INTERRELATIONSHIPS OF IRON AND ENDOTOXIC SHOCK IN SWINE AND THEIR EFFECTS ON HEMOGRAMS AND BLOOD SERUM ELECTROLYTES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Progress towards control of endotoxic diseases has been relatively slow in medicine and comparative medicine. This is due to a lack of understanding of the pathogenesis. Despite distinctive features, the outward clinical signs of endotoxic diseases can be confused with exotoxemia or infectious diseases. An important aspect, which also must be considered, is the variable response to endotoxin exhibited by different species of animals. Variations within species further complicate the study of endotoxins. Until the pathogenic mechanisms are understood, the prophylaxis and treatment of endotoxic maladies will be largely empirical and symptomatic.

There exists one disease entity which the veterinarian in swine practice should understand that appears to fall in the realm of endotoxemia. This entity is edema disease.

With knowledge of the pathogenesis of edema disease the veterinarian will be prepared to advise the husbandryman on management conducive to its prevention. A common procedure in swine production today is the injection of iron. That iron deficiency contributes to increased susceptibility to swine diseases is common knowledge to husbandrymen, but the specific part that iron plays in edema disease has not been elucidated.

To clarify the effect of parenteral iron on edema disease, a means of reproducing edema disease is needed. Because edema disease is sporadic, a logical approach to its study is by means of endotoxic shock.

The parameters to be studied should include routine hematology because both iron-deficiency anemia and endotoxic shock have been investi-

gated by this means. Since edema occurs in both edema disease and endotoxic shock, the serum electrolyte changes should also be investigated. Gross and microscopic lesions of edema disease and endotoxic shock have been described and, therefore, should be included in a study of endotoxic shock.

This investigation of experimental edema disease in swine involved the study of iron-deficiency anemia and endotoxic shock by means of pathology, hematology, and serum electrolyte determinations. The objectives were: (1) to note the effect of iron-deficiency anemia on endotoxic shock by investigating various hematologic parameters, (2) to consider serum electrolyte changes in endotoxic shock, and (3) to compare the results of this study with the edema disease syndrone.

II. LITERATURE REVIEW

A. Endotoxic Shock

1. Endotoxins

Endotoxins are very large molecules and exist as phosphorus - containing, polysaccharide - protein - lipid complexes (Tauber and Russell, 1961). Bacteria that produce endotoxins are of the Gram-negative type. Gram-negative bacteria need not be infectious to produce endotoxins (Braude, 1964). The endotoxins are extracted by several methods: normal saline (Pan et al., 1962), freeze-thaw (Pan et al., 1962), trichloroacetic acid (Braude, 1964), phenol-acetone extraction with lyophilization (Tauber and Russell, 1961), and sonic extraction (Davis et al., 1961). Endotoxins are thought to be situated on or near the surface of the bacterial cell (Weil and Spink, 1957).

There are major chemical differences between lipopolysaccharides obtained from different bacteria (Tauber and Russell, 1961). Yet endotoxins all produce the same general symptoms in experimentally exposed animals, regardless of what bacterium may have furnished the toxin (Carroll, 1965; Braude, 1964; Gilbert, 1960; Keiss et al., 1964). Despite the diverse origin and different methods of preparation, endotoxins may be considered collectively (Burrows, 1951; Thomas, 1954; Weil and Spink, 1957).

The lipid portion of endotoxins is pharmacologically and immunologically inert. The polysaccharide portion functions as a haptene and is responsible for the immunologic specificity of the complex. The protein moiety is an acidic conjugated protein, but not a nucleoprotein

and in combination with the polysaccharide does not give the ordinary qualitative tests for protein. It is stable to heat and proteolytic enzymes (Burrows, 1951). Enzymes which do not detoxify endotoxins are lysozyme, crystalline trypsin, chymotrypsin, pancreatic lipase and lipoprotein lipase (Tauber and Russell, 1961).

Ambiguities in the literature make it seem most likely that the entire molecule is essential for the action of the endotoxin in producing the disease (Braude, 1964).

2. Antigenic aspects of endotoxins

Certain symbiotic bacteria that live in the intestinal tract of mammals are known to contain endotoxin, and this may give rise to antibodies. Braude (1964) stated that he found antibodies to endotoxin in the blood of virtually all the mammals he examined. In an experiment in which endotoxin was given to rabbits, he found, after shock was produced, that the concentration of natural antibodies fell sharply as did the complement. Small doses of endotoxin, too small to produce shock, increased the antibody titer. It turns out that antibodies sometimes increase resistance to endotoxin and sometimes lower resistance. A possible answer is that the issue is decided by the relative proportion of endotoxin and antibody, i.e., if the amount of antibody exceeds the amount of toxin, it protects; if it does not, it may add to the toxicity (Braude, 1964).

Since endotoxin toxicity is often ineffectively neutralized by antibodies, endotoxins may resemble certain naturally occurring substances, such as enzymes, in their antibody-antigen behavior, e.g., catalase which

stimulates antibody formation and reacts *in vitro* with hemologous antibodies, but it is not neutralized *in vivo* by antibodies (Burrows, 1951).

Another possible explanation is that substances other than antibodies are involved (Braude, 1964). In such a study it was noted that peripheral vascular failure caused by endotoxin in the dog has an initial stage of vasoconstriction. Preliminary studies *in vitro* demonstrated that the constriction was due to the interaction of endotoxin with a heat labile serum or plasma factor and platelets, resulting in the liberation of histamine or histamine-like substances. *In vivo*, normal dogs were protected by transfusions of reconstituted blood in which the essential serum factor was depleted with heat or by injecting endotoxin into immune donor dogs. It was postulated that an enzyme or enzyme system is involved (Spink and Vick, 1961).

If endotoxic shock is an antibody-antigen reaction, it should resemble anaphylactic shock. It does in the dog. Both anaphylactic and endotoxin shock syndromes in dogs are the result of vascular stasis occurring in the portal venous system. They are similar histopathologically and hematologically. The similarity is due in part to the fact that both release histamine. There are small differences. Endotoxin tends to elevate body temperature as opposed to anaphylaxis; whereas anaphylaxis tends to prolong clotting time as opposed to endotoxin (Weil and Spink, 1957).

That endotoxins have antigenic properties has been alluded to by workers using single injections into superior mesenteric arteries of rabbits. Lesions in the colon were similar to the Shwartzman phenomenon, i.e., thrombosis of the small vessels, fibrinoid degeneration of vessels, and

necrosis. The omission of a preparatory dose was of interest and one could assume that the gut is in some way sensitized by previous contact with Escherichia coli (Chamovitz et al., 1962). Shwartzman-like reactions may occur in other organs, for example the kidney, upon endotoxin injection following a sensitizing dose (Thomas, 1954). Jensen et al., (1963) found germfree mice as well as specific pathogen free mice to be significantly more resistant to the lethal effect of endotoxin derived from Escherichia coli strains indigenous to the mouse, than conventional mice of the same genetic stock. Mice with greater coliform populations in their intestines were more susceptible. Conventionalization of germfree or specific pathogen free mice enhanced their susceptibility to endotoxin. Germfree or specific pathogen free mice contaminated with a single strain of Escherichia coli failed to become more susceptible to endotoxin. This experiment would also indicate an antibody-antigen reaction.

3. Toxic aspects of endotoxins

Just as endotoxic shock may be an antibody-antigen type reaction, it may well be a direct toxin. For example, Rubenstein et al., (1962) with the aid of immunofluorescence, found endotoxin distributed throughout the walls of blood vessels in endotoxin-injected dogs. It may be that endotoxin acts directly on blood vessels to produce peripheral collapse.

Chien et al., (1964) have observed one possibly direct action of endotoxin. They found that endotoxin caused an increase in capillary permeability to both the fluid and the macromolecules in plasma.

Greisman (1960) has shown that endotoxins probably activate histamine-releasing factors in rat plasma which accounts for the pathophysiologic alterations of the endotoxic response. On the other hand, failure of antihistamine in most instances to offset the detrimental effects of endotoxin does not obviate the role of histamine, but illustrates that endotoxic shock is complex and involves a variety of neurohumoral relationships (Hinshaw et al., 1961a). Chopra et al., (1964) and Chopra and Blackwood (1964) support the toxemia hypothesis by observing that repeated treatments of non-sensitized guinea-pig uterus with extracts of different hemolytic strains of Escherichia coli produce contractions of essentially the same magnitude. Considering that toxins produce edema in swine (Clawson et al., 1961), the purely toxic aspects of endotoxin must not be overlooked.

4. Effect of endotoxins on cardiovascular dynamics

Along with other dynamic effects of endotoxin there occurs a profound vasomotor disturbance (Thomas, 1954). Vick (1960) has shown in the dog that vessels contract due to neurohumoral substances when endotoxin is in the blood.

In discussing the mechanisms of hemodynamic shock due to endotoxin, Gilbert (1960, 1962) and Hinshaw et al., (1961c) attribute shock to a decline in cardiac filling and/or a decline in vascular resistance. Since cardiac output depends upon adequate cardiac filling and blood pressure equals cardiac output times total peripheral resistance, it can be understood why endotoxic shock results in hypotension as do other types of shock. Gilbert (1960) states that there probably exists no critical

reduction in total blood volume in uncomplicated endotoxic shock in man and dog. He does mention that vomiting, diarrhea, and edema may affect blood volume in the late stages of endotoxic shock. A diminished venous return, not myocardial failure, probably accounts for the decreased cardiac output (Gilbert, 1962). Weil and Spink (1957) have shown that evisceration prevents the immediate arterial blood pressure fall which is characteristic in the dog.

Kuida et al., (1961) have compared the reactions of different species to endotoxin. Hypotension does occur at some time after endotoxin injection in all species studied. Monkeys, rabbits, and cats were compared with dogs. Cats and dogs showed the earliest hypotension. The cat displays extraordinary pulmonary vasoconstriction and the dog excess splanchnic pooling as compared with the other animals. Infusion of endotoxin in sheep produced a late systemic hypotension which was attributed to vascular dilatation (Keiss et al., 1964).

Muller and Smith (1963) have shown in the dog a rise in pressure of the portal vein associated with a decrease in blood flow of the hepatic artery and portal vein. A reduced ratio of portal flow to hepatic artery flow indicated a relative maintenance of the hepatic artery circulation. Chien et al., (1964) felt that such findings indicate a marked constriction of hepatic venules or small veins after endotoxin.

Hinshaw et al., (1962a) have compared histamine, a histamine releaser, and endotoxin in the dog by recording hemodynamic events after injection. Early responses to endotoxin are altered if a histamine releaser is given prior to endotoxin. So it would appear that endotoxin

induces histamine release and that many of the vascular changes in endotoxic shock are due to histamine. Hinshaw et al., (1962b) have also shown why pressor agents are contraindicated in endotoxic shock and they have given a rationale for the protective action of adrenergic and histamine blocking drugs. Anderson et al., (1963) showed that antihistamines at the proper dosage reduced systemic hypotension, but augmented pulmonary hypertension.

In a similar experiment Tsagaris et al., (1963) gave an antihistamine and a drug that inhibits synthesis of serotonin prior to endotoxin injection. This pretreatment decreased pulmonary artery pressure elevation, portal pressure elevation, systemic arterial pressure drop, and the fall in cardiac output in the dog. This investigation shows that cardiovascular effects of endotoxin are mediated through the release of vasoactive agents such as histamine and serotonin. The pulmonary vasoconstrictive response to endotoxin was found amendable to selective blockade by alpha-methyl dihydroxyphenylalanine which itself has no effect on the pulmonary circulation (Koehler et al., 1963).

The activity of serotonin and histamine has also been studied in rabbits (Davis et al., 1963b). It was shown that warfarin, tolerance to endotoxin, and to a lesser extent heparin and immaturity lowered serotonin and histamine release. This work indicates that there are species differences in histamine and serotonin release. They compared rabbits with other laboratory animals. Histidine may be affected by localization of histidine decarboxylase, which is another unsolved complexity (Waton, 1964).

