intestinal mucous barrier was reduced during shock in the dog and that the intestine was accessible to proteolytic digestion. Dellenback *et al.* (1966) noted that the dog's central blood volume decreased while splanchnic blood increased during endotoxic shock. Lemperle (1966) found that injected endotoxin in mice is apparently taken up almost exclusively by the reticuloendothelial system and that death occurs after failure of this system to remove circulating endotoxin. McKay *et al.* (1966) gave an excellent microscopic study of the effects of the bacterial endotoxin on the blood vascular system of rats. They found that the first change was agglutination and sequestration of platelets in the pulmonary capillaries associated with a sequestration of polymorphonuclear leukocytes. One hour after injection, some of the

leukocytes were disrupted and then discharged their cytoplasmic and nuclear components into the plasma. Fibrin deposits appeared in the vicinity of platelets 4 hours later. They concluded that bacterial endotoxin induces damage by triggering the clotting mechanism *in vivo*. Tikoff *et al.* (1966) reported that the target organ for endotoxin in cats and sheep is the lung. The reaction of dog's liver to endotoxin was increased fibrillar material, then depletion of glycogen and pooling of blood (Boler and Bibighaus, 1967). Cortell and Conrad (1967) claimed that intestinal histology and the life span of mucosal cells is not changed by injecting *Escherichia coli* endotoxin in albino rats. Hinshaw *et al.* (1967) in their study with the coyote found a progressive increase in serum glutamic-oxaloacetic transaminase, constant blood urea nitrogen, hemoconcentration, systemic hypotension, depressed liver function, hepatic-renal and adrenal congestion, intestinal necrosis, and gall-bladder edema. The liver was the primary target organ. Levy *et al.* (1967) described the histologic changes in mouse liver after a single dose of endotoxin. Rebers *et al.* (1967) showed that mice and rabbits react similarly to *Pasteurella multocida* endotoxin. Granulocytopenia, followed by granulocytosis in the Rhesus monkey were noted by Sheagren *et al.* (1967). The chicken reacts to endotoxin by the development of yellowish, necrotic, and irregular foci of necrosis in the liver, as reported by Trauscott and Innis (1967).

Endotoxin in Large Animals

Little work has been done with endotoxin in large animals in contrast to the extensive work in small laboratory animals.

Physical signs. Schalm (1965) found that following the administration of endotoxin to many of the large animals there were evident responses characterized first by arteriolar constriction, fever, hypoglycemia, and leukopenia followed by leukosis. With a lethal dose, the animals became anorectic, progressively weak, ataxic, and highly feverish, with respiratory distress and blood-tinged foam at the nostrils. Vomition, diarrhea, convulsions, and death in 24 hours represented the terminal stages. Carroll *et al.* (1965) indicated that the immunologic, phsyiologic, and pathologic responses of animals to administration of endotoxin were the same regardless of the species of gram-negative organism from which the endotoxin was obtained.

Ruminants. Halmagyi *et al.* (1963) claimed that in sheep respiratory arrest is an important feature of endotoxic shock. Carroll *et al.* (1964) administered endotoxin into the udder of the cow and found the rectal temperature 7 hours postinoculation to be 106-107 F. Mullenax *et al.* (1966) reported a decrease in rumen motility, momentary apnea, and increased respiratory frequency in ruminants following the administration of endotoxin via the intravenous, subcutaneous, and intraruminal routes.

Horses. Schalm (1965) showed that after intraperitoneal injection of endotoxin in the horse, collapse resulted within an hour.

Hemodynamic and other tissue reactions to endotoxin. Lillehei and Maclean (1959) observed that during endotoxic shock in man, there was plasma loss, increase in hematocrit, increase in plasma hemoglobin, and necrosis of the bowel.

Ruminants. Halmagyi *et al.* (1963) indicated that in sheep the lung is the main target organ and that the response to endotoxic shock consists mainly of a marked rise in pulmonary arterial pressure and a fall in both cardiac output and in systemic arterial pressure. Davis and Simbert (1964) described the events in swine after *Escherichia coli* endotoxin injection as follows: glucose level initially dropped, then increased, but finally decreased below preinoculation levels. There was also a decrease in the number of white blood cells. Carroll *et al.* (1964) induced maximal local response in lactating mammary glands of the cow by intramammary inoculation of *Aerobacter aerogenes* endotoxin. Local edematous swelling was observed within 2 hours. After 3 hours, serum albumin was detected in milk and temporary hypoproteinemia resulted. The number of leukocytes in milk peaked at 6 hours. Hansen (1964) investigated an outbreak of toxic liver injury in ruminants. He found that the glutamic-pyruvic transaminase test gave the least correlation with liver injury, while the glutamic-oxaloacetic transaminase test was closely correlated, very sensitive, and may have been elevated before clinical symptoms were detectable. Schalm (1965) characterized anaphylactic shock in the cow by leukopenia and increased packed cell volume. Tikoff and Kuida (1965) stated: "Pulmonary vasoconstriction is a prominent feature of the hemodynamic responses to endotoxin in the calf." Mullenax *et al.* (1966) reported leukopenia in cattle in 1 to 4 hours, followed by leukocytosis about 24 hours after inoculation of an endotoxin prepared from rumen bacteria. They also found hyperglycemia in 1 to 3 hours, followed by hypoglycemia at 5 to 7 hours, and a decrease in serum beta-globulin 2 to 3 days later. Rebers *et al.* (1967) isolated from virulent encapsulated *Pasteurella multocida* a lipopolysaccharide-protein antigenic complex, which is a heat stable particulate. This, when injected in

fractional milligram amounts into mice, rabbits, and calves, produced toxic reactions which frequently resulted in death of the host. Surviving animals developed immunity to live organisms. In calves (13 to 18 weeks of age), after intravenous administration of this endotoxin, the sequence of events was described as: rapid shallow breathing within 5 to 15 minutes, depression, increased salivation, and lacrimation; 30 minutes later there was diarrhea which was mucoid and then watery. The rate of salivation was increased as the terminal phase of the shock approached and a nasal discharge appeared, followed by recumbency. One calf showed coma and died within 4 to 5 hours. They found also that in calves the signs following an injection of endotoxin resembled those which appear after challenge with the whole organism. Heddleston *et al.* (1967) reported that calves injected with *Pasteurella multocida* developed septicemia and died in 24-48 hours. They stated that

> "If the clinical signs and death are the result of an endotoxin, then sufficient toxin was released from infected lungs to produce clinical manifestations."

Rhoades *et al.* (1967) studied the gross and the microscopic lesions resulting from acute infections and from administration of endotoxin of *Pasteurella multocida* in 3 calves and 2 pigs. *Pasteurella multocida* endotoxemia is characterized by widely distributed hemorrhages, edema, general hyperemia, and pneumonia. These lesions were similar to those detected at necropsy in calves infected with *Pasteurella multocida* organisms. The calves which received endotoxin intravenously died within 4-1/2 hours, while those injected with the whole organisms died within 40 hours. The lungs of the endotoxemic calves were markedly hyperemic, hemorrhagic, and edematous, and a moderate degree of alveolar emphysema existed. There was slight hyperemia of the digestive tract, liver,

spleen, and pancreas, and moderate hyperemia of the kidneys. A slight degree of cloudy swelling in the liver and interlobular edema in the pancreas were also observed. Lymph nodes were hyperemic and edematous.

Horses. Rooney *et al.* (1963) reported a disease in the horse which they called Colitis "X". The etilogy of the disease was not clear, but its clinical and pathologic manifestations indicated that it was related to endotoxemia. Endotoxins prepared from several strains of *Escherichia coli* gave an identical disease syndrome when injected intravenously. Schalm (1965) described the effects of endotoxin in horses. Leukopenia was followed by an increased number of leukocytes. Packed cell volume initially rose, then dropped, only to increase again near completion of the experiment. Plasma protein concentration paralleled the packed cell volume. Petechial hemorrhages were observed in the lungs, heart, serosa of the gastrointestinal tract, mesentery, and cortex of the kidney. The small intestine was dilated and contained a yellowish material. Intestinal mucosa was congested and edematous, while the changes in the large intestine and cecum were not as marked. Carroll *et al.* (1965) indicated that the peracute form of endotoxemia in the horse resembles the generalized Shwartzman reaction.

Specific

Endotoxin from *Escherichia coli*

For many years, the endotoxin of *Escherichia coli* has been the preparation of choice in the study of the endotoxic shock in different species of animals. Extensive studies have been done with *Escherichia coli* endotoxin in laboratory animals, but very little work has been done in laboratory animals. Among those who used *Escherichia coli* endotoxin to

elucidate certain aspects of endotoxic shock are: Horwitz *et al.* (1962), studying the effect of endotoxin on platelets in rabbits; Harley and Johnson (1963), studying the clotting mechanism in endotoxic shock in dogs; Lawferd and White (1964), studying abnormal serum components after administration of *Escherichia coli* endotoxin in rats; Forbes (1965), studying induction of mitosis in macrophages by endotoxin in mice; and Starzecki *et al.* (1967), studying distribution of endotoxin ^{51}Cr after its administration in dogs.

Hemodynamic and other tissue reactions. Tikoff *et al.* (1966) studied the hemodynamic effects of endotoxin from *Escherichia coli* in anesthetized calves, ranging in age from 2 to 4 weeks. The dose of endotoxin administered was either 0.25 or 0.5 mg./kg. body weight, with most animals receiving the latter dose. Nine of 14 calves died 12 hours following receipt of endotoxin and 2 more died later (2nd and 4th day). They found the most striking effects to be pulmonary arterial hypertension and systemic arterial hypotension. Hemoconcentration, hepatosplanchnic pooling, and metabolic acidosis were either absent or minimal. The lung was the major target organ. At the time of necropsy, they found mild generalized visceral congestion which was easily visible in the liver but minimal in the bowel. Small endocardial hemorrhages were also observed. The lung was edematous with widespread areas of hemorrhage and atelectasis. Microscopically, the lesions were severe capillary engorgement, focal edema, and hemorrhage. Also, edema fluid and blood were found in the alveoli and bronchioles. Aside from congestion, all other tissues did not show significant changes.

Mode of Action of Endotoxin

The endotoxins, when administered in either animal or man, have a very wide spectrum of biological activity. But not all of the activities of endotoxin are related to each other. There are still many differences of opinion about the mode of action of the endotoxin. The following hypotheses seem to be quite well documented.

Lysosome Release Hypothesis

Weissmann and Thomas (1964) postulated that endotoxin affects the stability of the lysosomes with the subsequent release of acid hydrolase enzymes into cell sap or surrounding tissue, thus causing damage in the tissues. This gives rise to lactate, which lowers the intracellular pH and causes either the membrane of the lysosome or the autophagic vacuoles to become fragile.

This hypothesis links together many pieces of information presented by numerous investigators. Among these are: Berry *et al.* (1959), Nykiel and Galviano (1961), Atwood and Kass (1964), and Berry (1964).

Catecholamine Release Hypothesis

Fine and Minton (1966) observed that endotoxin is capable of acting directly on certain tissues like granulocytes and platelets. From the lethal effects of the endotoxin, they postulated that

> "The endotoxin increases the release of catecholamine in splanchnic tissues produced by the splanchnic sympathetic nerves, and this results in loss of the integrity of blood vessels in the splanchnic area and hence the lethal effect is produced."

This hypothesis also confirms scattered information presented by other investigators, among whom are: Thomas (1956), Nykiel and Glaviano (1961), and Palmerio *et al.* (1962).

Histamine Release Hypothesis

Hinshaw *et al.* (1960 and 1961) observed an increase in blood histamine and in the histamine-histidine ratio (H/Hd). Also, the activity of histidine decarboxylase enzyme is increased. They then postulated that "histamine plays an active role in endotoxic shock."

Miscellaneous Less Popular Hypotheses

Antigen-antibody reaction. Suter (1964) stated that "there are some observations that some of the biological manifestations of endotoxin action may be based on antigen-antibody reaction." However, Landy and Weidanz (1964) found natural antibodies against a variety of gram-negative organisms in the sera of different laboratory and large animals, which might play a part in the antigen-antibody reaction.

Generalized cell poison, key cells poison, or central nervous system effect. Kass *et al.* (1964) stated that

> "The wide variety of recorded physiological effects suggests either that endotoxin is a generalized cellular poison or that it has widespread effects on key cells that are widely distributed throughout the body (such as the vascular endothelium) or that there is a primary locus as in a major communications center such as the central nervous system that is specifically affected."

This hypothesis also agrees with the work of Alper *et al.* (1967).

Cessation of production of mucus. Bounous *et al.* (1966) observed that endotoxin administration results in cessation of the production of mucus by intestinal mucosa, and thus the intestine is accessible to proteolytic digestion.

Bioassay of Endotoxin

Braude (1964) suggested that the bioassay of endotoxin in tissues or body fluid should be based either on its toxicity, i.e., its ability to produce fever, abortion, dermal necrosis, and death, or on its antigenic properties. He found also that the body fluids may react with the endotoxin to reduce or accentuate their toxicity. The route of administration affects absorption. The lethality depends also on the persistence of the endotoxin in the blood.

Many investigators started looking at the possibility of using some laboratory animals for carrying out the bioassay of endotoxin. Smith and Thomas (1956) indicated that the inoculation of chorioallantoic membrane of a 10-day-old chick embryo with endotoxin of a gram-negative microorganism resulted in multiple hemorrhages and death of the embryo within a few hours. They noticed also that the susceptibility to the endotoxin was maximal in 10-day-old embryo, while younger or older embryos showed little or no response. The optimal inoculation temperature for effect of endotoxin was 39.5 C. Hook *et al.* (1961) gave intravenous injections of *Escherichia coli* endotoxin (0.1 μg.) to 15-day-old chick embryos and found extensive brain hemorrhages, hydrocephalus, and necrosis of some nerves. Injections of a sublethal dose increased the chick embryo's resistance. They concluded that the response of chicken embryo to bacterial endotoxin is determined by the route, dose, and age of the embryo. Finkelstein (1964) stated

> "The chick embryo may be regarded as essentially germ-free and immunologically virgin thus better in bioassay than animal hosts used."

He indicated that death of the embryo occurs within a matter of hours in susceptible embryos and that the chick embryo becomes refractory to intravenous administration of endotoxin during the 11th to 15th day of

of incubation. The magnitude of change of susceptibility was greater than 10,000-fold. Berczi *et al.* (1966) found the lethal dose for a chick weighing 1 gram to be greater than 50 mg./kg. body weight. Milner and Finkelstein (1966), comparing the bioassay of endotoxin in the chick to that in rabbits, concluded that the chick embryo test gives satisfactory reproducible results and is both cheaper and easier to perform than the pyrogen test in rabbits. Trauscott and Inniss (1967) explored the possibility of using chicks rather than embryos for the bioassay. They found that chicks generally demand a high dose of endotoxin, but 2-week-old chicks were susceptible to endotoxin provided it was prepared by sodium chloride extraction. The test is considered positive when 2 to 3 yellowish, necrotic, and irregular foci are found on the liver of these birds. They noted also that meat strain chicks were more susceptible than egg strain chicks. Keiss *et al.* (1963) tested the potency of *Pasteurella hemolytica* endotoxin in sheep by using both the rabbit dermal test and the hemodynamic effect test. Spink and Starzecki (1967) performed a bioassay of endotoxin by what they called the rabbit-epinephrine-skin test which employs an injection of 5 to 6 ml. of plasma (to be tested) intravenously and simultaneously with the intradermal injection of epinephrine. The test is considered positive if an area of hemorrhagic necrosis of 20 mm. or more is observed within 24 hours at the site where the epinephrine was injected.

Pasteurella Multocida

The subject of pasteurellosis in domestic animals has been extensively studied by many workers due to its economic implications in many countries.

History

Bain (1963) mentioned that pasteurellosis of cattle was first described in 1878 by Bollinger, and the causative agent was isolated by Kitt in 1885. A German pathologist (Hueppe) was the first to use the term hemorrhagic septicemia (HS).

Incidence

Hemorrhagic septicemia occurs in Southern Europe, including the U.S.S.R., North, Central, and East Africa, the Near East, and Southern and Southeast Asia.

Robert's (1947) serotype I or Carter's (1957) type B has caused hemorrhagic septicemia in bison in the U.S.A., and cultures of these still exist in various collections.

Wilson and Miles (1964) identified the *Pasteurella multocida* organism as a small, gram-negative, ovoid bacillus showing bipolar staining. The organism is aerobic and facultatively anaerobic, slightly ferments carbohydrate, is indol positive, but neither produces gas nor liquifies gelatin. The genus *Pasteurella* was fully described by Merchant and Packer (1967). Carter (1967) has reviewed comprehensively the whole subject of pasteurellosis and hemorrhagic septicemia. Rhoades *et al.* (1967) induced hemorrhagic septicemia experimentally in 3 calves and 2 pigs. They reported widely distributed hemorrhage, edema, and general hyperemia.

Physical Signs

Blood and Henderson (1968) stated that the disease is characterized by a sudden onset of fever (106-107 F.), profuse salivation, severe depression, and death in 24 hours. Localization may occur in subcutaneous tissues resulting in hot, painful swellings about the throat, dewlap,

brisket, or perineum, and severe dyspnea if respiration is obstructed. In the later stages of an outbreak of hemorrhagic septicemia there may be signs of pulmonary or alimentary involvement.

Hemodynamic and Other Tissue Reactions

Runnells *et al.* (1965) described pasteurellosis in cattle as an acute infectious disease caused by *Pasteurella multocida* and occasionally by *Pasteurella hemolytica*. These organisms are normal inhabitants of the bovine lung and are, on occasion, capable of invading the tissues and producing pneumonia. Usually the disease is associated with shipping of cattle, or the introduction of new cattle into a herd of animals. It is especially common in feedlot cattle. In most instances, *Pasteurella*, by themselves, do not produce pneumonia unless associated with or preceded by a viral infection. On the other hand, the viral manifestation by itself produces a very mild transitory disease. The principal lesions are those of an acute fibrinous pneumonia with generalized passive hyperemia, multiple hemorrhages in serous and mucous membranes, and degenerative alterations in the parenchymatous organs. In some animals, there is general edema due to passive hyperemia, hypoproteinemia, and shock. Smith and Jones (1966) stated that

> "Gross lesions in fatal cases of pasteurellosis are diverse and hardly specific. In cattle, losses following shipping ('shipping fever,' 'shipping pneumonia') are usually the result of pneumonia, consolidation of the lung being most frequent in the apical portions, less often extending to the diaphragmatic lobes. Interlobular edema may be present but is seldom prominent, nor are hemorrhages outstanding in the bovine disease. Organisms are reportedly numerous in the pneumonic areas of the lung."

Blood and Henderson (1968) described the lesions of hemorrhagic septicemia as: generalized petechial hemorrhage particularly under the

serosa and edema of the lungs and lymph nodes. Subcutaneous infiltration of gelatinous fluid may be present in a few animals. Lesions of early pneumonia and hemorrhagic gastroenteritis are also seen.

MATERIALS AND METHODS

Pasteurella multocida Organism

Pasteurella multocida strain 656 was used as one of the experimental inocula. The original culture was isolated from a buffalo suffering from an acute case of hemorrhagic septicemia in the Yellowstone National Park in 1922.

The organism was grown in brain heart infusion broth for 18 hours at 98.6 F. A laboratory mouse (Webster strain) was inoculated subcutaneously with 0.1 ml. of the broth culture. Eighteen hours later in the agonal stage of the infection blood was drawn aseptically from the heart and 0.5 ml. was inoculated subcutaneously into a male white New Zealand rabbit. The latter died in about 20 hours; immediately the liver was exposed aseptically and a small portion was removed which weighed 7.75 grams. It was cut into small pieces and then placed in a Ten Broeck tissue grinder and ground finely in human blood. Four milliliters of human blood (500 ml. blood in 40 ml. Alsever's solution) were used for each gram of liver substance. Immediately after grinding, 0.5 ml. portions were placed in small sterile test tubes and quickly frozen, using dry ice in alcohol. The frozen material was stored in a freezer (-75 F.) until used to inoculate animals in Group P.

Escherichia coli Endotoxin

Escherichia coli (*E. coli*) lipopolysaccharide (*E. coli* [026:B6]) was supplied by Difco Laboratories, Detroit, Michigan, U.S.A. It was

dissolved in sterile distilled water to make a final concentration of 1 mg./ml. and was stored at 37.4 F. (refrigerator) until used.

Chick Embryo Assay

White leghorn eggs were incubated in a humidified, self-turning incubator at a controlled temperature of 100.4 ± 1.8 F. At 11 days, a large vein in the chorioallantoic membrane of the chick embryo was identified by candling. A small triangular window was cut over the vein using a small carborundum disc. The soft shell membrane was then cleared by swabbing with sterile mineral oil, and an intravenous injection of serum (0.1 ml.) to be tested was made using a 27-gauge needle. Eggs observed to bleed significantly from the injected vein were discarded. A layer of melted paraffin was placed over the window and the eggs were returned to a non-turning tray in the incubator. Observations were conducted, by candling, at the 21st, 24th, and 40th hour postinoculation for death of the chick embryo. Prior to intravenous injection the serum was filtered through a 200 mμ membrane filter. Routine bacteriologic examinations of dead embryos included cultures on blood agar and smears for detection of bacterial contamination. Five embryos were used per serum sample to be assayed. Chick embryo assays were conducted for animals in Groups P and C.

Experimental Animals

Ten healthy Holstein bull calves, varying in age from 8 to 36 days and in weight from 64 to 140 pounds, were used. The animals were divided into 3 groups, P, E, and C, with the various ages and weights being represented in each group.

1. Group P was composed of 4 animals, P_1, P_3, P_4, and P_5. All the animals except P_3 were inoculated subcutaneously with 1 ml. of rabbit liver material (described before) containing approximately 4.75×10^5 viable *Pasteurella multocida* strain 656 organisms. Animal P_3 received 0.5 ml. of the same material. Blood samples were drawn from animals P_1 and P_3 by jugular venipuncture using suitable needles and syringes. From animals P_4 and P_5, the blood samples were drawn through an indwelling polyethylene catheter which had been fixed in the jugular vein 1 day before dosing the animal. Blood clotting inside the catheter was prevented by filling it with sodium heparin solution after each blood collection. Before each blood collection, heparin solution was withdrawn and discarded.

Physical observations and sample collections were made according to the following schedule:

Animals P_1 and P_3: Every 12 hours until each died.

Animals P_4 and P_5: At 6, 12, 14, 16, 18, 20, 22, 24, 25, 26, 28, and 30 hours postinoculation.

2. Group E was composed of 4 animals, E_1, E_2, E_3, and E_4. They were all dosed intravenously with *Escherichia coli* endotoxin as follows:

E_1 injected at the rate of 0.030 mg./lb. body weight, total dose being 3 mg.

E_2 injected at the rate of 0.036 mg./lb. body weight, total dose being 4 mg.

E_3 injected at the rate of 0.088 mg./lb. body weight, total dose being 8 mg.

E_4 injected at the rate of 0.057 mg./lb. body weight, total dose being 4 mg.

The blood samples from the animals in this group were drawn through an indwelling polyethylene catheter which had been fixed in the jugular vein 1 day before dosing the animal. Blood clotting inside the catheter was prevented by filling it with sodium heparin solution after each blood collection. The heparin solution was withdrawn and discarded before each sample collection.

Clinical observations and blood collections (postinoculation) for animals in Group E were as follows: at 5, 10, 15, 30, 45, 60 and 90 minutes, 3 hours, and at hourly intervals thereafter until the first signs of recovery.

3. Group C was the control group, composed of 2 animals, C_1 and C_2. Animal C_1 was treated in all respects as animals in Group E, except for the endotoxin injection. Animal C_2 was treated the same as animals P_4 and P_5 except that *Pasteurella multocida* organisms were not administered.