Hinshaw et al., (1962b), using isolated denervated dog legs perfused with blood containing endotoxin, noted edema as a result of increases in pressure in small veins. This agrees with Haddy et al., (1961) who observed that local administration of vasoactive agents varies the transfer of water across the capillary membrane by changing capillary hydrostatic pressure. Hydrostatic pressure in the capillary is related to the ratio of arterial to venous resistance. An imbalance results in excess water in the tissues and is responsible to an extent for hypovolemia, hemoconcentration, edema, and shock following injection of endotoxin. Chien et al., (1964) have also found that an intravenous injection of endotoxin into dogs causes an increase in capillary permeability and increased lymph flow in the thoracic duct.

The role of small mesenteric veins in the dog, after perfusion with blood obtained from intact dogs administered lethal injections of endotoxin, has been investigated by Hinshaw and Nelson (1962). They found an increase in resistance to blood flow and weight of intestine. Evidence indicated that the progressive development of splanchnic pooling is primarily due to active constriction of small veins in which responsiveness to epinephrine is enhanced by endotoxin.

Gillenwater et al., (1963) and Hinshaw et al., (1961b) have shown that the principal effect of endotoxin on the kidney is hemodynamic and not nephrotoxic. On the other hand, it is well to remember that Shwartzman-like reactions occur in the kidney after a second dose of endotoxin (Thomas, 1954). Assuming that a Shwartzman-like reaction does not take place, endotoxin markedly decreases all renal functions and

after the effects of endotoxin have ceased, the kidneys recover (Gillenwater et al., 1963).

5. Effect of endotoxins on body temperature

Mice, rats, and guinea pigs exhibit hypothermia in contrast to hyperthermia in man, rabbits, dogs, and cats after endotoxin injection (Atwood and Kass, 1964). Braude (1964) discussed the pyrogenic action of endotoxin and points out the two hypotheses advanced to explain the phenomenon. One is that the damaged white cells release a pyrogen and the other suggests that endotoxin itself acts on the hypothalamus.

Atwood and Kass (1964) found that fever induced in rabbits either by physical (increase in ambient temperature) or pharmacological means increased the susceptibility of rabbits to the lethal action of endotoxin. On the other hand, physical means could also protect rabbits from fever and the effects of endotoxin. Rabbits kept at room temperature had elevated rectal temperatures from 1.8 - 3.1°C due to endotoxin.

Fekety (1963) using shorn rabbits restrained in a supine position and exposed to the cold made an interesting observation. After intravenous administration of endotoxin, they had no fever. However, appropriate late physiological responses of heat dissipation were noted despite the lack of fever. As these animals were warmed they became febrile. Repeated endotoxin injections in shorn rabbits resulted in greater vasoconstriction over the trunk and fever developed.

Fukuda (1963a) has shown that endotoxic shock symptoms can be sedated by antipyretics in rabbits. Fukuda (1963b) has also demonstrated that the susceptibility of rabbits to endotoxin varies with the season.

Susceptibility was increased in the winter. Fukuda feels that the heat dissipating mechanism may be facilitated during the summer.

6. Adrenal function in endotoxic shock

Because the administration of adrenal cortical hormones usually improves the condition of human patients in endotoxic shock (Weil and Spink, 1958), it is pertinent to consider the role of the adrenal cortex in endotoxic shock. For example, an investigation by Melby et al., (1960) is enlightening. They found that the adrenal secretory activity in the dog which follows intravenous injection of lethal and pyrogenic doses of endotoxin is increased. This was determined by measurements of cortisol in the effluent of the cannulated lumboadrenal vein. The increase was of the same magnitude as the adrenal secretory response to a large dose of exogenous adrenocorticotropic hormone (ACTH). Hypophysectomy abolished the adrenal response to endotoxin. Secretory responses to exogenous ACTH diminished after the intravenous administration of a lethal dose of endotoxin. After a pyrogenic dose of endotoxin was given, the elevation of plasma cortisol appeared to be solely due to an increased adrenal secretion and not decreased cortisol metabolism. They felt that an intravenous dose of endotoxin did not produce adrenal secretory failure in their study. Evidently there is no lack of ACTH in endotoxic shock for Plager et al., (1963) found that endotoxin is more effective in stimulating ACTH release in the dog than 2-methyl-1, 2-bis-(3-pyridyl)-1-propanone[1] which blocks synthesis of cortisol.

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[1]Metapirone, Ciba Pharmaceutical Company, Summit, New Jersey.

Fukuda (1963a) used adrenalectomized rabbits and found that endotoxic shock still occurred with an intensified vagotonia which he considered important in endotoxic shock. He noted a hypoglycemia in both adrenalectomized and intact rabbits given endotoxin.

Weil (1961) stated there is no evidence of adrenal insufficiency as a significant factor in the progression of bacterial shock in man. He found that the value of corticosteroids in endotoxic shock is pharmacologic rather than physiologic. In mice, rats, and dogs he noted that pretreatment with corticosteroids prevented a fatal reaction to endotoxin.

Spink and Vick (1962) found that reversal of endotoxic shock was most impressive with an aldosterone-angiotension II combination. With the doses of aldosterone used, no alterations in serum sodium or potassium concentrations were detected. No hypothesis as to the action of exogenous aldosterone was made. In a similar study using cats Hayasaka et al., (1963) found pharmocological doses of aldosterone to be protective if given 30 minutes after the endotoxin. They felt that aldosterone had a direct effect on endotoxin itself.

Hinshaw et al., (1964) found that adrenalectomized dogs given endotoxin are more severely affected hemodynamically in the early phases of shock. Secretions of the adrenal cortex may, therefore, have a net beneficial action, although in several experiments, intravenous injections of cortisol after endotoxin did not influence the degree of systemic hypotension in adrenalectomized dogs.

At this point it is interesting to note that Austvoll (1958) reported reversal of edema disease in pigs using injections of cortisol

and a 5% sulfamethazine, 5% sulfamerazine solution intraperitoneally. He reported that cortisol alone was less successful than the sulfonamides alone.

The adrenal medulla has also been considered in endotoxic shock. Gourzis et al., (1961) found that rabbits pretreated with endotoxin showed significantly increased pressor responses to epinephrine and norepinephrine as compared with controls. The same relationship was demonstrated in vitro using rabbit aorta. They thought that the deleterious effects of endotoxin in shock were due to exaggeration of existing vasoconstriction in an already compromised animal. Increased levels of catecholamines in the post-endotoxin period have been determined by Rosenberg et al., (1959). However, Hinshaw et al., (1964) found no evidence which would assign a detrimental role to the sympathoadrenal system in the dog, cat, or monkey in endotoxic shock.

7. Effect of endotoxins on iron metabolism

Kampschmidt and Arredondo (1963) have shown that injections of endotoxin will produce a rapid decrease in plasma iron concentration in normal rats. Kampschmidt and Upchurch (1964) were able to show that endotoxin decreased the total iron-binding capacity of the serum of rats. Bound iron began to decrease shortly after injection of endotoxin. The total iron binding capacity decreased more slowly than the bound iron and did not reach a minimum until 24 hours after endotoxin injection. Kampschmidt et al., (1965) were able to show that the major reason for the decrease in plasma iron after injection of endotoxin in rats is an inhibited reutilization of iron from recently destroyed erythrocytes. In

this experiment, endotoxins blocked the removal of damaged red blood cells for the first 8 - 12 hours and then stimulated their removal.

8. The hematology of endotoxic shock

One of the distinctive features of the endotoxins is the characteristic change in the blood. Within a few minutes after intravenous injection of endotoxin 60 per cent of the white cells usually disappear from the bloodstream. The first to go are the granulocytes. During the next few hours the lymphocytes decline in number as do the platelets. Granulocytosis later results from movement of cells out of the bone marrow. A significant number of the original granulocytes which disappeared do not appear to recirculate. The number of white blood cells becomes abnormally high and this is accompanied by an increase in the immature erythrocytes (Braude, 1964; Herion et al., 1965; Weil and Spink, 1957). The leukocyte count also decreases in vitro (Davis and Smibert, 1963).

Blood glucose concentration is elevated within 1 to 2 hours after injection, and hyperglycemia may persist for several hours. In severe shock, hypoglycemia may occur prior to death (Davis and Smibert, 1963; Thomas, 1954). Acidosis occurs after intravenous injection of endotoxin (Hinshaw et al., 1964). Hemolysis occurs in the dog (Spink, 1962). Gillenwater et al., (1963) observed a decrease in serum potassium of the dog during the first hour after intravenous injection of endotoxin. The serum sodium remained unchanged.

During endotoxic shock in the dog Muller and Smith (1963) observed a venous packed cell volume increase which they felt was due to an acute

plasma reduction. Chien et al., (1964) differed in that they felt the increase in arterial packed cell volume, especially that seen during the 1st hour after endotoxin injection, was largely due to the contraction of the spleen in response to sympatheticoadrenal stimulation, for the initial rise was almost absent in splenectomized dogs. Weil and Spink (1957) observed little change in the packed cell volume and therefore felt there was in the dog a pooling of blood in distended veins and not leakage of plasma. They also noted no significant change in clotting time. Hinshaw et al., (1964) stated that hemoconcentration is not a common finding in all species given endotoxin. Packed cell volumes of monkeys and cats either remained constant or decreased.

An excellent study on endotoxic shock in swine was made by Davis and Smibert (1963). Hemolytic and nonhemolytic Escherichia coli endotoxins given intravenously caused similar alterations of blood constituents after inoculation. There was an increase in uric acid and bilirubin levels up to 5 hours. There was no significant change in total protein, calcium, chloride, or albumin serum levels. After inoculation of endotoxin, there was a decrease in the serum concentration of arginine, aspartic acid, glutamic acid, methionine, alanine, serine, proline, tyrosine, phenylalanine, leucine, isoleucine, and lysine. There was an increase in the levels of ammonia, ornithine, histidine, glycine, cystine, and urea. There was no significant change in the percentage of albumin and alpha, beta, and gamma globulins. However, in the serum there was a decrease in alpha and beta lipoproteins and an increase in high density lipoprotein. There was a marked decrease in the total

number of white blood cells 10 minutes after inoculation of endotoxin. The decrease was observed for all white blood cells rather than a specific type of cell. At 22 to 24 hours postinoculation, the number of white blood cells was higher than the normal level. There was an increase in stab cells and a marked decrease in lymphocytes. There was a slight increase in packed cell volume within 5 hours after inoculation of the endotoxin. The swine were specific-pathogen-free, nonanemic, 10 weeks old, and of Yorkshire-Duroc-Hampshire breeding.

9. Symptoms and lesions of endotoxic shock

Intravenous injection of endotoxins into experimental animals results in symptoms within one hour with symptoms at their peak at 2 - 8 hours post injection. The most obvious symptoms are dyspnea, tremor, vomition, diarrhea, convulsion, prostration, and paraplegia. Lethal doses result in death at 4 - 24 hours. Recovery from sublethal doses is rapid (Atwood and Kass, 1964; Burrows, 1951; Gilbert, 1960, 1962; Thomas, 1954; Weil and Spink, 1957, 1958).

The lesions seen at necropsy vary with the species, dose of endotoxin, and age, but in general the most prominent lesions are in the gastrointestinal tract. Localized edema and hemorrhage are found in the gastrointestinal tract and other organs. Congestion in most organs is the result of vascular stasis occurring in the portal system (Burrows, 1951; Gilbert, 1960; Weil and Spink, 1957, 1958). It is well to recall that secondary exposures result in hemorrhagic necrosis in many organs (Thomas, 1954). Considering the violence of the systemic reaction upon the first exposure, there is a paucity of anatomical changes (Thomas, 1954).

In reproducing experimental edema disease in swine Pan et al., (1962) reported vomiting, a staggering gait, paraplegia and convulsions. The inoculum consisted of supernatant of the extract from intestinal contents collected from pigs with edema disease. Gross lesions were edematous changes of the stomach, edema of the spiral portion of the colon, edematous and congested lungs, localized hemorrhage and congestion in the intestine, and swollen lymph nodes. Histopathological changes were fibrinoid necrosis, edema, and fibrinous thrombi seen in arteries of the kidney, liver, and heart. Pan et al., (1962) were unable to reproduce the above symptoms and lesions using a supernatant prepared as above from normal swine, nor were they able to reproduce the above using the freeze-thaw technique. Some pigs died, however, and necropsy revealed edema around the gall bladder.

Jones and Smith (1964) injected swine, under ordinary commercial conditions, intravenously with Escherichia coli and other Gram-negative endotoxins prepared by freeze-thaw and ultrasonic disintegrater techniques. The 37 pigs were 7 - 12 weeks of age. One pig died at 5 minutes and one 45 minutes post-injection. No macroscopic changes were observed. The remaining pigs showed immediate violent convulsions accompanied by screaming which ceased at 2 - 3 minutes. At 5 - 15 minutes the pigs clamped their jaws, salivated and vomited. Retching lasted until 45 minutes. Then the pigs became incoordinated and within an hour were recumbent. One-half of the pigs had diarrhea. Meanwhile, the skin over the ears, neck, shoulders, and snout became blue, rectal temperature usually increased $\frac{1}{2}$ - 1°C and by 6 hours had risen 1.5 - 2°C above

normal. During the first 6 hours no apparent difference was noted between survivors and those which ultimately died. After 6 hours the survivors gradually improved and those that died later showed paddling motions prior to death. Swelling of ears, snout, and reddening of the conjunctiva occurred. Near death, respiration became abdominal, shallow, and irregular. Extremities were cold and the rectal temperature was subnormal.

Principal lesions included submucosal edema of the stomach, reddening of the stomach mucosa, edema of the mesocolon, excess abdominal and/or thoracic fluid, congestion and hemorrhage of the kidneys. There was histological evidence of edema in the lungs, pancreas, liver, and adrenals.

B. Edema Disease of Swine

1. Clinical aspects

The author of this thesis is fortunate in that he can refer the reader to a recent and complete chapter (Bennett, 1964) on the historical and clinical aspects of edema disease. The various possibilities presented in Bennett's chapter as the etiological agent of edema disease include toxemia, anaphylaxis, non-specific reaction of the body to stress, and infectious agents.

Gregory (1957, 1959, 1960, 1962) and Timoney (1957, 1960) set the precedent of associating hemolytic Escherichia coli with edema disease and they have favored the toxemia theory. Because Escherichia coli is an endotoxin producer, edema disease may be a form of endotoxic shock.

To emphasize the diversity of thought concerning the etiology of edema disease, the work of Underdahl et al., (1959, 1963) should be mentioned. They reported the successful transmission of the disease by an infectious agent thought to be a virus.

Ohshima and Sadao (1961) reviewed the world-wide occurrence of edema disease. The pathology of edema disease is similar regardless of the country. Even the variations are similar.

Bomer (1963) stated, based on his clinical experience, only nutritional and psychic factors can be responsible for edema disease. He stated that the triggering cause of edema disease is the sudden massive protein feeding to swine hitherto fed a low protein diet. This stress results in an adrenal insufficiency. The edema is a sequela of pervious vessels associated with a gradual insufficiency of the adrenal cortex. In sudden insufficiency edemas to not develop. In adrenal insufficiency of humans a depletion of potassium and accumulation of calcium occurs in the myocardium. Bomer (1961) found such calcium deposits in swine with edema disease and in swine with apopletic heart failure. So another hypothesis is advanced concerning edema disease, namely, it is a characterization of the well-known symptoms of the collapse of the adrenal cortex.

In contrast Matthias (1963) by means of a histological study of the adrenal cortex found to a striking degree no change in the adrenals of 51 swine with edema disease as compared with normal swine. Therefore, he stated that adrenal insufficiency and adrenal hyperfunction should not be considered in edema disease. He further states that the myocardial changes in edema disease are characteristic in many other swine diseases.

Vesselinovitch (1955) recorded a dysproteinemia in edema disease, namely, a decrease in the albumin to globulin ratio, which is indicative of stress. This is a non-specific reaction and could occur after stress due to other factors. Vesselinovitch did not report the total protein value. Davis and Smibert (1963) reported no change in total protein in experimental edema disease and no change in the percentage of albumin and globulins. Vesselinovitch (1955) reported that the albumin to globulin ratio changes with age, but he gives no data from other diseases or stress conditions.

Edema disease is usually acute, progressing from weakness and incoordination to gradual paralysis or convulsions. Most affected animals do not show an increase in temperature. Both diarrhea and constipation have been observed. The lesions are variations of edema and hemorrhage. The variation occurs in respect to location and severity. Edema disease does not produce a marked leukopenia (Bennett, 1964).

The epizootiology of edema disease in swine has been studied by Kernkamp et al., (1965). Their study supported the view that edema disease is not a highly contagious and spreading disease among pigs in a herd. Certain litters do appear highly susceptible. Morbidity averaged 16 per cent with a mortality rate of 10 per cent. The disease never recurred in the same pig. Occurrence of edema disease could not be related to diet.

2. Relation to endotoxic shock

Intuitively, the injection of Gram-negative bacteria or disintegrated portions of Gram-negative bacteria into swine should produce endotoxic

shock. Such injections of bowel extract, freeze-thaw extract, and sonic extract have mimiced edema disease (Davis, 1961, 1963; Erskine, 1957; Gregory, 1960; Timoney, 1960). Therefore, because such injections produced endotoxic shock in other species (Burrows, 1951), the experimental edema disease must be endotoxic shock. Such is not the case according to Erskine (1957) and Gregory (1960). Intravenously administered freeze-thaw extracts, prepared from hemolytic Escherichia coli cultured from pigs not affected with edema disease, did not result in typical edema disease. Furthermore, Pan et al., (1962) were unable to produce edema disease with freeze-thaw extracts from clinical cases, although bowel extracts from these same cases did reproduce edema disease.

In contrast, Jones and Smith (1964), using intravenous freeze-thaw and ultrasonic disintegrator extracts, were able to reproduce experimental edema disease from both "edema" and "non-edema" strains of Escherichia coli. Clinical and pathological features of edema disease were not only produced by "non-edema" Escherichia coli, but by extracts of other Gram-negative bacteria. These findings are intuitively correct and would seem to place edema disease in the realm of endotoxic shock.

The study of the pathogenesis of endotoxic shock in swine as related to anaphylaxis (Buxton and Thomlinson, 1961; Smibert et al., 1962; Thomlinson and Buxton, 1962, 1963) and toxicity (Chopra and Blackwood, 1964; Gregory, 1964) should answer some of the questions concerning the pathogenesis of edema disease. In all probability both anaphylaxis and toxicity are involved in experimental endotoxic shock, but the pathogenesis of edema disease most likely does not involve anaphylaxis (Andersen, 1965).

The source of endotoxin in edema disease would be the intestinal Gram-negative bacteria. Ravin et al., (1960) have investigated gastrointestinal absorption of endotoxin in rabbits. They found a continuous but fluctuating absorption of bacterial endotoxin from the intestine.

C. Swine Hematology and Anemia

The author will refer the reader to recent and complete references in porcine hematology (Calhoun and Smith, 1964; Schalm, 1961; and Swenson, 1964).

Miller et al., (1961) have reported on swine hematology from birth to maturity. Reeder (1964) gave a brief review of the pathogenesis of swine anemia due to iron deficiency. Seamer (1956) had an excellent review of the incidence, economic importance, etiology, clinical signs, hematology, pathology, treatment, and prophylaxis of piglet anemia.

French and Bussell, (1963); McDonald et al., (1955); Ullrey et al., (1959); and Zimmerman et al., (1959) have investigated various iron treatments and the effect on hematology and growth.

Bush et al., (1955) have compared the blood volume in normal and anemic swine. They found the reduction in red blood cell volume in anemic swine was not compensated by a comparable increase in plasma volume. Therefore, there is a slightly lower blood volume in anemic swine.

Puller (1959) and Whitehair (1964) have reported on nutritional anemias and Puller elaborated on the relationship of other diseases to marginal deficiencies of iron and copper. Cartwright et al., (1948, 1950, 1951) have dealt with niacin, pteroylglutamic acid and vitamin B_{12}

deficiencies. Copper deficiency has been investigated by Brooksbank (1954); Coulson and Carnes (1963); Gubler et al., (1952); Lahey et al., (1952); and Shields et al., (1962).

III. MATERIALS AND METHODS

A. Procurement and Care of Swine

The sows were purchased from farmers in the vicinity of Ames. They were bought in pairs. The first pair was Hampshire-cross sows, carrying their second litter, and bred to a testing station Spotted Poland boar. Each sow weighed approximately 550 pounds.

The second pair was Hampshire-cross sows, carrying their first litter, and bred to a testing station Poland China boar. Each sow weighed approximately 295 pounds.

The sows were fed the following ration:

ground corn	46.5%
ground oats	20.0%
wheat bran	5.0%
50% soybean oil meal	16.5%
dried whey (70% lactose)	10.0%
dicalcium phosphate	1.0%
iodized salt	0.5%
vitamin premix	0.5%

Ingredients in the above ration were selected to contain a minimal amount of iron. The ration met the nutritional requirements for gilts and sows (National Research Council, 1959). Specifically, dietary requirements for crude protein, total digestible nutrients, phosphorus, salt, vitamin A, vitamin D, thiamine, riboflavin, niacin, pantothenic acid, and vitamin B_{12} were met. The ration was lacking

some calcium. It contained 0.5% calcium, and 0.6% was required.

The vitamin premix was manufactured by Vet-A-Mix, Inc., Shenandoah, Iowa. The premix contains the following:

	per pound
vitamin A U.S.P. units	200,000
vitamin D U.S.P. units	50,000
riboflavin	500 mg
calcium dl - pantothenate	2,400 mg
niacin	2,000 mg
vitamin B_{12}	2 mg

Sows were fed the ration free choice and water was always available. Prior to parturition, the sows were placed in farrowing crates. The sows were released from the crates twice a day for feed, water, and exercise. The crates were washed with water twice a day.

The first two sows were delivered March 5, 1964. The second two sows were delivered April 24, 1964. Their pens were located in a semi-isolated basement area. Procedures were conducted to prevent introduction of infectious diseases, e.g., antiseptic foot baths and hand washing facilities.

The first sow farrowed April 1, 1964; the second April 9, 1964; the third May 18, 1964; and the fourth May 22, 1964. Pigs were numbered by an ear notching code. Litters, sexes, and weights were as follows:

First litter

pig number	sex
1	F
2	F
3	F
4	F
5	M
6	M
7	F
8	M
9	M
10	F
11	F
12	F
13	F
14	M

Second litter

pig number	sex
15	M
16	F
17	M
18	F
19	M
20	F
21	F
22	F
23	F
24	F

Third litter

		Birth weight (pounds)
25	M	2.75
26	M	3.50
27	F	3.25
28	F	3.25
29	F	2.75
30	F	1.50

Fourth litter		Birth weight (pounds)
31	M	2.50
32	F	2.50
33	F	2.50
34	F	2.25
35	M	2.75
36	M	2.75

The following pigs died:

pig number	apparent cause	death date
2	hypoglycemia	April 4, 1964
3	starvation	April 14, 1964
7	hypoglycemia	April 7, 1964
8	hypoglycemia	April 6, 1964
9	hypoglycemia	April 4, 1964
13	laid on by sow	April 15, 1964
14	euthanized	April 2, 1964
19	unknown	April 11, 1964
24	laid on by sow	April 9, 1964

Pigs 16, 22, and 23 were injected intraperitoneally with a therapeutic solution[1] and sterile water for hypoglycemia. This solution contains calcium gluconate, dextrose, magnesium hypophosphite, and d-amphetamine hydrochloride. Pig 22 received 5 ml of the solution and 5 ml of sterile water. Pigs 16 and 23 received 2.5 ml of the solution and 2.5 ml of sterile water.

The second sow had a rectal temperature of 106°F on the morning of April 9, 1964. She had farrowed prior to the taking of the temperature. She was injected intramuscularly with 11 ml of dihydrostreptomycin

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[1]Amcal Solution, Diamond Laboratories, Des Moines, Iowa.

(0.25 gm/ml) both morning and evening, 5 ml of procaine penicillin G (300,000 units per ml) both morning and evening, and 2.5 ml of purified oxytocic principle[1], (20 U.S.P. units per ml) in the evening.

On April 10, 1964, her temperature remained at 106°F and she was anorexic. The dihydrostreptomycin and penicillin were repeated as before. In the evening she was injected intramuscularly with 20 ml of oxytetracycline HCl[2] (50 mg per ml) and her uterus was infused with tetracycline HCl, neomycin, and dihydrostreptomycin[3], and nitrofurozone[4].

On April 11, 1964, her temperature was 104.5°F. She was injected with oxytetracycline morning and evening as described before. Her uterus was infused in the evening as described before. She drank some water.