Clinical Observations

The clinical signs, rectal temperature, and heart rate, determined by a stethoscope over the heart area, were recorded in all the animals at the time of blood sampling.

Necropsy and Histopathology

Animals in Group C were euthanatized with an electric shock (110 voltage) at the termination of the experiment. Two animals in Group E (E_2, E_4) were likewise euthanatized shortly after recovery from endotoxic shock. Animal E_3 died as a result of the endotoxin. All animals in Group P died as a result of the *Pasteurella* infection.

A complete necropsy was conducted on all the animals except E_1, and specimens from the brain, kidney, adrenals, liver, intestine, lymph nodes, and lungs were taken for histopathologic and bacteriologic examinations. The specimens for histopathologic examination were fixed in 10% buffered neutral formalin, paraffin* embedded, and cut at 6 μ. The sections were stained with hematoxylin and eosin, as described by the Armed Forces Institute of Pathology (1960).

Bacteriologic Examination

The blood samples, drawn aseptically, and the specimens taken at necropsy referred to above, were subjected to routine bacteriologic examination. Blood was inoculated into diphasic blood culture bottles and organs and tissues were inoculated onto blood agar. Routine procedures were employed for the identification of those bacteria isolated.

Hematologic Determinations

Approximately 2.5 ml. of blood were collected in a small test tube containing 0.05 ml. of 7.5% tripotassiumethylenediaminetetraacetate (K_3EDTA) for the hematologic determinations.

Total erythrocyte and total leukocyte counts were performed on an electronic cell counter.**

Packed cell volume (PCV) was performed using the microhematocrit method.

Hemoglobin (Hb.) was determined by the cyanmethemoglobin method using a Coleman Junior spectrophotometer.

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*Paraplast, Scientific Products, Evanston, Illinois.

**Electronic Coulter Counter, Model A.

Platelets were counted using the method described by Schweizer and Gerarde (1964).

Smears for differential leukocyte counts were made from the test tube blood soon after collection, promptly dried, stained with Wright's stain, and 100 cells differentiated.

Biochemical Determinations

About 20 ml. of blood were drawn at the time of each observation. This blood was placed in 2 test tubes and was centrifuged in a refrigerated IEC international centrifuge at 19 x 10^2 rpm for 20 to 30 minutes. The serum was then transferred, using a Pasteur pipette, to a plastic vial which was stoppered and stored in a freezer (-76 F.) until the tests were run.

Sugar Determination

The sugar content of the serum was determined using the Folin-Wu method (Folin and Wu, 1919).

Enzyme Determinations

Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were determined using the standard method described by SIGMA.*

Cholesterol Determination

Serum cholesterol was determined by the Ferro and Ham modified procedure (Ferro and Ham, 1960).

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*SIGMA Technical Bulletin No. 505, Reissued September, 1967.

Statistical Analysis

Where appropriate, the mean, the standard error of the mean, and the level of significance were determined. Analysis of variance was conducted on the hematologic and biochemical findings of both Groups P and E.

RESULTS

Physical Signs

Pasteurella multocida, Group P

Within 7 to 16 hours after inoculation, there were signs of depression accompanied by respiratory distress, salivation and infrequent nasal discharges. The degree of depression progressed continually throughout the course of the disease, to the point where the animals were unable to eat and remained recumbent 18 to 20 hours before death occurred. Painful swellings around the throat, dewlap, and the brisket developed about 24 to 26 hours postinoculation. During the terminal stages of the disease, there was mild diarrhea, the animal rested its head more or less permanently on its shoulder, and the tongue, which became progressively bluish in color, partially protruded from the mouth (Figure 1).

The rectal temperature (F.) and the heart rate (per minute) are given in Table 1 and Figure 2. The temperature rose from 101.2 F. at 6 hours postinoculation to 104.4 F. at the 18th hour and remained within less than one degree of that peak until death. The heart rate was elevated throughout the course of the disease with peaks reached at the 14th, 24th, and 30th hours after inoculation.

Escherichia coli, Group E

Within 5 minutes after the injections of the endotoxin, all animals were lying down and were experiencing difficulty in respiration. Animal

Figure 1. Prostration and twisting of the neck prior to death in *Pasteurella*-inoculated calf.

Table 1. Mean values for body temperature and heart rate for animals inoculated with *Pasteurella multocida* organisms (Group P) as compared to the control animal

Time (hrs.)	Group P		Control (C_2)	
	Temp. (F.)	Heart rate/min.	Temp. (F.)	Heart rate/min.
6	102.8	79	101.2	68
12	103.8	86	101.2	68
14	104.1	96	102.0	76
16	104.3	76	101.2	68
18	104.4	84	101.2	68
20	104.1	93	101.2	64
22	104.7	120	101.2	64
24	104.5	140	102.0	64
25	103.8	85	102.0	64
26	104.9	137	102.0	64
28	104.7	132	102.0	60
30	104.4	167	102.0	64
32	104.6	164	---	---

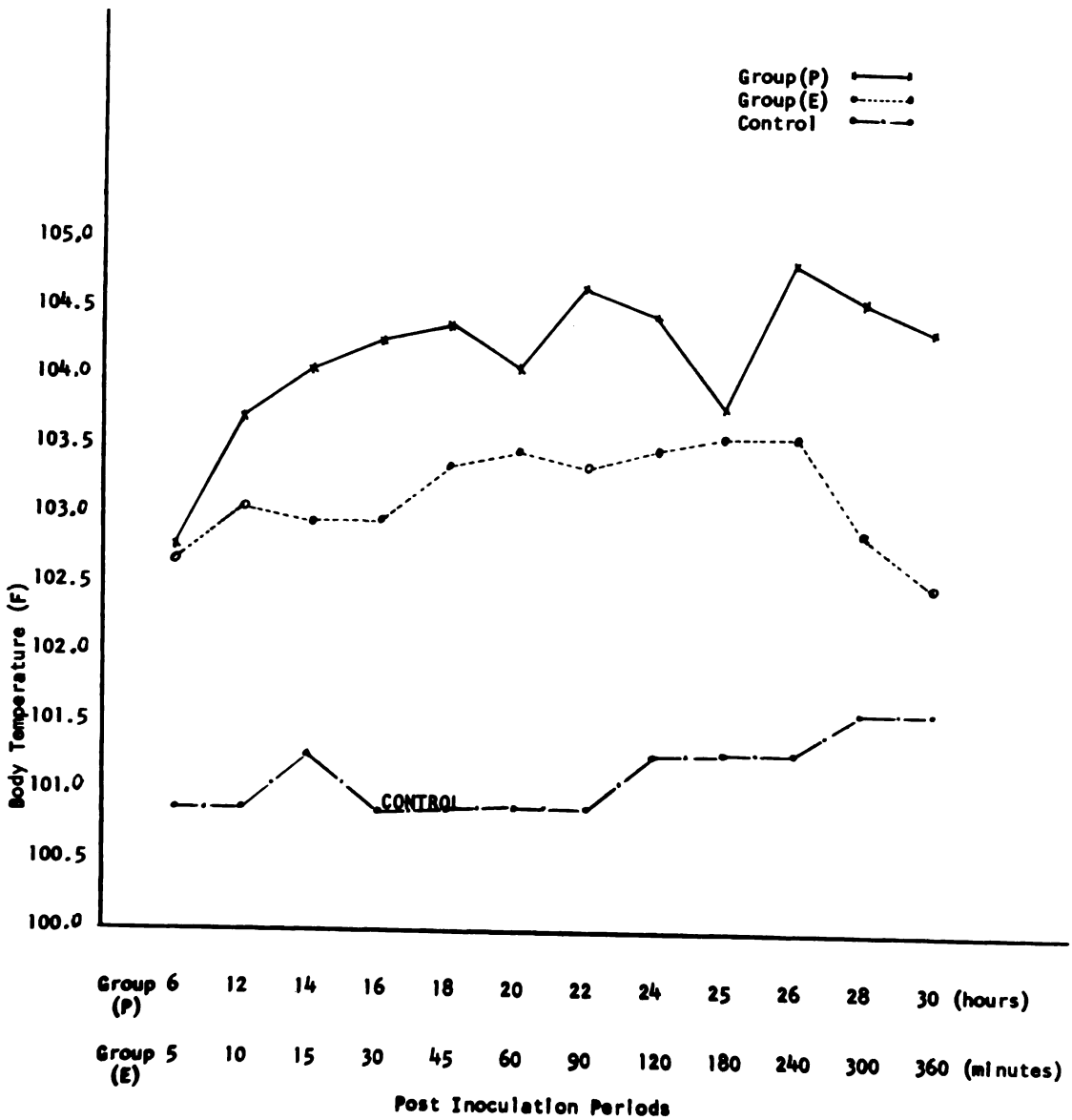

Fig.2 --- Mean Body Temperature Values for Experimental Calves.

E_3 showed the most difficulty and, in the next 5 minutes, began to struggle and then died 15 minutes postinoculation. Respiratory distress continued to progress in the other 3 animals and, in about 45 minutes after the endotoxin administration, the animals were "panting" with their tongues partially protruding. They frequently rested their heads on the shoulder region or on the floor. It was at approximately this time that soft feces were passed. These signs continued during the period of endotoxic shock, after which the character and rate of respiration gradually returned to a normal pattern. Passing of feces ceased and the animal resumed an alert attitude. For animals E_1 and E_4, recovery occurred at 6 hours postinoculation and, for animal E_2, at 7 hours postinoculation.

Body temperature and heart rate remained elevated throughout the course of shock (Table 2 and Figure 2). For each determination, there was a sharp rise within the first 5 minutes. The highest readings were reached at 3 hours postinoculation and then, after 5 hours, they gradually declined.

Bacteriologic Examination of Jugular Blood

Pasteurella multocida, Group P

Pasteurella multocida was recovered from the 6th hour after inoculation until all animals died. No other organisms were detected.

Escheriohia coli Endotoxin, Group E

No organisms were isolated from the blood.

Control Group C

No organisms were isolated from the blood.

Table 2. Mean values for body temperature and heart rate for animals receiving *Escherichia coli* endotoxin (Group E) as compared to the control animal

Time (min.)	Group E		Control (C_1)	
	Temp. (F.)	Heart rate/min.	Temp. (F.)	Heart rate/min.
5	102.7	61	100.6	96
10	103.1	67	100.6	96
15	103.0	69	100.6	96
30	103.0	60	100.6	96
45	103.4	60	100.6	96
60	103.5	72	100.6	96
90	103.4	94	100.6	96
120	103.5	110	100.6	96
180	103.6	124	101.7	60
240	103.6	112	101.7	60
300	102.9	110	101.2	60
360	102.5	94	101.2	76

Necropsy Findings

Gross Lesions

Pasteurella multocida, Group P. Petechial and ecchymotic hemorrhages were widely distributed throughout the pectoral and sternal muscles and on the outer surface of the trachea, thymus, pericardium, epicardium, endocardium, spleen (Figure 3), forestomach, and the mesentery. Congestion was evident in the lungs, small intestine, lymph nodes, the medulla of the kidneys, the adrenals, and the brain. The liver was pale. Edema was detected in the ventral portion of the neck and around the thoracic inlet. The ruminal contents were dry and both the urinary bladder and gallbladder were full. Calf number P_5 showed, in addition to these findings, hemorrhage in the tricuspid and bicuspid valves of the heart.

Escherichia coli, Group E. The organs in the abdominal and thoracic cavities were congested in all the animals. In all calves, except E_3, there was petechial hemorrhage on the outer surface of the trachea, lung, and the spleen (Figure 4). The adrenals were hemorrhagic. The trachea and the bronchi contained some frothy material. Both the urinary bladder and the gallbladder were full.

Control Group C. No gross lesions were observed in the control animals.

Bacteriologic Examination at Time of Necropsy

From animals in Group P, *Pasteurella multocida* was isolated in pure culture from the liver, lung, spleen, kidney, and lymph nodes, but not from the bile or urine. *Escherichia coli* was isolated from the intestines.