On April 12, 1964, her temperature was 105° F and she was injected as before with oxytetracycline. Her cervix was closed. She ate and drank small amounts of water. On April 13, 1964, her temperature was 105° in the morning. She regained her appetite and she drank water. She was not treated and her evening temperature was 103°F.

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[1]Purified Oxytocic Principle, Armour-Baldwin Laboratories, Omaha, Nebraska.

[2]Chas. Pfizer and Co., Inc., New York City, New York.

[3]Polyotic, American Cyanamid Co., Princeton, New Jersey.

[4]Furacin, Eaton Laboratories, Norwich, New York.

B. Design of the Experiment

One-half of the pigs were randomly injected with iron-dextran[1] at three days of age. Each pig was injected intramuscularly with 2 ml (75 mg of elemental iron per ml) in a hind leg. Pigs receiving iron were 1, 3, 4, 5, 7, 11, 20, 21, 22, 23, 27, 29, 30, 31, 34, and 35.

At 1, 2, and 3 weeks of age 6 - 10 ml of blood were drawn from the anterior vena cava from each pig for hematological examination.

After the three-week blood samples were taken, endotoxin was injected into the anterior vena cava of all pigs except one iron-injected pig and one iron-deficient pig from each litter.

The following data indicate dosage of endotoxin and pig weight:

Pig number	Dosage (mg/kg)	Weight (kilograms)
11	control	5.8
4	2	5.3
5	4	4.9
1	6	5.9
12	control	4.8
6	4	2.8
10	6	5.0
20	control	4.3
21	1	5.6
22	2	3.3
23	4	3.1
15	control	5.7
16	1	3.5
17	2	2.6
18	4	4.8
30	control	3.4
27	4	5.0
29	2	5.5

[1]Ferrextran, supplied by Fort Dodge Laboratories, Fort Dodge, Iowa.

Pig number	Dosage (mg/kg)	Weight (kilograms)
25	control	3.9
26	2	5.1
28	4	5.1
35	control	3.2
31	4	2.6
34	2	3.4
32	control	3.9
33	2	2.9
36	4	3.1

C. Endotoxin Preparation

The endotoxin used in this investigation was prepared by Dr. John R. Andersen, Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa. Andersen (1965) modified a method of Tauber and Russell (1961) in preparing the endotoxin. A culture of Escherichia coli sero-group 0138, which was isolated by Dr. Paul C. Bennett of the Iowa Veterinary Diagnostic Laboratory from the intestine of a pig with a clinical case of edema disease, was utilized in the endotoxin preparation.

D. Hematology

The disodium salt of ethylenediaminetetracetic acid was used as an anticoagulant. The packed cell volume was determined by the microhematocrit method. Plain capillary tubes were employed. Paired samples were run for five minutes at 11,500 r.p.m. The packed cell volume was read directly by means of a hematocrit capillary tube reader. The hemoglobin determinations were made by the cyanomethemoglobin method.[1]

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[1]Method described in pamphlet (revised, 1962) from Hycel Inc., Houston, Texas.

The Wintrobe hematocrit tube was used to determine the sedimentation rate of erythrocytes. Millimeters of sedimentation were recorded at 0.25, 0.5, 1, and 2 hours.

Enumeration of erythrocytes and leukocytes was accomplished using pipettes with $\pm$1% accuracy and a Neubauer Bright Line counting chamber. Hayem's solution was used for erythrocyte enumeration and 0.1 N HCl for leukocyte enumeration. The leukocyte count was corrected by the following formula:

$$\frac{100 \text{ leukocytes}}{100 \text{ leukocytes} + \text{no. of nucleated erythrocytes}}$$

X uncorrected leukocyte count/cu mm blood = corrected leukocyte count

One count was made per sample. For the differential leukocyte count, 100 cells were counted. A blood stain[1] was used. The number of nucleated erythrocytes encountered while making a differential count of 100 leukocytes in the stained blood film was recorded.

The mean corpuscular volume (cubic microns) was calculated by multiplying the packed cell volume by 10^7 and dividing by the erythrocyte count. The mean corpuscular hemoglobin (micromicrograms) was calculated by multiplying the grams of hemoglobin by 10^7 micromicrograms and dividing by the erythrocyte count. The mean corpuscular hemoglobin concentration (per cent) was calculated by dividing the grams of hemoglobin

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[1]Tetrachrome, Hartman-Leddon Co., Philadelphia, Pennsylvania.

in 100 ml of blood by the packed cell volume and multiplying the quotient by 100.

Sodium and potassium were determined by using a Baird-Atomic flame photometer model KY-2.[1] The blood serum mode was employed as directed in the instruction manual revised October 25, 1963. The blood was drawn and the serum stored in utensils rinsed in ion free water. The ion free water was obtained by filtering distilled water through an exchange column filled with Amberlite MB-1.[2] Sodium and potassium standard and the lithium internal standard were purchased from Baird-atomic Inc.,[1] Cambridge, Massachusetts. All solutions were stored in polyethylene containers. The serum was frozen in capped polyethylene culture tubes. Hemolysis was avoided when the serum was taken from the clotted samples. Five hundredth ml of serum was utilized for the electrolyte analysis. Electrolyte determinations were made in duplicate.

E. Necropsy and Histopathological Procedures

The necropsy was conducted after death and gross lesions were recorded. Portions from the brain, gastrointestinal tract, liver, kidneys, adrenals, thymus, lymph nodes, and lungs were fixed in 10% buffered formalin solution. They were dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin. The sections were cut at 6 or 7 microns and stained with Delafield's hematoxylin and ethyleosin.

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[1]Baird-Atomic Inc., Cambridge, Massachusetts.

[2]Fisher Scientific Co., Fair Lawn, New Jersey.

F. Statistical Analysis

The statistical analysis consisted of a multiple regression and analysis of variance. The calculations included 17 variables. They were litters (3), treatments (3), packed cell volume, hemoglobin, sedimentation rate, red and white blood cell counts, nucleated red blood cell counts, Wintrobe erythrocytic indexes (3), sodium and potassium. The data analyzed appears in Appendix A. Significance was determined by the F-test. Significance of variation due to litters (1 - 3 weeks of age), iron, endotoxin, and iron by endotoxin was tested. No statistical analysis was made for the 24 hour and 120 hour data because of the small number of surviving pigs. Means recorded and shown on the graphs (Appendix B) and in Tables 1 - 11 are corrected for variation due to litters. Statistical analysis of the sedimentation rate employed data from the 1 hour observations. The significance of the effect of hemolysis on serum Na and K was determined by use of Student's t-test using the data of serum samples collected prior to injection of endotoxin. Blood samples marked with an H in the tables (Appendix A) were hemolized.

The variance ratio was declared significant at the 0.01 and 0.005 probability levels for both differences and interaction. Student's t-test was declared significant at 0.05 probability on a two tailed test.

IV. RESULTS AND DISCUSSION

A. Total Erythrocyte Number

Table 1. Means of total erythrocytes per cu mm of blood (10^6) from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	3.49	3.19			0.42
2 wk.	3.85	3.05			0.52
3 wk.	3.91	2.75			0.41
Postinjection time					
1 hr.	3.20	2.64	3.06	2.26	0.25
5 hr.	3.38	2.50	3.44	2.80	0.32
24 hr.	4.39	--	3.67	--	--
120 hr.	3.72	--	3.22	--	--

The erythrocyte counts (Table 1) are below the ranges reported by Calhoun and Smith (1964). The values are also below the ranges reported by Schalm (1961), but in 9 Duroc-Jersey pigs at 10 days after birth on concrete, Schalm (1961) reports one erythrocyte count of 2.1 million/cu mm. Talbot and Swenson (1963) also report higher mean count values than the results of this investigation. The values in the table above come closest to the values reported by Doyle et al., (1927) from an anemic farm herd. The iron-injected pigs have lower counts than iron-injected pigs cited by Miller et al., (1961) at 1 and 3 weeks of age. Schalm (1961) points out that an experienced technician will show little variation, but that variation among technicians can be significant.

The litter differences for erythrocyte counts were significant (1.0% level) for weeks 1, 2, and 3. With the exception of the first week there exists significant differences between iron-injected and iron-deficient groups throughout the experiment. Endotoxin caused a significant drop (Figure 1) in both iron-injected and iron-deficient groups in the 1-hour samples. Packed cell volume (PCV) and hemoglobin show a nonsignificant drop at 1 hour for the iron-injected group, but a nonsignificant rise in the iron-deficient pigs. At 5 hours the erythrocyte count fails to show a significant rise when the PCV and hemoglobin values show a significant rise. An increase in cell volume could account for the increase in PCV, but not the increased hemoglobin. Increased hemoglobin might be accounted for by hemolysis due to endotoxin. These data indicate a lack of full correlation of the erythrocyte counts with PCV and hemoglobin values. This lack of agreement is attributed to the inherent variation in total erythrocyte counts.

Techniques used for the counts were routine and no technical reason for low counts can be ascertained. Swenson et al., (1955) have shown that the sow's ration during gestation affects the erythrocyte counts. Because these sows were for sale during the gestation period, it is possible that their ration did not meet dietary requirements prior to their purchase. Unequal distribution of erythrocytes in large and small vessels would account for a difference in total erythrocyte counts among pigs bled at different sites. In most of the references cited the anterior vena cave was the vessel from which blood was drawn.

Although no statistical analysis was made, it is worth observing that at 24 hours, when the PCV and hemoglobin decreased, the erythrocyte count also decreased.

B. Hemoglobin

Table 2. Means of hemoglobin values (gm/100 ml) of blood from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	10.0	8.2			1.1
2 wk.	11.4	6.5			1.1
3 wk.	11.0	5.5			1.1
Postinjection time					
1 hr.	10.9	5.2	10.7	5.4	1.1
5 hr.	11.0	5.3	11.8	5.9	0.7
24 hr.	11.6	---	10.4	---	---
120 hr.	9.5	---	10.0	---	---

The hemoglobin values (Table 2) extended beyond the maximum and minimum values cited by Schalm (1961). Hemoglobin values in this investigation followed the pattern outlined in Calhoun and Smith's (1964) review. The values also compare favorably with Talbot and Swenson (1963), with the iron-injected pigs somewhat higher and the iron-deficient pigs somewhat lower. The iron-injected pigs compare favorably with hemoglobin values cited by Miller et al., (1961).

The statistical analysis yielded a significant difference (Figure 2) between the iron-injected pigs and the iron-deficient pigs throughout the experiment. The 5-hour postinjection samples showed a significant rise

in hemoglobin due to endotoxin. The changes in hemoglobin values are also reflected in the PCV (Table 3). The litter differences for hemoglobin values were significant (1.0% level) during the 3 week period prior to endotoxin injection.

C. Packed Cell Volume

Table 3. Means of packed cell volumes (%) from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	35.0	28.0			3.3
2 wk.	38.5	23.5			3.1
3 wk.	37.0	22.5			3.5
Postinjection time					
1 hr.	35.5	20.5	35.0	22.5	3.1
5 hr.	36.0	20.0	39.2	22.6	1.7
24 hr.	37.0	--	36.0	--	---
120 hr.	32.0	--	32.0	--	---

Mean packed cell volumes (Table 3) exceeded those cited by Schalm (1961). He cited a mean of 26.7 at 6 days after birth in Duroc-Jersey pigs on concrete. The mean was derived from a litter of 9 pigs which were not injected with iron. It is not valid to compare values in this case due to breed differences and the small number of pigs involved. Comparison of the experimental PCV values to values found in the literature, as summarized by Calhoun (1964), shows the experimental values to be in the physiological range. The experimental values are just over the values cited by Miller et al., (1961) for the iron-injected pigs.

The experimental values are higher than those reported by Talbot and Swenson (1963).

The statistical analysis yielded a significant difference between the iron-injected and iron-deficient pigs throughout the experiment. The 5-hour postinjection samples showed a significant hemoconcentration due to endotoxin (Figure 3). The litter differences were significant (1.0% level) for weeks 1, 2, and 3.

Evidently, swine is a species which shows hemoconcentration during endotoxic shock. The experiment gives no indication as to whether this is due to plasma loss or splenic contraction. Splenectomized pigs would lend themselves to solving this ambiguity. Hemoconcentration in dogs occurs during the first hour of endotoxic shock (Chien et al., 1964). It appears to occur later in swine (Davis and Simbert, 1963).