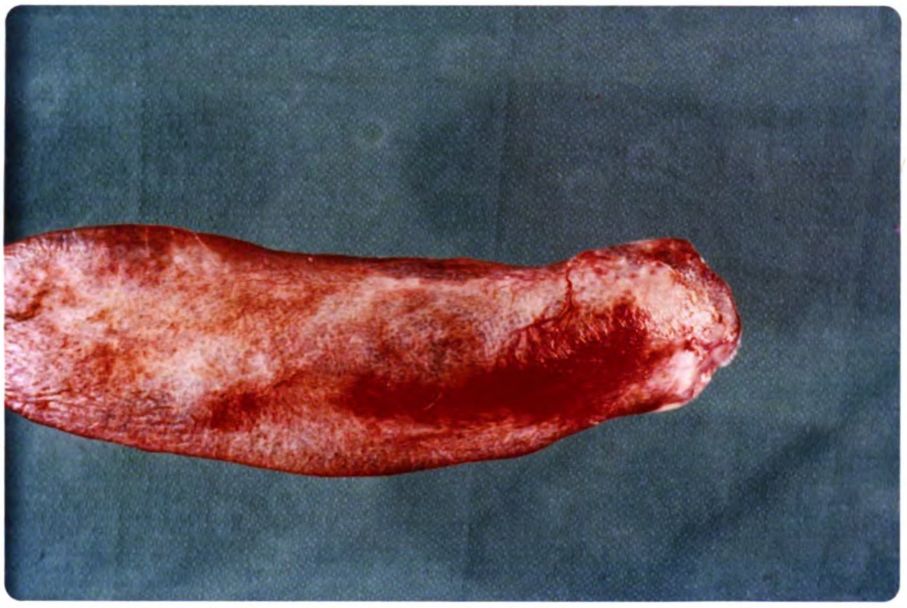

Figure 3. Subcapsular ecchymotic hemorrhage in the spleen of *Pasteurella*-inoculated calf (dorsal view).

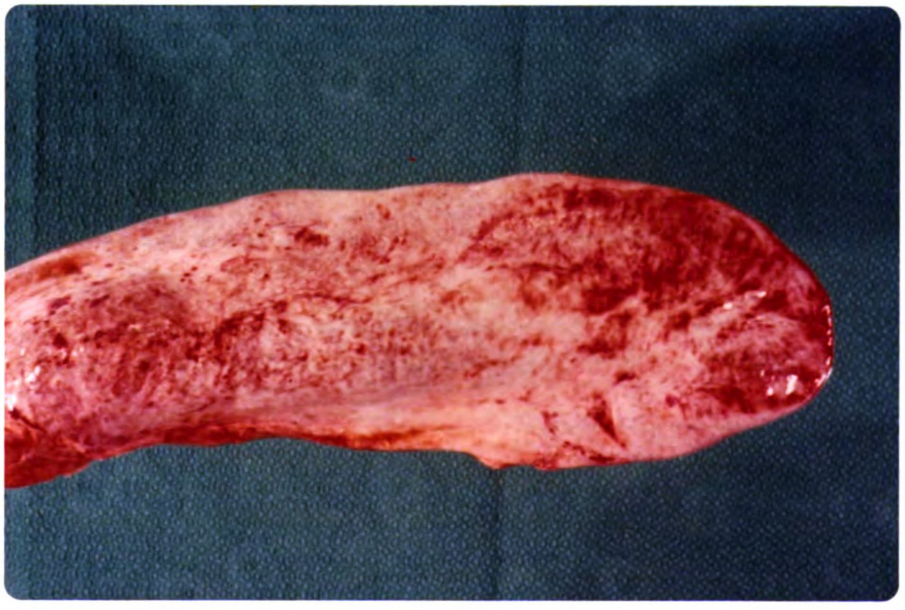

Figure 4. Subcapsular petechial hemorrhage in the spleen of endotoxin-treated calf (dorsal view).

From animals in Group E, no organisms were isolated from the liver, spleen, kidneys, and lungs. *Escherichia coli* was isolated from the intestines.

From animals in Group C, *Escherichia coli* was isolated from the intestines. No organisms were cultured from the liver, spleen, kidneys, or lungs.

Histopathologic Examination

Pasteurella multocida, Group P

Examination of the lung revealed widespread hyperemia, edema, and interstitial pneumonia, which was characterized by proliferation of septal cells and infiltration with many lymphocytes and a few neutrophils (Figures 5 and 6). The lumen of some of the bronchioles contained an inflammatory exudate.

There was a mild degree of hemorrhage in the epicardium and the endocardium. In addition, there was myocardial hemorrhage in animal P_1.

The intestinal submucosa was edematous, the villi were hyperemic, and goblet cells were very few in number.

The liver was hyperemic with a slight degree of cloudy swelling and fatty degeneration.

There was hyaline degeneration of the capsule of the spleen along with subcapsular hemorrhage, hyperemia, and a mild depletion of Malpighian corpuscles.

The lymph nodes were hyperemic. There was a moderate degree of cloudy swelling in the renal tubular epithelium.

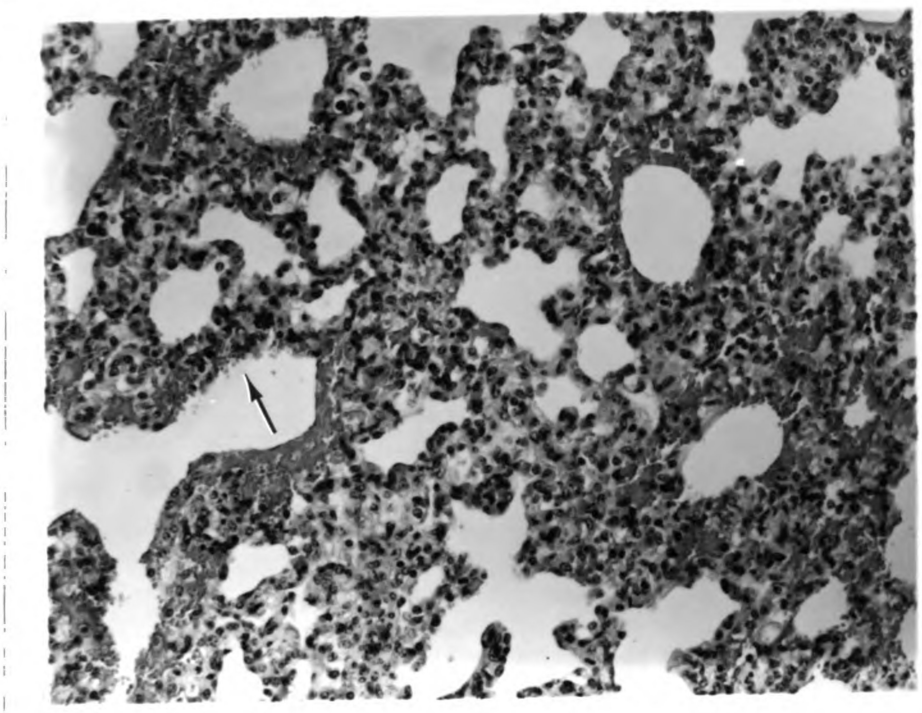

Figure 5. Photomicrograph of the lung of *Pasteurella*-inoculated calf. Congestion and hemorrhage (arrow) in the alveoli. H & E stain. x 190.

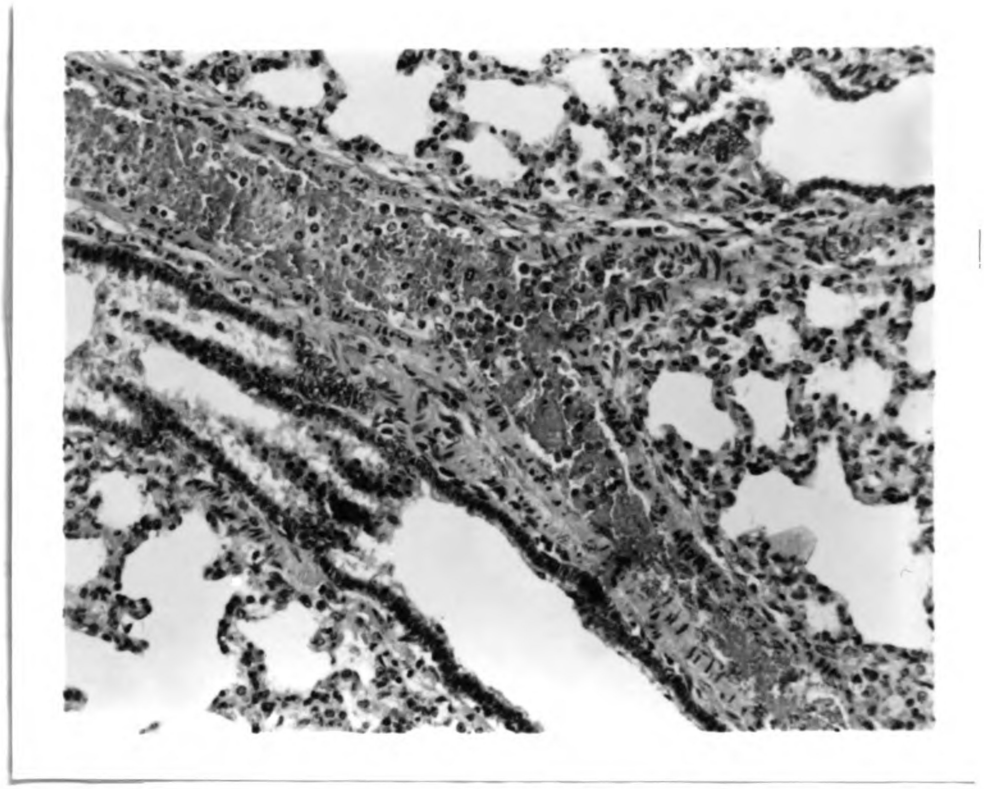

Figure 6. Photomicrograph of the lung of *Pasteurella*-inoculated calf. Congestion of the blood vessels. H & E stain. x 190.

The dura mater of the brain was hemorrhagic and infiltrated with leukocytes and neutrophils. The cerebrum, medulla, and cerebellum revealed hyperemia and a slight degree of hemorrhage.

Escherichia coli, Group E

The lung parenchyma was hyperemic and infiltrated with neutrophils, and the capillary bed of the lung contained numerous leukocytes, mainly neutrophils. Some of the alveoli contained erythrocytes, neutrophils, and fibrin clots. The interlobular and intralobular tissues were edematous. A large portion of the bronchioles were collapsed, the tunica adventitia and tunica media of the blood vessels were frequently hemorrhagic, and some thrombi were observed in them. The pleura of animal E_4 was also hemorrhagic and infiltrated with neutrophils (Figures 7 and 8).

The heart was moderately hyperemic. Examination of the intestine revealed a high degree of congestion in the submucosa and, in the villi, there was moderate congestion, occasional hemorrhage, edema, and infiltration of neutrophils.

The liver was slightly congested with a moderate degree of cloudy swelling and occasional hemorrhage.

The spleen was hyperemic with subserosal hemorrhage, and the Malpighian corpuscles were surrounded by some neutrophils and macrophages.

The rate of mitosis was increased in the lymph nodes, and the medulla was slightly hyperemic.