No explanation can be given for the generally higher PCV values during this study. The 5-minute centrifugation at 11,500 r.p.m. will trap a minimum of plasma and is routine (Schalm, 1961). The disodium salt of ethylenediaminetetracetic acid was used in place of oxalate, but probably this would make little difference (Schalm, 1961).

D. Mean Corpuscular Volume

Table 4. Means of the mean corpuscular volumes (cubic microns) of erythrocytes from four groups of pigs

Age	Treatments				Standard error
	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	
1 wk.	102.0	88.4			11.5
2 wk.	100.9	80.0			9.6
3 wk.	96.8	83.8			9.8
Postinjection time					
1 hr.	111.4	89.1	114.3	99.1	9.8
5 hr.	106.0	80.4	114.9	80.4	8.8
24 hr.	85.4	--	96.2	--	---
120 hr.	84.7	--	98.5	--	---

Due to the low total erythrocyte counts the mean corpuscular volume (MCV) values are much higher (Table 4) than those reported by Miller et al., (1961); Schalm (1961); Talbot and Swenson (1963); and Ullrey et al., (1959).

The greater drop at 1 hour in erythrocyte counts in the iron-deficient plus endotoxin group (Figure 1) results in a significant interaction. That is to say, the iron-deficient plus endotoxin pigs showed greater rise in MCV than the iron-injected plus endotoxin pigs (Figure 4). This may be due to the uptake of fluid by the erythrocytes which correlates with the nonsignificant rise in PCV, but an intake of fluid does not explain the nonsignificant rise in hemoglobin. Variation due to litters was significant at weeks 1 and 2 (5.0% level) and week 3 (1.0% level).

The significant difference in MCV throughout the experiment between iron-injected and iron-deficient pigs agrees with previous statements of Schalm (1961), Seamer (1956), Talbot and Swenson (1963), and Whitehair (1964) in that the iron-deficient pigs show a smaller mean corpuscular volume, but is, relatively speaking, in contrast to the above investigators because all the pigs have macrocytic erythrocytes. This is due, for the most part, to the low red blood cell counts. Schalm (1961) defines microcytocis as a MCV more than two standard deviations below the normal mean, in this case iron-injected swine, as determined by the same method on the blood samples of healthy animals of the same species. Thus, with the exception of the first and third weeks, there exists a microcytosis.

E. Mean Corpuscular Hemoglobin

Table 5. Means of the mean corpuscular hemoglobin values (micromicrograms) of erythrocytes from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	29.2	26.5			3.6
2 wk.	29.9	22.2			2.4
3 wk.	28.4	20.5			2.2
Postinjection time					
1 hr.	34.3	19.2	34.7	24.1	3.0
5 hr.	32.4	21.0	34.3	20.4	2.9
24 hr.	26.6	--	28.3	--	---
120 hr.	25.6	--	30.6	--	---

Due to low total erythrocyte counts the mean corpuscular hemoglobin (MCH) values (Table 5) are higher than those reported by Miller et al., (1961), Schalm (1961), and Talbot and Swenson (1963). With the exception of the first week the MCH differed significantly (Figure 5) when iron-injected and iron-deficient pigs were compared. Endotoxin showed no significant effect. This is intuitively correct.

The 1-hour postendotoxin injection blood samples from the endotoxin injected pigs reflect the significant decrease in erythrocytes by a nonsignificant rise in MCH (Figure 5). MCH fluctuations due to endotoxin might occur if hemolysis (Spink, 1962) lowered the erythrocyte number and the hemoglobin remained constant. Litter differences in MCH values were significant (1.0% level) in the second and third week.

F. Mean Corpuscular Hemoglobin Content

Table 6. Means of the mean corpuscular hemoglobin content (%) of erythrocytes from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	29.3	29.4			2.0
2 wk.	29.6	27.8			1.9
3 wk.	29.4	24.6			2.5
Postinjection					
1 hr.	30.8	25.2	30.3	24.8	2.8
5 hr.	30.5	26.2	29.9	25.5	1.4
24 hr.	31.2	--	29.3	--	---
120 hr.	30.2	--	31.0	--	---

With the exception of the first week there was a significant difference (Figure 6) between iron-injected and iron-deficient pigs throughout the experiment (Table 6). Endotoxin had no effect on mean corpuscular hemoglobin content (MCHC).

Hypochromic anemia exists when a MCHC and usually a MCH fall more than two standard deviations below the mean normal determined by the same method on blood of healthy animals (Schalm, 1961). If the iron-injected pigs serve as the normals, hypochromic anemia exists in the controls at 1 and 5 hours postinjection, with the 3-week samples on the borderline.

Previous literature (Schalm, 1961; Seamer, 1956; Whitehair, 1964) reports iron-deficiency anemia as hypochromic. Talbot and Swenson (1963) reported a normochromic iron-deficiency anemia in pigs up to 8 weeks of age. Ullrey et al., (1959), utilizing data from hematology recorded from birth to 5 weeks, recorded a significant difference (5.0% level) in MCHC between iron-deficient and iron-injected pigs only at 17 days of age. The standard deviations were not given. It seems that iron-deficiency anemia can be hypochromic or normochromic depending upon the circumstances.

G. Total Leukocyte Number

Table 7. Means of total leukocytes per cu mm of blood (10^3) from four treated groups of pigs

Age	Treatments				Standard error
	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	
1 wk.	7.5	7.9			3.0
2 wk.	10.3	8.0			2.9
3 wk.	8.2	6.2			2.1
Postinjection time					
1 hr.	7.9	5.0	2.2	1.6	1.8
5 hr.	8.9	5.4	1.7	1.0	1.2
24 hr.	8.8	--	13.5	--	--
120 hr.	7.8	--	7.2	--	--

A significant difference (Figure 7) between iron-injected and iron-deficient pigs existed at the second and third week in total leukocyte per cu mm of blood (Table 7). The literature varies as to whether a slight leukopenia should accompany iron-deficiency anemia (Seamer, 1956). Ullrey et al., (1959) reported no significant differences the first 5 weeks of age. The failure to report a significant difference is probably because the leukocyte counts were not corrected for nucleated erythrocytes.

At one week neutrophils outnumbered lymphocytes. This picture was reversed the second and third week (Figure 8). This is the same leukocyte picture described by Schalm (1961).

Endotoxin caused a significant drop in leukocytes at 1 and 5 hours postendotoxin injection. Some of the cells are driven into the tissues, for example the eosinophils, and some are destroyed. Evidence of

destruction is given by the increased serum lysozyme levels after endotoxin injection (Andersen, 1965). The increase in immature leukocytes gives further evidence of leukocyte destruction. At 24 hours the iron plus endotoxin injected pigs rebounded and displayed a leukocytosis. This was reported by Davis and Smibert (1963). The 24 hour leukocytosis was primarily neutrophils. This agrees with Herion et al., (1965) who account for the increased neutrophils by the movement of these cells out of the marrow.

Endotoxin caused a general leukopenia with the majority of circulating leukocytes being lymphocytes. This is different than the picture during experimental stress as produced by Luke (1953). Adrenocorticotrophic hormone and adrenal cortical extract injected into normal pigs caused a marked decrease in lymphocytes and an increase in neutrophils. At the same time there was a sharp increase in the total WBC count. These changes were apparent within 2 hours postinjection.

At 24 hours neutrophils dominated the picture in endotoxin-injected and control pigs (Figure 8). Litter differences were significant (1.0% level) only for the first week.

H. Nucleated Red Blood Cells

Table 8. Means of nucleated red blood cells per 100 leukocytes in the blood of four treated groups of pigs

Age	Treatments				Standard error
	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	
1 wk.	41	43			53
2 wk.	6	23			13
3 wk.	1	24			14
Postinjection time					
1 hr.	7	45	32	256	115
5 hr.	1	28	41	364	107
24 hr.	3	--	18	---	---
120 hr.	4	--	2	---	---

After the first week, differences in nucleated red blood cells between iron-injected and iron-deficient pigs were significant (Table 8). Endotoxin significantly raised the number of nucleated erythrocytes in iron-injected and iron-deficient pigs at 1 hour postendotoxin injection. At 5 hours after endotoxin injection the iron-deficient plus endotoxin pigs showed a significantly greater rise than the iron-injected plus endotoxin pigs (Figure 9). The rise in nucleated erthrocytes after endotoxin could be expected. Braude (1964) reports a rise in the immature red cells in experimental animals after endotoxin injection. In this study, litter differences were not significant.

Ullrey et al., (1959) made no mention of nucleated red blood cells and evidently did not correct their white blood cell counts. Doyle, et al., (1927) felt that nucleated red blood cells were not especially numerous in anemic pigs. Perhaps the lack of difference in leukocyte

counts between iron-injected and iron-deficient pigs, in the literature, is due to uncorrected leukocyte counts. Swenson, et al., (1955) showed statistically that maternal rations affected the number of nucleated erythrocytes.

The increase in nucleated erythrocytes in anemic pigs is probably due to their release from hemopoietic foci. Contraction of the spleen probably plays a part. Again, splenectomies would help answer this question.

No reticulocyte counts were made in this study. Ullrey et al., (1959) found no significant reticulocyte count differences between iron-injected and iron-deficient pigs. Drs. Talbot and Swenson, Department of Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University of Science and Technology, in a private communication on July 7, 1965, found that iron-injected pigs had a significantly (0.1% level) higher number of reticulocytes at 1 week of age when compared with pigs not injected with iron. At 2 and 3 weeks the iron-injected pigs had lower reticulocyte counts. The counts were not significantly lower.

Reticulocytes and nucleated erythrocytes are very prominent in young swine (Schalm, 1961). The relationship between these immature erythrocytes in swine should be investigated. Both methods of counting (number per 100 white blood cells and number per 1,000 mature red blood cells) should be employed.

I. Sedimentation Rate

Table 9. Means of erythrocyte sedimentation rate in mm per hour of four treated groups of pigs

Age	Treatments				Standard error
	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	
1 wk.	0	3			1
2 wk.	1	14			5
3 wk.	2	16			7
Postinjection time					
1 hr.	2	20	2	15	8
5 hr.	1	14	1	10	6
24 hr.	1	--	27	--	--
120 hr.	2	--	9	--	--

There exists a significant difference throughout the experiment between iron-injected and iron-deficient pigs (Table 9). Endotoxin had no significant effect (Figure 10). However, at the 24 hour postendotoxin injection sampling the iron-injected plus endotoxin group displayed a large increase in sedimentation rate. This has been reported by Davis and Smibert (1963). The litter differences were significant (1.0% level) at week 1 and 3.

The sedimentation rate of erythrocytes in their own plasma is dependent upon the size of the cell aggregates and the number of erythrocytes (Schalm, 1961). Decreased total erythrocytes would tend to increase the sedimentation rate. Microcytosis would tend to decrease the sedimentation rate. So, the smaller cells are not greatly influencing sedimentation rate, but the decreased erythrocyte number (Table 1) of iron-

deficient pigs may account for the increased sedimentation rate. As anemia progresses, plasma protein concentration is decreased and the reduced blood viscosity (Schalm, 1961) may also account for the increased sedimentation rate.

J. Serum Potassium

Table 10. Means of serum potassium in mEq/L from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	7.4	6.1			1.0
2 wk.	6.2	6.3			0.8
3 wk.	8.6	7.5			0.6
Postinjection time					
15 min.	8.7	8.0	8.6	6.8	0.6
1 hr.	8.7	7.2	7.3	6.2	0.7
5 hr.	8.4	7.7	8.9	7.4	0.8
8 hr.	8.6	7.2	10.6	8.3	1.7
24 hr.	6.0	--	7.2	--	--
120 hr.	9.2	--	8.1	--	--

With the exception of the second week, there exists a significant difference between iron-injected pigs and iron-deficient pigs (Figure 12) with the iron-deficient pigs displaying a hypokalemia in the experiment (Table 10). Variation due to litters was not significant.

Endotoxin significantly affected the iron-deficient pigs at 15 minutes after injection. At 1 hour after injection endotoxin caused a significant hypokalemia in iron-injected pigs and iron-deficient pigs.

At 8 hours after endotoxin injection the endotoxin-injected pigs displayed a significant over compensation (hyperkalemia).