There was slight hyperemia, subcapsular edema, cloudy swelling, and vacuolar degeneration, especially in the cortical convoluted tubules of the kidney. The adrenal glands were moderately congested and slightly hemorrhagic. The pia mater, medulla, and the cerebellum of the brain

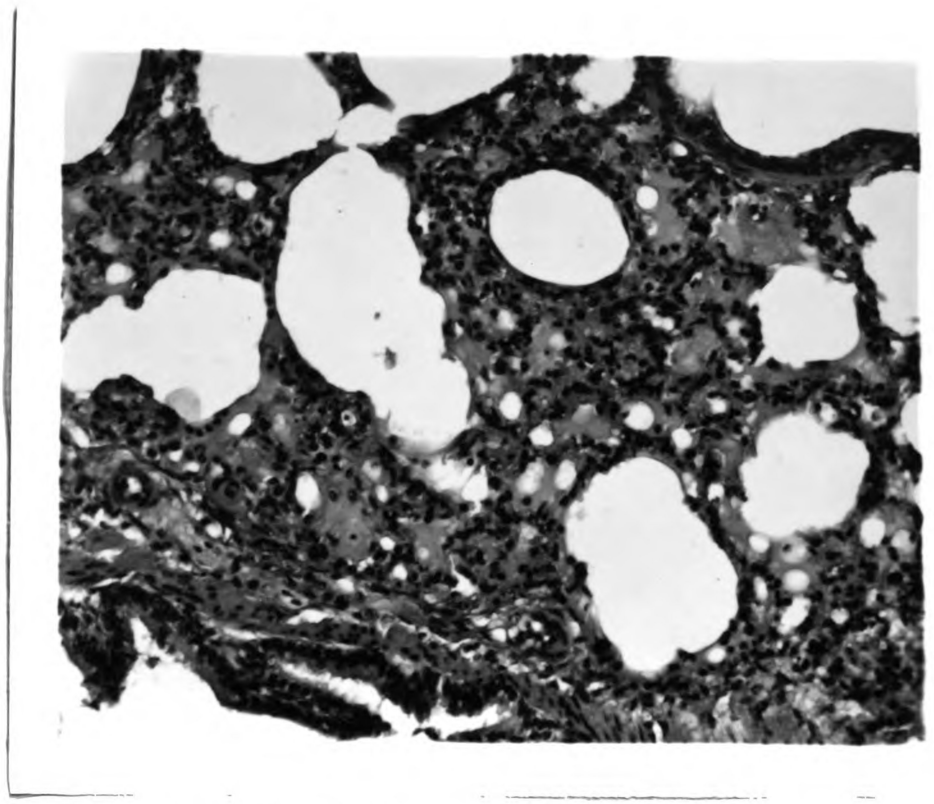

Figure 7. Photomicrograph of the lung of a calf 6 hours after endotoxin inoculation showing pneumonic areas. H & E stain. x 190.

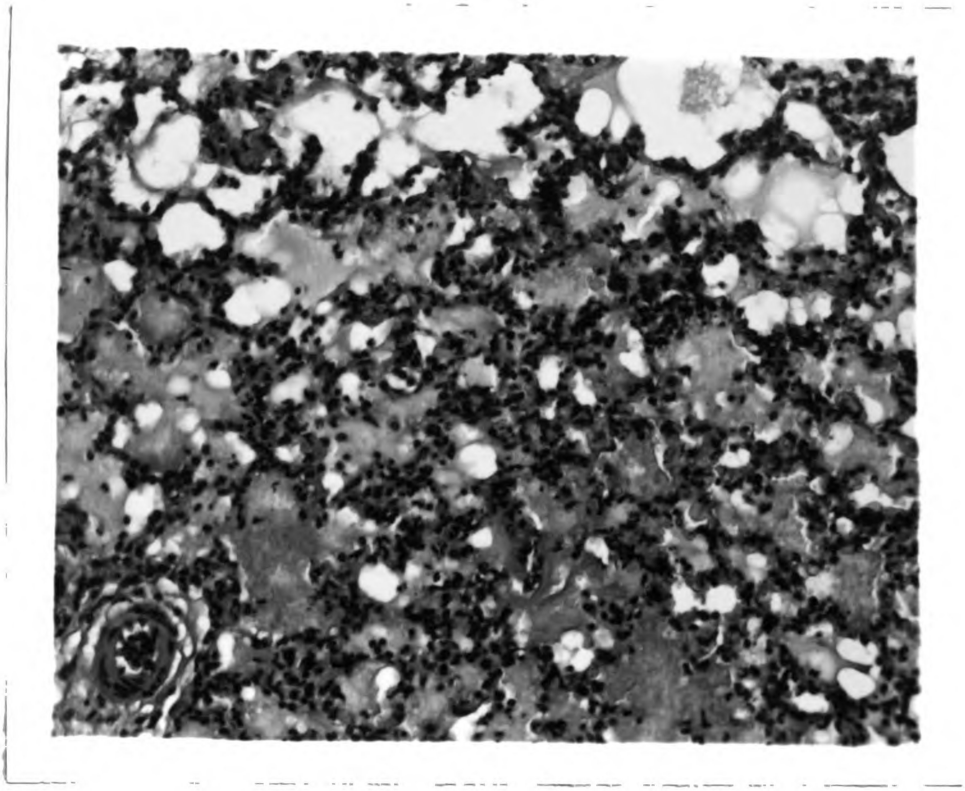

Figure 8. Photomicrograph of the lung of a calf 6 hours after endotoxin inoculation. Notice the infiltration of serous fluid into the alveoli. H & E stain. x 190.

were slightly hyperemic. Small patches of hemorrhage were detected in the medulla and the cerebellum.

Hematologic Examination

The mean and standard error of the mean values for the various hematologic studies (glucose, cholesterol, serum glutamic-oxaloacetic transaminase, and serum glutamic-pyruvic transaminase) for animals in Groups P, E, and C are presented in Tables 3, 4, and 5 and Appendix 1.

Erythrocyte counts for animals in Group P were essentially normal; however, the number of erythrocytes was elevated ($P < 0.01$) for animals in Group E throughout the shock period (Tables 4 and 5).

The packed cell volume dropped ($P < 0.01$) in Group P during the first 16 hours and was also low on the 22nd and 25th hours (Table 4). In Group E the packed cell volume was elevated ($P < 0.01$) during the first 45 minutes of the shock period (Table 5).

For mean corpuscular volumes there were no significant changes between control and inoculated animals.

For animals in Group P, the mean hemoglobin values were significantly decreased ($P < 0.01$) from the mean value for the control animal (Table 4) during the first 14 postinoculation hours and periodically thereafter, whereas for Group E there was a significant increase ($P < 0.01$) over the value for the control animal (Table 5) during the first 45 minutes and at several readings thereafter.

Starting with the 18th postinjection hour, there was a definite trend for the platelet count to decrease ($P < 0.01$) as the disease progressed (Group P, Table 4 and Figure 9). A similar trend ($P < 0.01$) was noted for the endotoxin group (E) starting 60 minutes after dosing and lasting throughout the shock period (Table 5 and Figure 9).

Table 3. Mean hematologic and biochemical values for the various groups of animals during the period of experimentation

Factors	Mean and Standard Error of the Mean			
	C_1 (control)	Group E	C_2 (control)	Group P
RBC x 10^6 mm^3	7.3±0.0	7.9±0.2**	9.8±0.9	9.2±0.3
PCV %	30.5±0.2	33.5±0.1**	44.2±0.5	38.2±0.5**
MCV μ^3	41.4±0.4	42.2±0.4	42.2±0.1	40.6±0.5
Hb. gm.%	9.5±0.0	10.5±0.0**	14.8±0.2	11.2±0.3**
MCHC %	31.3±0.2	31.5±0.0	33.6±0.1	31.0±0.2**
Platelet/mm^3	570454± 44920	349895**± 28298	729318± 32550	428845**± 70580
WBC/mm^3	5529±818	2761±420**	6045±386	7370±796
Lymphocyte A	2175±317	2455±412	2866±255	2662±280
Neutrophil A	2878±504	286±62**	3004±200	4837±665**
Eosinophil	0.0±0.0	30±9	5±5	1±1
Glucose mg.%	113±7	100±13	107±7	135±11*
Cholesterol mg./dl.	134.5±15	114.2±7	104±10	95±11
SGOT	52±5	80±5**	52±4	65±6
SGPT	17.1±8	14.1±1	8±1	18±1

A = Absolute number

SGOT = Serum glutamic-oxaloacetic transaminase expressed as Sigma-Frankel units/ml.

SGPT = Serum glutamic-pyruvic transaminase expressed as Sigma-Frankel units/ml.

C_1 = Control for endotoxin Group E.

C_2 = Control for *Pasteurella multocida* Group P.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

Table 4. Hematologic and blood chemical values[1] for animals in the *Pasteurella multocida* group (P)

Factors	Control: Mean value of 12 consecutive determination for each factor	Hours After Injection of Organisms: 6	12	14	16
RBC x $10^6/mm^3$	9.8±0.9	8.4±1	7.8±1	8.1±1	8.7±2
PCV %	44.2±0.5	35.7±3	35.2±4	35.7±4	36.7±4
MCV μ^3	42.4±0.1	39.0±2	41.0±9	40.3±2	40.0±3
Hb. gm.%	14.8±0.2	10.1±1	10.3±1	10.3±1	12.0±1
MCHC %	33.6±0.1	31.2±0.4	32.4±0.7	32.0±0.8	31.3±1
Platelet/mm^3	729318± 32550	722250± 215250	795750± 416750	702000± 323000	645750± 166750
WBC/mm^3	6045± 386	5816± 1994	10766± 2730	9133± 1483	8666± 2602
Lymphocyte A	2866± 255	3126± 1226	3187± 639	4371± 1671	2458± 895
Neutrophil A	3004± 200	2655± 801	7525± 2059	6870± 967	6208± 1741
Eosinophil A	5±5	17±17	0.0±0	0.0±0	0.0±0
Glucose mg.%	107±7	170±9	166±33	178±26	169±24
Cholesterol mg./dl.	104±10	73±17	93±3	104±3	146±31
SGOT	52±4	48±4	51±4	55±10	52±1
SGPT	8±1	13±2	15±3	13±2	10±3

Table 4--(Cont'd.)

Factors	Hours After Injection of Organisms				
	18	20	22	24	25
RBC x $10^6/mm^3$	10.2±2	10.1±2	11.4±6	9.3±2	7.6±0.8
PCV %	40.8±5	41.5±5	37.7±9	39.6±4	36.1±3
MCV μ^3	39.8±3	42.3±4	39.1±11	42.8±5	43.1±1
Hb. gm.%	12.5±2	10.8±3	11.6±3	11.7±1	10.1±1
MCHC %	31.5±7	30.9±1	30.2±2	31.4±0.1	30.8±0.5
Platelet/mm^3	317500± 32500	239400± 45600	92500± 0.0	545000± 155000	286000± 104000
WBC/mm^3	5183± 1708	7783± 4538	6025± 3875	7850± 2853	12800± 1200
Lymphocyte A	2458± 895	1679± 154	1783± 276	1219± 328	2915± 1393
Neutrophil A	6208± 1741	3503± 1642	5656± 4011	4805± 4203	4935± 2357
Eosinophil A	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Glucose mg.%	97±27*	97±0.6**	191±76	141±45	188±12**
Cholesterol mg./dl.	95±40	109±88	132±26	85±18	136±13
SGOT	51±8	46±2	51±21	53±20	76±25
SGPT	14±2	16±4	17±9	17±3	22±8

Table 4--(Cont'd.)

Factors	Hours After Injection of Organisms 26	28	30
RBC x $10^6/mm^3$	10.0±2	10.7±2	8.5±0.0
PCV %	39.1±3	41.6±3	38.2±3
MCV μ^3	43.2±1	38.8±6	38.3±3
Hb. gm.%	11.7±1	13.1±2	10.1±0.0
MCHC %	30.7±0.6	31.3±0.0	28.9±0.0
Platelet/mm^3	207000± 183000	163000± 0.0	430000± 0.0
WBC/mm^3	7150± 2741	3075± 425	4200± 0.0
Lymphocyte A	3029± 1327	1672± 587	2478± 0.0
Neutrophil A	4069± 1776	1419± 995	1638± 0.0
Eosinophil A	0.0±0.0	0.0±0.0	0.0±0.0
Glucose mg.%	128±19	76±48*	80±52
Cholesterol mg./dl.	82±19	53±31	132±0.0
SGOT	99±48	120±75	83±0.0
SGPT	28±8	30±14	20±0.0

A = Absolute number.