The serum potassium levels are considerably higher than those reported in the plasma of 6 pregnant sows and 12 fetal pigs by Cummings and Kaiser (1959). Their range was 4.0-4.7 mEq/L for the means and 2.6-5.2 for the individual observations. Widdowson and McCance (1956) reported means of 17.5 for fetal pigs, 8.6 for newborn pigs, and 6.0 for adult swine. Both references used flame photometry. Cummings and Kaiser (1959) used heparin for plasma determinations, while Widdowson and McCance (1956) used serum.

The hypokalemia of iron-deficient pigs could be explained if plasma volumes made up for the decrease in RBC's. Such is not the case according to Bush et al., (1955).

Correlating with symptoms, the hypokalemia after endotoxin is probably due to vomition. No reason can be given for the greater effect of endotoxin on the iron-deficient pigs. With the hypokalemia one can expect less aldosterone production (Davis, 1963; Gann et al., 1962). One must keep in mind that the kidneys are probably non-functional during the potassium fluctuation (Gillenwater et al., 1963). In addition, the pig probably has a low degree of pituitary-adrenal response (Link, 1953) and the electrolyte fluctuations seen in the above data are probably better explained by some other means, e.g., fluid transfer.

Because hemolysis was noted in many samples, a statistical analysis was made to ascertain its effect upon the electrolyte content. No attempt was made to find the degree of hemolysis. Hemolysis was noted

in samples other than those receiving endotoxin; therefore the hemolysis was not solely the result of endotoxin. In addition the serum potassium dropped in endotoxin samples which is the reverse of effects expected due to hemolysis. Statistical results showed no significance due to hemolysis during the first 3 weeks. Perhaps the pig has a low potassium content in the erythrocyte as is the case in other animals (Berstein, 1954; Hendricks, 1964; Meier, 1963). No particular reason for the hemolysis can be given other than technique. Pigs have relatively non-fragile erythrocytes, espcially young pigs (Perk et al., 1964).

K. Serum Sodium

Table 11. Means of serum sodium in mEq/L from four treated groups of pigs

	Treatments				
Age	Fe-injected	Fe-deficient	Fe-injected & endotoxin	Fe-deficient & endotoxin	Standard error
1 wk.	141.2	142.5			3.5
2 wk.	141.7	140.7			4.0
3 wk.	143.4	141.2			6.3
Postinjection time					
15 min.	147.0	148.0	138.0	142.0	4.4
1 hr.	147.0	146.0	145.0	145.0	3.7
5 hr.	139.7	143.5	144.0	139.2	5.8
8 hr.	140.5	142.5	140.3	140.2	3.7
24 hr.	143.5	--	145.0	--	--
120 hr.	143.0	--	139.0	--	-

Variation due to litters was significant (1.0% level) only for week 3. Iron injections did not affect serum sodium (Table 11). Endotoxin

injection caused a hyponatremia at 15 minutes postinjection (Figure 11). Meier (1963) includes a loss of sodium into the tissues as a sign of shock and this could account for the hyponatremia as well as the concurrent vomition. Because a hypokalemia also occurred at the same time, vomition is the probable cause of the hyponatremia.

Hemolysis had no significant effect on serum sodium during the first three weeks. Significance was tested by use of Student's t-test.

Cummings and Kaiser (1959) reported a range of 139.1-148.1 mEq/L in the plasma of 6 pregnant sows and 12 fetal pigs. They found that the sodium to potassium ratio was about equal in the sow and fetus. Widdowson and McCance (1956), in contrast, found a fall in serum potassium and a possible rise in sodium as the swine grew older. For the newborn pig they reported a range of 132 to 149 mEq/L with a mean of 141 mEq/L. The data were taken from 11 newborn pigs from different litters.

It would be interesting to know the adrenal response to the low serum sodium at 15 minutes postinjection. Normally hyponatremia stimulates aldosterone production (Davis, 1963a). Also hyperkalemia stimulates aldosterone (Davis, 1963a). In this investigation a hyponatremia occurred at about the same time as hypokalemia. The affect this had on aldosterone secretion is open to conjecture. Even if aldosterone is secreted, it would be speculative as to its action, because, the kidney is relatively inactive (Gillenwater, 1963). The measurement of plasma renin might give some clue as to the aldosterone activity during endotoxic shock (Binnion, 1965). Kalant (1962) using experimental nephrosis in rats showed that edema need not be associated with hyperaldosterone secretion, but can be due to decreased ability of the kidney to secrete sodium.

When the decrease in electrolytes is compared with the ranges of serum electrolytes in normal swine (Appendix A), it does not seem that the electrolyte decreases caused an edematous condition. Widdowson and McCance (1956) reported a range of 135-152 mEq/L for sodium in healthy adult swine. Both hypernatremia and hyponatremia may be associated with a variety of states of hydration (Bland, 1963).

L. Clinical Symptoms of Endotoxic Shock

The iron-injected control pigs were normal throughout the experiment. The iron-deficient control pigs were normal except for signs of anemia. Their mucous membranes were pale and they tired easily.

Table 12. Symptoms and time of symptoms during endotoxic shock in pigs

Pig no.	Vomiting (minutes)	Diarrhea	Temperature (rectal)	Time of death
Iron-injected				
4	12	1 hr.	--	survived
5	10	1 hr.	--	8 hr.
1	10	1 hr.	--	18 hr.
21	15	1 hr.	104°F 30 min.	30 hr.
22	20	50 min.	100°F 30 min.	survived
23	10	1 hr.	102°F 30 min.	survived
29	7	none	101°F 1 hr.	3 hr.
27	8	none	104°F 1 hr.	3 hr.
34	6	1 hr.	--	8 hr.
31	14	1 hr.	--	14 hr.
Iron-deficient				
6	8	1 hr.	--	9 hr.
10	15	1 hr.	--	9 hr.
16	10	50 min.	101°F 30 min.	4 hr.
17	15	1 hr.	102°F 30 min.	4 hr.
18	15	1 hr.	103°F 30 min.	4 hr.
26	8	none	--	50 min.
28	8	none	102°F 1 hr.	1.5 hr.
33	7	1 hr.	100°F 6 hr.	6.5 hr.
36	8	1 hr.	--	10 hr.

Pig 34 died during the bleeding procedure at 8 hours postinjection and death was attributed to hemorrhage into the lungs. The first sign of endotoxin toxicity, observed just prior to vomiting, was frequent clamping of the jaws accompanied by salivation. Intermittent vomition continued for as long as 30 minutes. The pigs developed incoordination and within an hour many were recumbent and unable to rise. At about 1 hour the respiratory rate increased markedly and dyspnea was observed. Diarrhea occurred in most of the pigs. At approximately 3 hours, many of the pigs that eventually died appeared to make a partial recovery, but they soon passed into a phase of shallow respiration and paddling motions. The extremities of such pigs were cold. Pigs which recovered appeared normal after about 24 hours. No correlations of dosage and symptoms were determined.

M. Necropsy and Histopathology

Routine necropsy procedures revealed the gross lesions described below.

Of the iron-injected pigs, pig 1 had edematous lungs, a small area of hyperemia and edema on the stomach wall in the cardiac region, and mild edema of the gall bladder. Pig 5 had edematous lungs, fluid in the thoracic cavity, and an area of edema and hemorrhage 2 cm in diameter in the cardiac stomach, edema of the gall bladder, swollen and hyperemic mesenteric lymph nodes, and the colon was hyperemic. Pig 21 had only a mild enteritis. Pig 27 had edematous lungs, excess pericardial and thoracic fluid, an area of edema and hemorrhage in the cardiac stomach, some edema of the gall bladder, edematous and hyperemic mesenteric lymph

nodes, and the pig had a mild enteritis. Pig 29 had edematous lungs, a mild hyperemia in the cardiac stomach, an edematous gall bladder, edematous and hyperemic mesenteric lymph nodes, an enlarged spleen, and the kidneys showed some hemorrhage on the surface. Pig 31 had a mild edema of the lungs, a mild edema of the gall bladder, hyperemic lymph nodes, and a severe enteritis. Pig 34 had a mild edema of the gall bladder and a mild hyperemia of the mesenteric lymph nodes.

Of the iron-deficient pigs, pig 6 had edema of the lungs, an edematous area in the cardiac stomach 2 cm in diameter, edema of the gall bladder, enteritis, and hemorrhagic spots on the colon. Pig 10 had edematous lungs, edema of the gall bladder, and an enteritis. Pig 16 had some edema of the lungs, edema of the gall bladder, a hemorrhagic and edematous area in the stomach wall, enteritis, a few hemorrhagic foci on the colon, and the anterior portion of both kidneys were hemorrhagic. Pig 17 had edematous lungs, excess pleural fluid, a hemorrhagic edematous area in the stomach wall, edema of the gall bladder and enteritis. Pig 18 had edematous lungs, excess pleural and pericardial fluid, a mild edema of the stomach wall, and edema of the gall bladder. Pig 26 was quite anemic and had hemorrhages in the lung, a mild hyperemic area of the stomach wall, and excess fluid around the brain. Pig 28 had edematous lungs with some hemorrhage and edema of the gall bladder. Pig 33 had some interlobular edema of the lungs, a great excess of fluid in the thoracic cavity, an enlarged heart probably due to the effects of anemia, a mild edema of the gall bladder, hyperemic mesenteric lymph nodes and

enteritis. Pig 36 was quite anemic and had edema of the lungs, mild edema of the gall bladder, edematous mesenteric lymph nodes, and an enteritis.

Controls from litter four were euthanized with electricity. Pig 35 was an iron-injected control and had no gross lesions. Pig 32 was an iron-deficient control and had an enlarged heart as a result of anemia. No other gross lesions were detected.

Upon microscopic examination the lesions described below were noted in iron-injected pigs.

The sectioned adrenal of pig 1 was highly congested, especially in the zona arcuata. The small intestine was highly congested, especially around the glands of Lieberkuhn. The kidneys were essentially normal except for mild congestion in the medulla and cortex. The liver and lungs were slightly congested.

The stomach of pig 5 had localized edema and hemorrhage in the submucosa. The small intestine was highly congested, especially around the glands of Lieberkuhn, and certain areas were edematous in the submucosa. The liver, lungs, and mesenteric lymph nodes were congested.

In pig 21 the kidneys had generalized congestion with some local hemorrhages. The liver and lymph nodes were congested. The lungs were slightly edematous with some highly congested areas.

In pig 27 the adrenals, anterior and posterior pituitary, small intestine, kidneys, liver, lungs, lymph nodes, and thymus were congested. The lungs were also mildly edematous. The stomach had localized hemorrhages in the submucosa.

In pig 29 the adrenals, anterior and posterior pituitary, small intestine, kidneys, liver, lungs, and lymph nodes all were congested. The lungs had a mild interlobular edema.

The kidneys of pig 31 were congested, especially in the medulla. The liver, lungs, and lymph nodes were congested. The lungs also were mildly edematous.

No tissues were taken from pig 34 due to its premature death during the drawing of a blood sample.

Of the following iron-deficient pigs, the sectioned adrenal of pig 6 was congested in the zona reticularis and medulla. The small intestines were congested around the glands of Lieberkuhn and in the submucosa. Edema appeared in the submucosa of the stomach and small intestine. The kidneys, liver, lungs, and lymph nodes were all congested.

The sectioned adrenal of pig 10 was congested in the cortex. The small intestines were congested around the glands of Lieberkuhn. The kidneys, liver, lungs, and lymph nodes were congested.

In pig 16 the small intestine and stomach were edematous in the submucosa. The intestinal glands of Lieberkuhn were surrounded by congestion. The kidneys, liver, lungs, and lymph nodes were highly congested.

In pig 17 the adrenal, anterior pituitary, small intestine, kidneys, liver, lungs, and lymph nodes were congested. The edema around the gall bladder was quite evident microscopically.

In pig 18 the adrenal, small intestine, kidneys (mostly in the medulla), liver, lungs, and lymph nodes were congested. The zona arcuata

of the adrenal gland appeared disrupted. The separated cells may have resulted from autolysis or the histological technique. Slight and localized interlobular edema of the lungs was present.

In pig 26 the small intestine, kidneys, liver, lungs, lymph nodes, and thymus were congested. Again the adrenal zona arcuata appeared disrupted.

In pig 28 except for interlobular edema of the lungs, all organs sectioned appeared normal. Again the adrenal zona arcuata appeared disrupted.

In pig 33 the medulla of the kidney was mildly congested. The lymph nodes were congested. The lungs were mildly edematous.

The brain of pig 36 was mildly congested. The kidney and lungs were highly congested.

Control pigs 35 and 32 revealed no significant microscopic changes. Pig 32, an iron-deficient pig, had hemopoietic areas in the liver.

All the livers of iron-deficient pigs contained numerous hemopoietic foci as opposed to the iron-injected pigs.

Edema of the lungs was interlobular, not fluid filled alveoli. Edema, in general, was not as apparent microscopically as might be expected from the gross description.

The histopathology could be summarized as passive congestion of the viscera. This is in general agreement with the work of Weil and Spink (1958). The passive congestion connotes a hemodynamic mechanism in endotoxic shock. This is in agreement with the work of Hinshaw and Nelson (1962). Certain porcine lesions described by Pan et al., (1962)

were not observed, namely, fibrinoid necrosis, edematous swelling and fibrin thrombi in arterioles and arteries. These lesions were usually found in the kidney, liver, heart, lymph nodes, stomach, and intestines. The lack of fibrinoid lesions and an extensive eosinophilia may be due to the young age of the pigs. Aggregations of eosinophils were found in most all lymph nodes, so a mild eosinophilia did exist. At 3 weeks of age the antigen-antibody aspects of endotoxic shock are probably at a minimum.

Sections of the adrenals revealed no hyper- or hypoplasia of the adrenal cortex. No difference was noted upon gross examination which would be the first indication of adrenal malfunction (Selye, 1956). With no indications of malfunction grossly, weights of the adrenals might well have been ambiguous, had they been weighed, considering the variable degrees of congestion.

In reviewing the histopathology described in this investigation, it is well to remember that the lesions were usually localized or, as in the case of congestion, varied from area to area in degree.

V. SUMMARY AND CONCLUSIONS

One-half of the pigs from each of 4 litters of swine were injected with iron. Each week, for 3 weeks after birth, blood samples were drawn.

Packed cell volume, hemoglobin content, sedimentation rate, total erythrocyte and leukocyte cell counts, and differential leukocyte counts were determined. In addition serum was stored for determination of sodium and potassium with a flame photometer. After the third week blood sample was drawn, Escherichia coli endotoxin was injected intravenously into the pigs with the exception of one iron-injected and one iron-deficient control from each litter. After the endotoxin injection, blood samples were drawn on a schedule for 5 days and the above hematologic parameters determined.

Hematologic responses generally resembled the hemograms previously reported by other investigators of iron deficiency and endotoxic shock in other mammals and swine. One exception was the lower total erythrocyte counts in the pigs throughout the experiment. This, along with some high packed cell volumes, and hemoglobin values, resulted generally in large mean corpuscular volumes and large mean corpuscular hemoglobin values. Another exception was a hypokalemia in the iron-deficient pigs throughout most of the experiment.

During endotoxic shock a decrease in serum potassium occurred in both iron-injected and iron-deficient pigs during the first hour of endotoxic shock. Also a decrease in serum sodium occurred at 15 minutes

after endotoxin injection in both iron-injected and iron-deficient pigs.

Although a greater mortality occurred in the iron-deficient pigs, all pigs given a lethal dose of endotoxin exhibited a passive congestion of the viscera upon histopathologic examination. It would seem that the effect of iron-deficiency anemia on endotoxic shock is one of over-strain on the cardiovascular system due to venous pooling of blood. The cardiovascular system is strained due to the anemia and is further strained by endotoxic shock. The serum electrolyte changes did not indicate that they contributed to the edema. The necropsies and the histopathology indicated that endotoxic shock is similar to edema disease as described by other workers. Therefore, the pathogenesis of endotoxic shock probably applies to edema disease.

VI. REFERENCES

Andersen, J. R.
1965 The response of young pigs to Escherichia Coli endotoxin. M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Anderson, F. L., Kuida, H., and Hecht, H. H.
1963 Influence of promethazine on circulatory response to endotoxin in dogs. Am. J. Physiol. 204: 983-986.

Atwood, R. P. and Kass, E. H.
1964 Relationship of body temperature to lethal action of bacterial endotoxin. J. Clin. Invest. 43: 151-159.

Austvoll, J.
1958 Gut edema in pigs. Vet. Med. 53: 349-350.

Bennett, P. C.
1964 Edema disease. In Dunne, Howard W., ed. Diseases of swine. 2nd ed. pp. 612-624. Ames, Iowa, The Iowa State University Press.

Bernstein, R. E.
1954 Potassium and sodium balance in mammalian red cells. Science 120: 459-460.

Binnion, P. F., Davis, J. O., Brown, T. C., and Olichney, M. J.
1965 Mechanisms regulating aldosterone secretion during sodium depletion. Am. J. Physiol. 208: 655-661.

Bland, J. H.
1963 Clinical metabolism of body water and electrolytes. Philadelphia, Pennsylvania, W. B. Saunders Company.

Bomer, H.
1961 Topochemische Kalziumbestimmungen im Herzmuskel von gesunden and an Odemkrankheit erkrankten Schweinen. Mh. Tierheilk 13: 222-225.

Bomer, H.
1963 Observations on the etiology and experiences with the therapy of edema disease. Prakt. Tierarzt 44: 318-320 + 323-326.

Braude, A. I.
1964 Bacterial endotoxins. Scientific American 210: 36-45.

Brooksbank, N. H.
1954 Anaemia in piglets associated with a copper deficiency. Vet. Rec. 66: 322-324.

Burrows, W.
1951 Endotoxins. Annual Review of Microbiology 5: 181-196.

Bush, J. A., Jensen, W. N., Cartwright, G. E., and Wintrobe, M. M.
1955 Blood volume studies in normal and anemic swine. Am. J. Physiol. 181: 9-14.

Buxton, A. and Thomlinson, J. R.
1961 The detection of tissue-sensitizing antibodies to Escherichia coli in oedema disease, haemorrhagic gastro-enteritis and in normal pigs. Res. Vet. Sci. 2: 73-88.

Calhoun, M. L. and Smith, E. M.
1964 Hematology and hematopoietic organs. In Dunne, Howard W., ed. Diseases of swine. 2nd ed. pp. 37-61. Ames, Iowa, The Iowa State University Press.

Carroll, E. J., Schalm, O. W., and Wheat, J. D.
1965 Endotoxemia in a horse. J. Am. Vet. Med. Assoc. 146: 1300-1303.

Cartwright, G. E., Tatting, B., and Wintrobe, M. M.
1948 Niacin deficiency anemia in swine. Arch. Biochem. 19: 109-118.

Cartwright, G. E., Palmer, J. G., Tatting, B., Ashenbrucker, H. and Wintrobe, M. M.
1950 Experimental production of nutritional macrocytic anemia in swine. III. Further studies on pteroylglutamic acid deficiency. J. Lab. Clin. Med. 36: 675-693.

Cartwright, G. E., Tatting, B., Robinson, J., Fellows, N. M., Gunn, F. D. and Wintrobe, M. M.
1951 Hematologic manifestations of vitamin B_{12} deficiency in swine. Blood 6: 867-891.

Chamovitz, R., Valdes-Dapena, A., and Vilardell, F.
1962 Shwartzman-like phenomenon of the rabbit colon induced by a single injection of endotoxin. J. Proc. Soc. Exptl. Biol. Med. 109: 527-529.

Chien, S., Sinclair, D. G., Dellenback, R. J., Chang, C., Peric, B., Usami, S., and Gregersen, M. I.
1964 Effect of endotoxin on capillary permeability to macromolecules. Am. J. Physiol. 207: 518-522.

Chopra, S. L. and Blackwood, A. C.
1964 Toxic action of haemolytic Escherichia coli isolated from pigs. Nature (London) 203: 667.

Chopra, S. L., Blackwood, A. C., and Dale, D. G.
1964 Emteritis of early weaned pigs. II. Pathogenesis Can. J. Comp. Med. Vet. Sci. 28: 272-278.

Clawson, A. J., Smith, F. H., Osborne, J. C., and Barrich, E. R.
1961 Effect of protein source, autoclaving, and lysine supplementation on gossypol toxicity. J. An. Sci. 20: 547-552.

Coulson, W. F. and Carnes, W. H.
1963 Cardiovascular studies on copper-deficient swine. Am. J. Pathol. 43: 945-954.

Cummings, J. N. and Kaiser, I. H.
1959 The blood gases, pH, and plasma electrolytes of the sow and fetal pig at 106 days of pregnancy. Am. J. Obst. Gynecol. 77: 10-17.

Davis, J. O., Urquhart, J., and Higgins, J. T., Jr.
1963a The effects of alterations of plasma sodium and potassium concentration on aldosterone secretion. J. Clin. Invest. 42: 597-609.

Davis, J. W., Allen, R. C., and Smibert, R. M.
1961 Studies on hemolytic Escherichia coli associated with edema disease of swine. 1. Separation and properties of a toxin of hemolytic Escherichia coli. Am. J. Vet. Res. 22: 736-740.

Davis, J. W. and Smibert, R. M.
1963 Studies on hemolytic Escherichia coli associated with edema disease of swine. III. The effect of hemolytic and non-hemolytic Escherichia coli endotoxin on the blood of swine. Am. J. Vet. Res. 24: 324-328.

Davis, R. B., Bailey, W. L., and Hanson, N. P.
1963b Modification of serotonin and histamine release after Escherichia coli endntoxin administration. Am. J. Physiol. 205: 560-566.

Doyle, L. P., Mathews, F. P., and Whiting, R. A.
1927 Anemic in young pigs. J. Am. Vet. Med. Assoc. 72: 491-510.

Erskine, R. G., Sojka, W. J., and Lloyd, M. K.
1957 The experimental reproduction of a syndrone indistinguishable from edema disease. Vet. Rec. 69: 301-303.

Fekety, F. R., Jr.
1963 Heat balance and reactivity to endotoxin. Am. J. Physiol. 204: 719-722.

French, C. T. and Bussell, B. W.
1963 The effects of iron supplementation in piglets on haemoglobin levels and growth rates. Aust. Vet. J. 39: 41-45.

Fukuda, T.
1963a On the mechanism of endotoxin intoxication in rabbits. Jap. J. Physiol. 13: 155-168.

Fukuda, T.
1963b Seasonal variations in endotoxin susceptibility of rabbits. Jap. J. Physiol. 13: 240-245.

Gann, D. S., Cruz, J. F., Casper, A. G. T., and Bartter, F. C.
1962 Mechanism by which potassium increases aldosterone secretion in the dog. Amer. J. Physiol. 202: 991-996.

Gilbert, R. P.
1960 Mechanisms of the hemodynamic effects of endotoxin. Physiol. Rev. 40: 245-279.

Gilbert, P.
1962 Endotoxin shock in the primate. Proc. Soc. Exptl. Biol. Med. 111: 328-331.

Gillenwater, J. Y., Dooley, E. S., and Frohlich, E. D.
1963 Effects of endotoxin on renal function and hemodynamics. Am. j. Physiol. 205: 293-297.

Gourzis, J. T., Hollenberg, M. W., and Nickerson, M.
1961 Involvement of adrenergic factors in the effects of bacterial endotoxin. J. Exptl. Med. 114: 593-604.

Gregory, D. W.
1957 Hemolytic Escherichia coli in edema disease of pigs. N. Amer. Vet. 38: 40.

Gregory, D. W.
1959 Edema disease in swine. J. Am. Vet. Med. Assoc. 135: 321-323.

Gregory, D. W.
1960 Experimental edema disease (hemolytic Escherichia coli toxemia) in pigs and in mice. Am. J. Vet. Res. 21: 88-94.

Gregory, D. W.
1962 Hemolytic Escherichia coli enteritis of weanling pigs. J. Am. Vet. Med. Assoc. 141: 947-949.

Gregory, D. W.
1964 The pathogenesis of pastroenteritis associated with Escherichia coli. Vet. Rec. 76: 485.

Greisman, S. E.
1960 Activiation of histamine-releasing factor in normal rat plasma by Escherichia coli endotoxin. Proc. Soc. Exptl. Biol. Med. 103: 628-632.

Gubler, C. J., Lahey, M. E., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M.
1952 Studies on copper metabolism III. The metabolism of iron in copper deficient swine. Blood 7: 1075-1092.