SGOT = Serum glutamic-oxaloacetic transaminase expressed as Sigma-Frankel units/ml.

SGPT = Serum glutamic-pyruvic transaminase expressed as Sigma-Frankel units/ml.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

[1]Expressed as the mean value and standard error of the mean.

Table 5. Hematologic and blood chemical values[1] for animals in the endotoxin group (E)

Factors	Control: Mean value of 12 consecutive determination for each factor	Minutes After Injection of Endotoxin: 5	10	15	30
RBC x 10^6/mm^3	7.3±0.0	8.2±1	7.9±0.8	8.1±0.6	5.6±2
PCV %	30.5±0.2	34±1	33.6±0.4	33.6±0.5	34.3±0.4
MCV μ^3	41.4±0.4	43.1±4	43.6±4	42.1±3	44.2±5
Hb. gm.%	9.5±0.0	10.6±0.3	10.7±0.2	10.5±0.1	10.7±0.1
MCHC %	31.3±0.2	31.3±0.5	31.9±0.3	31.4±0.1	31.3±0.7
Platelet/mm^3	570454± 44920	545000± 60449	471250± 88867	452500± 100778	400000± 108282
WBC/mm^3	5229± 818	6212± 1303	4025± 818	3887± 444	3250± 236
Lymphocyte A	2175± 217	5630± 1120	3633± 698	3729± 358	3157± 262
Neutrophil A	2878± 504	582± 238	373± 129	145± 92	81± 40
Eosinophil A	0.0±0.0	0.0±0.0	68±35	17±17	16±16
Glucose mg.%	113±7	109±11	156±47	180±58	176±41
Cholesterol mg./dl.	134±15	121±14	100±21	121±24	133±25
SGOT	52±5	59±8	72±11	63±4	66±19
SGPT	17±8	12±0.4	10.7±1	12±1	11±2

Table 5--(Cont'd.)

Factors	Minutes After Injection of Endotoxin				
	45	60	90	120	180
RBC x $10^6/mm^3$	8.1±0.5	8.1±1	7.7±1	7.9±1	8.6±1
PCV %	33.8±0.4	33.1±0.6	33.1±0.1	33.3±0.1	34.3±0.7
MCV μ^3	41.8±3	42.2±6	44.4±5	43.5±6	40.9±4
Hb. gm.%	10.7±0.0	10.3±0.2	10.2±0.2	10.6±0.3	10.3±0.1
MCHC %	31.8±0.4	31.3±0.1	31.4±1	31.6±0.5	31.7±0.7
Platelet/mm^3	395000± 90046	326666*± 60850	358333*± 40034	338333*± 32446	313333*± 26822
WBC/mm^3	3066± 387	2716± 356	2483± 224	1600± 702	1366± 667
Lymphocyte A	2751± 250	2527± 410	2250± 159	1492± 682	1284± 627
Neutrophil A	279± 116	181± 57	215± 74	87± 0.5	83± 40
Eosinophil A	107±0.0	25±0.0	56±0.0	15±15	0.0±0.0
Glucose mg.%	123±38	99±16	127±0.0	76±10	52±19*
Cholesterol mg./dl.	143±33	155±46	74±0.0	108±27	105±33
SGOT	64±4	64±11	99±0.0	82±15	77±5
SGPT	12±2	15±4	20±0.0	15±2	11±0.0

Table 5--(Cont'd.)

Factors	Minutes After Injection of Endotoxin 240	300	360
RBC x $10^6/mm^3$	8.7±0.7	8.1±0.7	8.2±0.9
PCV %	33.3±0.7	33.1±1	32.5±1
MCV μ^3	38.7±4	41.4±3	40.1±4
Hb. gm.%	10.6±0.5	10.3±0.5	10.3±0.4
MCHC %	31.5±0.7	31±0.6	31.7±0.4
Platelet/mm^3	231666**± 32188	255000**± 35000	231666**± 31797
WBC/mm^3	1483± 712	1216± 669	1833± 845
Lymphocyte A	1233± 645	716± 318	1063± 313
Neutrophil A	182± 55	483± 358	753± 550
Eosinophil A	41±23	13±12	12±12
Glucose mg.%	61±15*	62±2*	64±3*
Cholesterol mg./dl.	130±11	78±17	104±33
SGOT	95±13	92±18	128±30*
SGPT	15±2	18±2*	14±3

A = Absolute number.

SGOT = Serum glutamic-oxaloacetic transaminase expressed as Sigma-Frankel units/ml.

SGPT = Serum glutamic-pyruvic transaminase expressed as Sigma-Frankel units/ml.

*Significant ($P < 0.05$).

**Highly significant ($P < 0.01$).

[1]Expressed as the mean value and standard error of the mean.

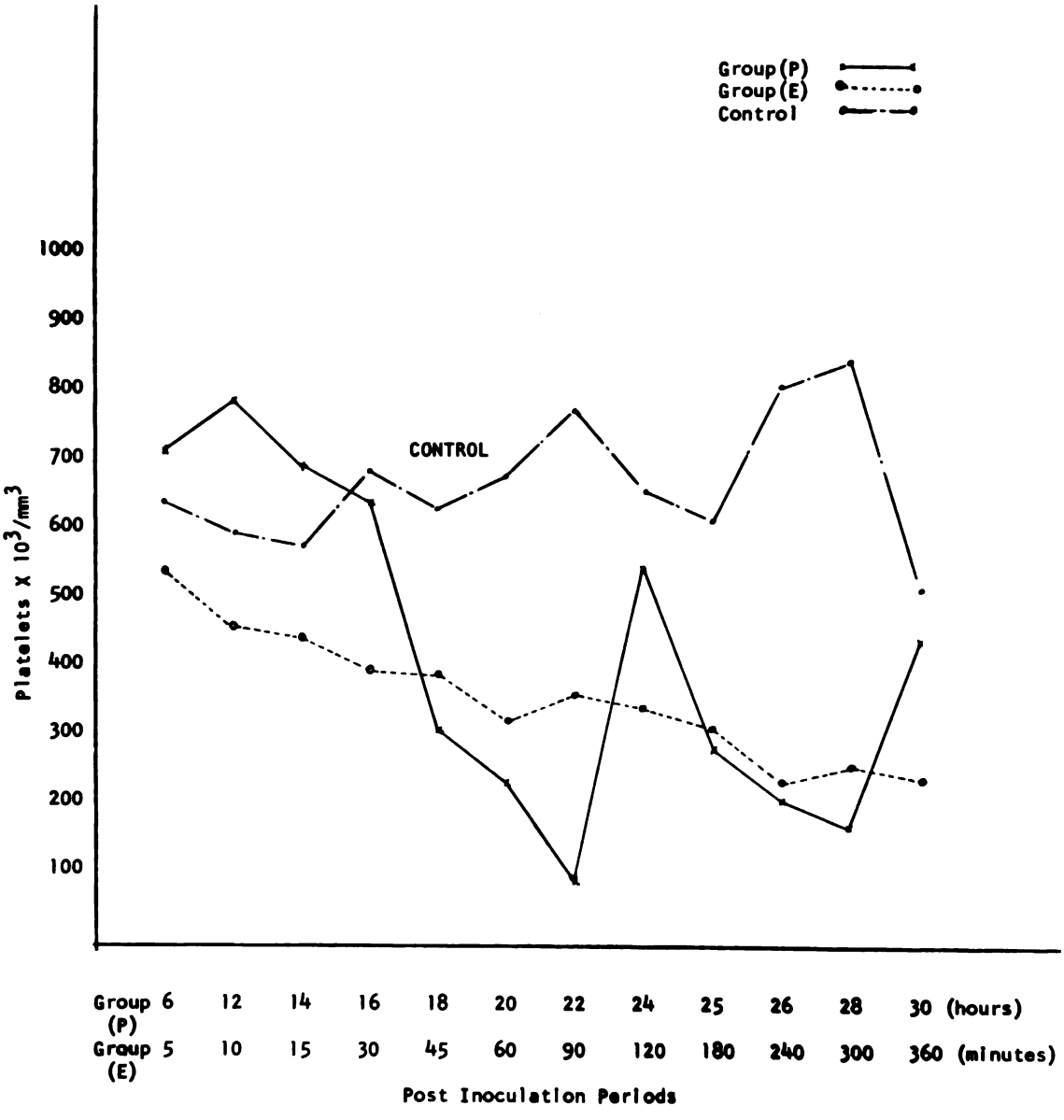

Fig. 9--- Mean Blood Platelet Counts for Experimental Calves.

The total leukocytic counts for Group P were more or less increased during the first 16 hours following inoculation and decreased thereafter (Table 4), but a significant reduction in the leukocytic counts ($P < 0.01$) was observed in Group E (Table 5). This reduction in leukocytes was observed about 60 minutes following the administration of the endotoxin and persisted throughout the period of shock.

The total absolute numbers of lymphocytes for Group P were somewhat reduced during the middle phase and near the terminal stage of the disease, while in Group E the lymphocytes were elevated during the first 90 minutes following dosing of the animals but were reduced thereafter.

There was nearly a complete absence of eosinophils throughout the period of the disease in Group P, but their level remained fluctuating following endotoxin injections (Tables 4 and 5).

The differential leukocytic count revealed a significant neutrophilia ($P < 0.01$) between the 12th and 25th postinjection hours in Group P (Table 4 and Figure 10). Within the first 15 minutes of the shock period, neutropenia was evident in Group E. Neutropenia persisted in this group throughout the shock period (Table 5 and Figure 10).

Blood glucose levels were significantly higher ($P < 0.05$) than those for the control in Group P during the first 16 hours and between the 22nd and 25th hours. The amount of glucose in the blood was very low just prior to death (Table 4). An elevation in blood glucose was observed between 10 and 30 minutes; thereafter, a significant reduction ($P < 0.05$) in blood glucose was observed to begin 3 hours following endotoxin injection (Group E, Table 5) and to continue until the end of the shock period.

Cholesterol levels in both Groups P and E indicated periodic fluctuations (Tables 4 and 5). However, when compared with the control

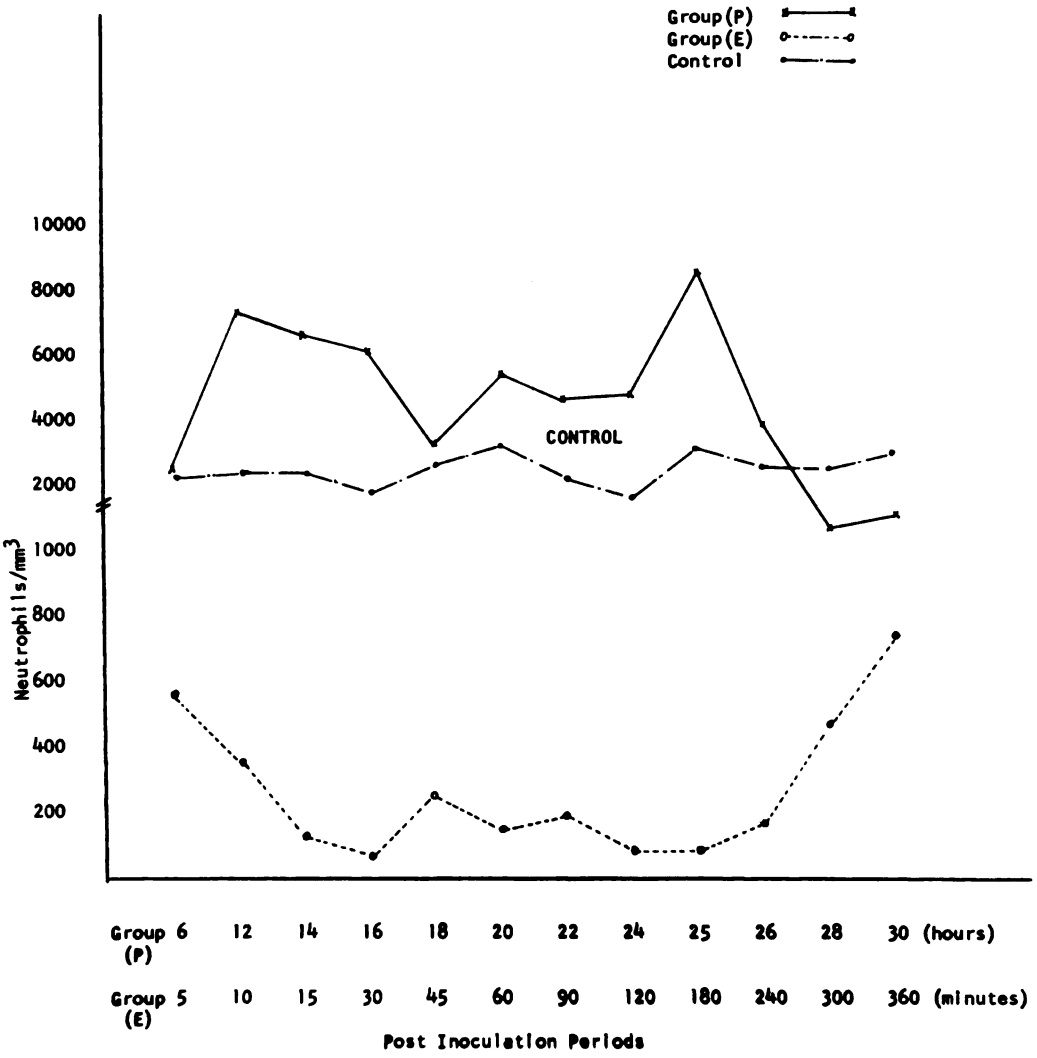

Fig.10--- Mean Values for Absolute Neutrophil Counts for Experimental Calves.

animals, levels of cholesterol were especially lower at the beginning and near the end of the experiment.

There was no significant change in the serum level of the enzyme glutamic-oxaloacetic transaminase in Group P. In Group E the enzyme was increased in the blood ($P < 0.01$) starting 1.5 hours following administration of the endotoxin until the end of the shock period. The glutamic-pyruvic transaminase was significantly elevated in the blood of animals in Group P ($P < 0.01$) from the 25th hour postinoculation until the animals died; but in Group E the enzyme showed a transitory rise ($P < 0.05$) at the 5th hour.

In comparing all the parameters for the *Pasteurella multocida* group and the *Escherichia coli* endotoxin group, an analysis of variance indicated that there was no significant difference between the 2 groups, except in a very few instances which are indicated by asterisks (Appendix 1).

The results of the chick embryo bioassays are given in Table 6. Sera collected from the *Pasteurella* group at the 6th and 32nd postinoculation hours, as well as sera from the control animals, were not lethal to chick embryos. Sera collected 26 hours postinoculation resulted in the death of only 1 of the 5 embryos.

Table 6. Summary of results of the chick embryo bioassays

Animal Number	Sample Collection Times*	Hours After Inoculation of the Embryos: 21 Alive	21 Dead	21 Traumatized	24 Alive	24 Dead	24 Traumatized	40 Alive	40 Dead	40 Traumatized
C_1	5 min.	3	0	2	3	0	2	3	0	2
C_2	32 hr.	3	0	2	3	0	2	3	0	2
P_1	6 hr.	4	0	1	4	0	1	4	0	1
P_4	26 hr.	3	1	1	3	1	1	3	1	1
P_5	6 hr.	4	0	1	4	0	1	3	1	1
P_5	26 hr.	0	1	4	0	1	4	0	1	4
P_5	30 hr.	3	1	1	3	1	1	3	1**	1
P_5	32 hr.	-	-	-	1	2	2	0	3**	2
P_5	32 hr.	4	0	1	4	0	1	4	0	1

C_1 and C_2 were the control animals.

P_1, P_4, and P_5 were animals inoculated with *Pasteurella multocida* organisms.

*After inoculation of the animal or, in the case of the controls, after initiation of the experiment.

**Embryos contaminated with *Pasteurella multocida* organisms, which were considered the cause of death.

DISCUSSION

The physical signs shown by the calves inoculated with *Pasteurella multocida* organisms, Group P, were nearly identical to those described by Blood and Henderson (1968) for bovine pasteurellosis. The signs observed in the group injected with *Escherichia coli* endotoxin, Group E, were more or less similar to those in Group P, but they differed in the time of onset and severity.

Both groups had elevated rectal temperatures, depression, respiratory distress, alimentary involvement, and recumbency. These signs, except for the alimentary involvement, were seen 7 to 16 hours after inoculation for calves in Group P, and 5 minutes after injection for calves in Group E. Diarrhea was observed in Group P during the terminal stages of the disease, while in Group E it was observed 45 minutes after injection and was not as severe as described by Schalm (1965). The severity of diarrhea (Group E) may be dependent upon amount of endotoxin given. Animals in Group E did not have the local painful swellings as seen for Group P. This might be due to the relatively short period of stress characteristic of endotoxic shock. The heart rate decreased during the first hour following administration of endotoxin (Group E). Whether this was due to a direct effect of endotoxin on the cardiovascular system or was mediated through other substances, like catecholamines (Fine and Minton, 1966), is not certain. Higher thermal reactions were attained for animals in Group P than for those in Group E. It is probable that the thermal reaction is dependent upon the dose of

endotoxin. The physical signs observed in the calves during endotoxic shock differed from those described in small laboratory animals inasmuch as there was no biphasic fever (Osborne, 1965), no hypothermia (Zahi and Hutner, 1944; Berry *et al.*, 1959; Sheagren *et al.*, 1967), and no vomition (Berczi *et al.*, 1966).

Pasteurella multocida organisms were recovered from the blood of calves in Group P from about 6 hours after inoculation until death occurred. At necropsy, *Pasteurella multocida* was present in the liver, lung, spleen, kidney, and lymph nodes, but not in the bile or urine. *Escherichia coli* was isolated from the intestine. No organisms were found in the blood of calves that received endotoxin, and cultures after necropsy did not reveal the presence of bacteria from the liver, spleen, kidneys, and lungs. Coliform organisms were isolated from the intestine of all calves in Group E. It is known that *Escherichia coli* organisms are normal inhabitants of the digestive tract of calves. Usually these organisms multiply rapidly in the digestive tract of mice following endotoxin administration (Ushiba *et al.*, 1962). Probably *Escherichia coli* organisms in the digestive tract play an important role in aggravating the existing endotoxic shock, as it has been found that mice free from ordinary pathogens as well as *Escherichia coli* were highly resistant to the lethal effects of endotoxin (Schaedler and Dubos, 1964).

The gross lesions observed in calves of both Group P and Group E included congestion and widespread hemorrhage of visceral organs. The hemorrhage was in general more widespread in Group P than in Group E. The edema detected in the ventral portion of the neck and around the thoracic inlet (Group P) was absent from animals in Group E. It is

possible that the lack of edema was due to the relatively short period of shock for animals in Group E.

Microscopic examination indicated that for both groups, the lung had the most prominent changes. Pulmonary lesions in Group P calves were widespread hyperemia, edema, and interstitial pneumonia, while for Group E calves they were hyperemia, leukocytic infiltration, petechial hemorrhage, edema, and thrombosis. In both groups there were varying degrees of petechial hemorrhage and hyperemia in the heart, intestine, liver, spleen, lymph nodes, and the brain. Early cellular degeneration was also detected in the liver and the kidney of both groups.

Histopathologic findings in Group P animals indicated that this organism was capable of producing pneumonia somewhat similar to pasteurellosis described by Runnells *et al.* (1965) and Smith and Jones (1966). However, the lung was also edematous, a finding which was a prominent feature of hemorrhagic septicemia as reported by Blood and Henderson (1968). Those in Group E were in partial agreement with Tikoff *et al.* (1966), in that the lung was the major target organ after administration of *Escherichia coli* endotoxin. In this respect calves are similar to sheep (Halmagyi *et al.*, 1963), cats (Tikoff *et al.*, 1966), and most small laboratory animals (Osborne, 1965) but differ from dogs (Alican, 1962), chickens (Trauscott and Inniss, 1967), and coyotes (Hinshaw *et al.*, 1967). The gross and microscopic lesions (Group E) observed in this study were similar to those described by Rhoades *et al.* (1967) in calves following *Pasteurella multocida* endotoxin injections. This striking similarity suggests that endotoxin from *Pasteurella multocida* organisms or *Escherichia coli* organisms behaves similarly in the body systems. Such a suggestion confirms the earlier observation of Carroll *et al.* (1965) that the pathologic

responses of animals to administration of endotoxin were the same regardless of the species of gram-negative organisms from which the endotoxin is obtained. Furthermore, the histopathologic findings for both groups in the present study were more or less similar suggesting that the fatal outcome in calves with hemorrhagic septicemia might be due to an endotoxin released sometime during the course of the disease. Although the lung was the major target organ in both groups, other important organs (for example, the brain, liver, kidney, and spleen) were also involved and might contribute to the final outcome of hemorrhagic septicemia or endotoxic shock.

Published reports for erythrocytic counts and indexes, for normal young calves, usually show a marked disparity (Holman, 1956; Greatorex, 1954 and 1957). To minimize the day-to-day variations in blood counts, and possibly in other determinations, the figures reported herein for each of the 2 control animals (Tables 3, 4, and 5) represent an average (mean) of 12 consecutive observations made on samples collected throughout the course of the experiment.

The red cell count did not change in Group P but increased following administration of *Escherichia coli* endotoxin. Whether endotoxin has a stimulating effect on the erythropoietic system is not known, nor is there an explanation for the mechanisms involved.

The packed cell volume dropped in Group P during the first 16 hours. As the disease progressed, there was a mild degree of hemoconcentration and a rise in the packed cell volume. This might be due to the slowly developing edema in the subcutaneous tissues of the neck region. In Group E there was an initial rise in the packed cell volume, a finding in common agreement with that of Tikoff *et al.* (1966). In this respect endotoxemic calves were similar to endotoxemic horses (Schalm, 1965),

cows (Schalm, 1965), man (Lillehei and Maclean, 1959), and dogs (Alican, 1962; Muller and Smith, 1963). The mean corpuscular volume was not altered following injection of either *Pasteurella multocida* organisms or *Escherichia coli* endotoxin.

No explanation is offered for the initial drop in hemoglobin concentration (Group P). Animals in Group E had an initial rise in hemoglobin. In this respect, endotoxemic calves were similar to endotoxemic man (Lillehei and MacLean, 1959) and dogs (Alican, 1962). The mean corpuscular hemoglobin, on the other hand, was slightly increased in Group P, particularly during the terminal stages of the disease, while it remained unchanged in Group E throughout the shock period.

The rapid decrease in numbers of blood platelets following *Pasteurella multocida* inoculation or *Escherichia coli* endotoxin administration may be a result of platelet sequestration in the capillaries of some of the body's organs (McKay *et al.*, 1966), or it might be a result of other mechanisms. However, the widespread hemorrhage observed in both groups (P and E) probably is due to injury of vascular endothelium (Kass *et al.*, 1964), coupled with failure in the clotting mechanisms as a result of the removal of platelets from the circulation.

The total leukocytic counts of the calves in Group P were initially elevated but, as the disease progressed, they were decreased. Calves in Group E showed a reduction in the leukocytic counts 1 hour following the administration of the endotoxin and persisting until the end of the shock period.

The differential leukocytic count revealed a reduction in the lymphocytic counts of the animals in Group P and E during the terminal stages of either hemorrhagic septicemia or *Escherichia coli* endotoxic shock.

The neutrophil counts of the calves in Group P followed more or less the same pattern as observed with the total leukocytic counts of these animals. The neutropenia detected in Group E, in contrast to that observed in Group P, persisted throughout the shock period and was accompanied by a concurrent leukopenia. Endotoxemic calves in this respect were similar to endotoxemic cows (Schalm, 1965; Mullenax *et al.*, 1966), horses (Schalm, 1965), and rabbits (Bennett and Beeson, 1953). Eosinophils were almost absent in calves with hemorrhagic septicemia, but they were present, in normal numbers, during endotoxic shock. If eosinophils played a role in either of the 2 conditions, it was not reflected by any significant changes in numbers circulating in peripheral blood. Their absence during *Pasteurella* infection is perhaps noteworthy.

The normal level of blood glucose in young calves is more or less similar to that of the monogastric animals and represents the main source of energy during calfhood. However, following inoculation of *Pasteurella multocida* organisms, the level of glucose was observed to increase initially, but during the second half of the disease period, blood glucose values were somewhat reduced. Since the animals were unable to feed during this time, the source of glucose may have been endogenous or from the body reserves; hence, it is probable that the terminal drop in the blood glucose observed in Group P may be due to lack of mobilization of these reserves or to their depletion. In Group E a drop in blood glucose was detected during the terminal stage of the shock period. Endotoxemic calves in this respect were similar to endotoxemic mice (Berry *et al.*, 1959) and dogs (Boler and Bibighaus, 1967).

It is known that cholesterol is the precursor of the steroid compounds produced in the animal's body. These steroid compounds include a variety of substances, such as vitamin D, gonadal hormones, and

adrenal cortical hormones. In the present study, the levels of cholesterol were initially and terminally lower (in both Groups P and E), than the control levels. This might be due to a depressed steroidogenesis or rapid removal of cholesterol from the circulation following either of the 2 treatments adopted.

Glutamic-oxaloacetic transaminase (SGOT) enzyme is widely distributed in many tissues of normal animals, being especially abundant in the liver, myocardium, and skeletal muscles. When these tissues are damaged, the enzymes diffuse across the damaged cell wall and enter the blood stream. Glutamic-pyruvic transaminase is not as widely distributed in normal calves as is SGOT. The blood of healthy animals usually contains minimal amounts of these enzymes. The level of SGOT was not changed in Group P but was elevated in Group E from 1.5 hours after endotoxin administration until the end of the shock period. Serum glutamic-pyruvic transaminase was elevated in Group P during the last third of the disease but showed only a transitory rise in Group E on the 5th hour following the administration of the endotoxin. These results indicate that injury accompanying *Pasteurella multocida* infection characteristically elevates levels of serum glutamic-pyruvic transaminase while injury due to *Escherichia coli* endotoxin characteristically elevated levels of serum glutamic-oxaloacetic transaminase. Hence, endotoxemic calves were similar to endotoxemia in other ruminants (Hansen, 1964) and the coyote (Hinshaw *et al.*, 1967).

There were a few significant differences between Group P and Group E in total leukocyte, neutrophil, or lymphocyte counts, and in blood glucose concentrations. The significant differences observed in leukocytes, neutrophils, and in lymphocytes between the 2 groups were accounted for by a more marked neutropenia and initially elevated lymphocytes in

Group E than in Group P. The preinjection levels of blood glucose were higher in Group E than in Group P. This may, in part, account for the few significant differences between the 2 groups during the course of experimentation.

Apart from these, all the other hematologic findings, as well as glucose, cholesterol, serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase levels, were not significantly different between Groups P and E, indicating that physiological responses were more or less similar, following either *Pasteurella multocida* inoculation or *Escherichia coli* endotoxin injection. Further, this may indicate that during the course of hemorrhagic septicemia, an endotoxin similar to *Escherichia coli* endotoxin may be released.

No lethal effect was produced in the 11-day-old chick embryos following intravenous administration of serum from either the control animals or from Group P animals. Hook *et al.* (1961) and Berczi *et al.* (1966) described the lethal dose of endotoxin for the chick embryo, which may be higher than the amount of endotoxin present in the 0.1 ml. of serum from animals in Group P, if such an endotoxin was present. On the other hand, the chick embryo might be refractory to intravenous administration of the endotoxin at this particular age (Smith and Thomas, 1956; Finkelstein, 1964).

It is apparent that in performing chick embryo bioassays for endotoxin, it would be advisable to concentrate the amount of serum that can be inoculated or possibly to inject these embryos at other ages which might be more suitable.

SUMMARY AND CONCLUSIONS

1. Inoculation of *Pasteurella multocida* organisms or *Escherichia coli* endotoxin into experimental animals gave the same physiologic and pathologic responses. This possibly indicates that in hemorrhagic septicemia an endotoxin similar to *Escherichia coli* endotoxin is released.

2. Although the lung had the most prominent changes during either *Pasteurella multocida* infection or *Escherichia coli* endotoxemia, other important organs in the body were affected as well. Hence, the fatal outcome following either treatment may be due to a sum of multiple factors rather than a single one.

3. The primary effect of *Escherichia coli* endotoxin seems to be on the blood platelets, neutrophils, and the vascular system. This may be a direct effect of the lipopolysaccharide or may be mediated through other substances and systems.

4. The marked reduction in number of blood platelets may be an important factor in the widespread hemorrhage seen in both hemorrhagic septicemia and *Escherichia coli* endotoxemia.

5. Hypoglycemia was observed during the terminal stages of either *Pasteurella multocida* infection or *Escherichia coli* endotoxemia.

6. Blood cholesterol levels were initially and terminally lowered in inoculated animals.

7. Tissue injury accompanying *Pasteurella multocida* infection characteristically elevated levels of the serum glutamic-pyruvic

transaminase, while injury accompanying *Escherichia coli* endotoxin characteristically elevated the level of serum glutamic-oxaloacetic transaminase.

8. The bioassay for endotoxin using chick embryos produced negative results for animals in the hemorrhagic septicemia group. Suggested improvements in this test include a method for concentration of the endotoxin in serum or the injection of chick embryos at ages that might be more sensitive than at 11 days.

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APPENDIX

Appendix 1. Hematologic and blood chemistry data[1] used for analysis of variance between Group P and Group E

Factors	Time After Inoculation							
	6 hr. (P)	5 min. (E)	12 hr. (P)	10 min. (E)	14 hr. (P)	15 min. (E)	16 hr. (P)	20 min. (E)
RBC x $10^6/mm^3$	8.4±1	8.2±1	7.8±1	7.9±0.8	8.1±1	8.1±0.6	8.7±2	8.0±1
PCV %	33±3	34±1	32±4	34±0.5	32±5	32±5	34±0.5	34±0.4
MCV μ^3	39±3	43±5	41±9	44±4	40±2	42±5	50±3	44±6
Hb. gm.%	10±1	11±0.3	10±1	11±0.1	10±1	11±0.1	11±1	11±0.7
MCHC %	31±0.4	31±0.5	32±0.7	32±0.3	32±0.8	31±0.1	31±1	31±0.7
Platelet/mm^3	722250± 215250	545000± 60449	795750± 416750	471250± 88867	702000± 323000	452500± 100778	645750± 266750	400000± 108282
WBC/mm^3	5816±1994	6212±1303	10766±2730	4025±818*	6133±3178	3887±444	8666±2602	3246±237
Lymphocyte A	3126±1121	5631±1227	3188±639	3633±639	4372±1672	3730±358	2458±895	3157±262
Neutrophil A	2655±802	582±239*	7526±2055	373±129**	6870±968	145±93**	6208±1742	80±40*
Eosinophil A	13±13	0.0±0.0	0.0±0.0	19±19	0.0±0.0	12±12	0.0±0.0	12±12
Glucose mg.%	170±9	108±11**	166±33	155±47	178±26	179±58	177±17	176±41
Cholesterol mg./dl.	72±17	120±14	103±11	99±21	103±30	120±24	146±31	133±25
SGOT	47±4	59±8	50±4	71±11	55±10	63±4	52±1	66±19
SGPT	13±2	12±0.4	14±3	10±1	13±2	12±1	9±3	10±2

Appendix 1--(Cont'd.)

Factors	Time After Inoculation							
	18 hr. (P)	45 min. (E)	20 hr. (P)	60 min. (E)	22 hr. (P)	90 min. (E)	24 hr. (P)	120 min. (E)
RBC x $10^6/mm^3$	10.2±2	8.1±0.5	10.1±2	8.1±1	11.3±6	7.7±1	9.3±2	7.9±1
PCV %	40±7	34±0.4	41±7	33±0.6	38±10	33±0.1	37±5	33±0.1
MCV μ^3	40±3	42±3	42±5	43±7	39±12	44±6	43±5	44±6
Hb. gm.%	13±3	11±0.0	13±3	10±0.2	12±4	10±0.4	12±2	11±0.2
MCHC %	32±0.7	32±0.4	31±1	31±1	30±2	31±1	31±0.1	32±0.5
Platelet/mm^3	317500± 32500	395000± 90046	239400± 45600	326666± 60850	392500± 0.0	358333± 40034	545000± 155000	338333± 32446
WBC/mm^3	5183±1708	3066±387	7783±4538	2716±356	6025±3875	2483±224	7850±2853	1600±702
Lymphocyte A	1680±155	2752±251*	1783±276	2527±410	1219±328	2250±159*	2915±1393	1492±682
Neutrophil A	3504±1643	297±117	5656±4012	181±57	4806±4204	215±74	4935±2358	87±15
Eosinophil A	0.0±0.0	35±35	0.0±0.0	8±8	0.0±0.0	18±18	0.0±0.0	20±20
Glucose mg.%	80±12	122±12	103±12	99±16	180±88	127±0.0	147±47	75±10
Cholesterol mg./dl.	95±40	142±33	109±88	155±46	131±26	73±0.0	85±18	107±27
SGOT	50±8	64±4	46±2	63±11	51±21	99±0.0	52±20	82±15
SGPT	17±3	11±2	12±1	14±4	17±9	20±0.0	16±3	15±2

Appendix 1--(Cont'd.)

Factors	Time After Inoculation							
	25 hr. (P)	180 min. (E)	26 hr. (P)	240 min. (E)	28 hr. (P)	300 min. (E)	30 hr. (P)	360 min. (E)
RBC x $10^6/mm^3$	7.9±0.8	---	10.0±2	8.7±0.7	10.7±2	8.1±0.7	8.5±0.0	8.2±0.9
PCV %	33±3	34±0.7	38±5	33±0.7	42±7	33±1	35±0.0	33±1
MCV μ^3	43±1	41±4	40±4	39±4	40±5	41±3	41±0.0	40±4
Hb. gm.%	10±1	11±0.4	12±2	11±0.4	13±2	10±0.5	10±0.0	10±0.4
MCHC %	31±0.5	32±0.7	31±0.6	32±0.7	31±0.0	32±0.6	29±0.0	32±0.4
Platelet/mm^3	286000± 104000	313333± 26822	207000± 183000	231666± 32188	163000± 0.0	255000± 35000	430000± 0.0	231666± 31797
WBC/mm^3	12800±1200	1366±667**	7150±2741	1483±712	3075±425	1216±669	4200±0.0	1833±845
Lymphocyte A	4032±1652	1284±627	3029±1327	1233±645	1672±587	716±318	2478±0.0	1063±313
Neutrophil A	8768±2852	83±41*	4069±1780	182±56	1420±996	483±359	1638±0.0	753±551
Eosinophil A	0.0±0.0	0.0±0.0	0.0±0.0	68±35	0.0±0.0	17±17	0.0±0.0	16±16
Glucose mg.%	111±16	51±19	107±39	60±15	76±48	62±2	133±0.0	64±3
Cholesterol mg./dl.	136±13	105±33	81±19	130±11	52±31	77±17	131±0.0	104±33
SGOT	75±25	77±5	99±48	95±13	119±75	92±18	82±0.0	127±30
SGPT	22±8	11±0	28±8	14±2	30±14	18±2	20±0.0	13±3

A = Absolute number.

SGOT = Serum glutamic-oxaloacetic transaminase expressed as Sigma-Frankel units/ml.

SGPT = Serum glutamic-pyruvic transaminase expressed as Sigma-Frankel units/ml.

(P) = Animals inoculated with *Pasteurella multocida* organisms.

(E) = Animals injected with *Escherichia coli* endotoxin.

*Significant difference ($P < 0.05$).

**Highly significant difference ($P < 0.01$).

[1]Expressed as mean and standard error of the mean.