Haddy, F. J., Molmar, J. I., and Campbell, R. W.
1961 Effects of denervation and vasoactive agents on vascular pressures and weight of the dog forelimb. Am. J. Physiol. 201: 631-638.

Hayasaka, H., O'Malley, J. F., and Howard, J. M.
1963 Antitoxic effect of aldosterone on cats in endotoxic shock. Archives of Surgery 87: 861-865.

Hendricks, S. B.
1964 Salt transport across cell membranes. Am. Scientist. 52: 306-333.

Herion, J. C., Walker, R. I., Herring, W. B. and Palmer, J. G.
1965 Effects of endotoxin and nitrogen mustard on leukocyte kinetics. Blood 25: 522-540.

Hinshaw, L. B., Jorden, M. M., and Vick, J. A.
1961a Mechanism of histamine release in endotoxin shock. Am. J. Physiol. 200: 987-989.

Hinshaw, L. B., Spink, W. W., Vick, J. A., Mallet, E., and Finstad, J.
1961b Effect of endotoxin on kidney function and renal hemodynamics in the dog. Am. J. Physiol. 201: 144-148.

Hinshaw, L. B., Vick, J. A., Wittmers, L. E., Worthen, D. M., Nelson, D. L., and Swenson, O. P.
1961c Changes in total peripheral resistance in endotoxin shock. Proc. Soc. Exptl. Biol. Med. 108: 24-27.

Hinshaw, L. B. and Nelson, D. L.
1962 Venous response of intestine to endotoxin. Am. J. Physiol. 203: 870-872.

Hinshaw, L. B., Emerson, T. E., Jr., Iampietro, P. F., and Brake, C. M.
1962a A comparative study of the hemodynamic actions of histamine and endotoxin. Am. J. Physiol. 203: 600-606.

Hinshaw, L. B., Vick, J. A., Jordon, M. M., and Wittmer, L. E.
1962b Vascular changes associated with development of irreversible endotoxin shock. Am. J. Physiol. 202: 103-110.

Hinshaw, L. B., Brake, C. M., Emerson, T. E., Jr., Jordan, M. M., and Musucci, F. D.
1964 Participation of the sympathoadrenal system in endotoxin shock. Am. J. Physiol. 207: 925-930.

Jensen, S. B., Mergenhagen, S. E., Fitzgerald, R. J., and Jordon, H. V.
1963 Susceptibility of conventional and germ free mice to lethal effects of endotoxin. Proc. Soc. Exptl. Biol. Med. 113: 710-714.

Jones, J. E. T. and Smith, H. W.
1964 The effect of intravenous injection of extracts of Escherichia coli in pigs and mice. J. Path. Bact. 87: 113-122.

Kalant, N., Das Gupta, D., Despointes, R., and Giroud, C. J. P.
1962 Mechanisms of edema in experimental nephrosis. Am. J. Physiol. 202: 91-96.

Kampschmidt, R. F. and Arredondo, M. I.
1963 Some effects of endotoxin upon plasma iron turnover in the rat. Proc. Soc. Exptl. Biol. Med. 113: 142-145.

Kampschmidt, R. F. and Upchurch, H. F.
1964 Effect of endotoxin upon total iron-binding capacity of the serum. Proc. Soc. Exptl. Biol. Med. 116: 420-422.

Kampschmidt, R. F., Upchurch, H. F., and Johnson, H. L.
1965 Iron transport after injection of endotoxin in rats. Am. J. Physiol. 208: 68-72.

Keiss, R. E., Will, D. H., and Collier, J. R.
1964 Skin toxicity and hemodynamic properties of endotoxin derived from Pasteurella hemolytica. Am. J. Vet. Res. 25: 935-942.

Kernkamp, H. C. H., Sorensen, D. K., Hanson, L. J., and Nielson, N. O.
1965 Epizootiology of edema disease in swine. J. Am. Vet. Med. Assoc. 146: 353-357.

Koehler, J., Albrecht, T., Theofilos, J., Kuida, H., and Hecht, H. H.
1963 Inhibition of endotoxin-induced pulmonary vasoconstriction in dogs by alpha-methyl dopa. Am. J. Physiol. 204: 987-990.

Kuida, H., Gilbert, R. P., Hinshaw, L. B., Brunson, J. G., and Visscher, M. B.
1961 Species differences in effect of Gram-negative endotoxin on circulation. Am. J. Physiol. 200: 1197-1202.

Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M.
1952 Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7: 1053-1074.

Link, R. P.
1953 A study of the effect of repeated intraperitoneal injections of glucose in pigs. Am. J. Vet. Res. 14: 150-159.

Luke, D.
1953 The effect of adrenocorticotrophic hormone and adrenal cortical extract on the differential white count in the pig. Brit. Vet. J. 109: 434-436.

Matthias, D.
1963 Das Verhalten der Nebennieren bei der Odemkrankheit der Schweime und die Beziehumgen dieser Krankheit zum sog. plotzlichen Herztod. Monatshefte fur Veterinarmedizin 18: 856-860.

Mayerson, H. S., Wolfrom, C. G., Shirley, H. H., Jr., and Wasserman, K.
1960 Regional differences in capillary permeability. Am. J. Physiol. 198: 155-160.

McDonald, F. F., Dunlop, D., and Bates, C. M.
1955 An effective treatment for anemia of piglets. Brit. Vet. J. 111: 403-407.

Meier, H.
1963 Fluids and electrolytes. In Cornelius, C. E. and Kaneko, Jiro J., ed. Clinical biochemistry of domestic animals. pp. 500-553. New York, Academic Press.

Melby, J. C., Egdahl, R. H., and Spink, W. W.
1960 Secretion and metabolism of cortisol after injection of endotoxin. J. Lab. Clin. Med. 56: 50-62.

Miller, E. R., Ullrey, D. E., Ackermann, I., Schmidt, D. A., Luecke, R. W., and Hoefer, J. A.
1961 Swine hematology from birth to maturity. II. Erythrocyte population, size and hemoglobin concentration. J. An. Sci. 20: 890-897.

Muller, W. and Smith, L. L.
1963 Hepatic circulatory changes following endotoxin shock in the dog. Am. J. Physiol. 204: 641-644.

National Academy of Sciences. National Research Council
1959 Nutrient requirements of swine. National Research Council Publication 648.

Ohshima, K. I. and Sadao, M.
1961 Pathological study on an occurrence of swine edema disease in Iwate prefecture in 1959. Jap. J. Vet. Sci. 23: 111-123.

Pan, In-Chang, Wang, Chun-Tsuen, Yeh, Yau-Chin, Pan, In-Jen, and Chen, Hsien-Chuan.
1962 Experimental reproduction of edema disease of swine. Bull. Inst. Zool. Academic Sinica. 1: 73-87.

Perk, K., Frei, Y. F., and Herz, A.
1964 RBC osmotic fragility of young and mature domestic and laboratory animals. Am. J. Vet. Res. 25: 1241-1248.

Plager, J. E., Bray, G. A., Jackson, J. E.
1963 Pituitary-ACTH response to metopirone and endotoxin administration in the dog. Endocrinology 72: 876-883.

Puller, E. M.
1959 Mineral deficiencies in pigs. I. Natural cases in Victoria. Australian Vet. J. 35: 203-209.

Ravin, H. A., Rowley, D., Jenkins, C., and Fine, J.
1960 On the absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. J. Exptl. Med. 112: 783-792.

Reeder, D. G.
1964 Iron deficiency anemia of swine. The Illinois Veterinarian 7: 12-15.

Rosenberg, J. C., Lillehei, R. C., Moran, W. H., and Zimmerman, B.
1959 Effect of endotoxin on plasma catecholaminize and serum serotonin. Proc. Soc. Exptl. Biol. Med. 102: 335-337.

Rubenstein, H. S., Fine, J., and Coons, A. H.
1962 Localization of endotoxin in the walls of the peripheral vascular system during lethal endotoxemia. Proc. Soc. Exptl. Biol. Med. 111: 458-467.

Schalm, O. W.
1961 Veterinary hematology. Philadelphia, Pennsylvania, Lea and Febiger.

Seamer, J.
1956 Piglet anaemia. Veterinary Review and Annotations 2: 79-93.

Selye, H.
1956 The stress of life. New York, McGraw-Hill Book Company, Inc.

Shields, G. S., Coulson, W. F., Kimball, D. A., Cannes, W. H., Cartwright, G. E., and Wintrobe, M. M.
1962 Studies on copper metabolism. Cardiovascular lesions in copper-deficient swine. Am. J. Pathol. 41: 603-621.

Smibert, R. M., Davis, J. W., and Allen, R. C.
1962 Studies on hemolytic Escherichia coli associated with edema disease of swine. II. An immunologic study of a toxic factor. Am. J. Vet. Res. 23: 460-464.

Spink, W. W. and Vick, J.
1961 A labile serum factor in experimental endotoxin shock: cross-transfusion studies in dogs. J. Exptl. Med. 114: 501-508.

Spink, W. W. and Vick, J.
1962 Canine endotoxin shock: reversal with aldosterone and angiotensin. II. (hypertensin). Proc. Soc. Exptl. Biol. Med. 109: 521-522.

Spink, W. W.
1962 Endotoxin shock. Ann. Internal Med. 57: 538-552.

Swenson, M. J., Goetsch, D. D., and Underbjerg, G. K. L.
1955 The effect of the sow's ration on the hematology of the newborn pig. Am. Vet. Med. Assoc. Proc. 92: 159-162.

Swenson, M. J.
1964 Composition of body fluids. In Dunne, Howard E., ed. Diseases of swine. 2nd ed. pp. 81-106. Ames, Iowa, The Iowa State University Press.

Talbot, R. B. and Swenson, M. J.
1963 Normochromic, microcytic anemia of baby pigs. Am. J. Vet. Res. 24: 39-41.

Tauber, H. and Russell, H.
1961 General methods for isolation of endotoxins. Exptl. Med. and Surg. 19: 161-170.

Thomas, L.
1954 The physiological disturbances produced by endotoxins. Annual Review of Physiology 16: 467-490.

Thomlimson, J. R. and Buxton, A. A.
1962 A comparison of experimental anaphylactic shock in guinea pigs with naturally occurring edema disease and haemorrhagic gastro-enteritis in pigs. Res. Vet. Sci. 3: 186-202.

Thomlimson, J. R. and Buxton, A.
1963 Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastro-enteritis associated with Escherichia coli. Immunology 6: 126-139.

Timoney, J. F.
1957 Oedema disease in swine. Vet. Rec. 69: 1160-1171.

Timoney, J. F.
1960 An alternative method to repeated freezing and thawing for the preparation of an extract from a strain of haemolytic Escherichia coli which on intravenous injection in pigs mimics the syndrome of oedema disease. Vet. Rec. 72: 1252.

Tsagaris, T. J., Koehler, J. Albrecht, J., Kuida, H., and Hecht, H. H.
1963 Drup inhibition of circulatory response to endotoxin in dogs. Am. J. Physiol. 204: 991-996.

Ullrey, D. E., Miller, E. R., West, D. R., Schmidt, D. A., Seerley, R. W., Hoefer, J. A., and Lvecke, R. W.
1959 Oral and parenteral administration of iron in the prevention and treatment of baby pig anemia. J. Animal Sci. 18: 256-263.

Underdahl, N. R., Blore, I. C., and Young, G. A.
1959 Edema disease of swine. A preliminary report on experimental transmission. J. Am. Vet. Med. Assoc. 135: 615-617.

Underdahl, N. R., Stair, E. L., and Young, G. A.
1963 Transmission and characterization of edema disease of swine. J. Am. Vet. Med. Assoc. 142: 27-30.

Vesselinovitch, S. D.
1955 Electrophoretic studies of oedema disease in swine. Brit. Vet. J. 111: 398-403.

Vick, J.
1960 Studies on the trigger mechanism involved in the vascular alterations induced by endotoxin. J. Lab. Clin. Med. 56: 953-954.

Waton, N. G.
1964 Uptake and formation of histamine by cat tissues. J. Physiol. 172: 475-481.

Weil, M. H. and Spink, W. W.
1957 A comparison of shock due to endotoxin with anaphylactic shock. The J. Lab. Clin. Med. 50: 501-515.

Weil, M. H. and Spink, W. W.
1958 The shock syndrome associated with bacteremia due to Gram-negative bacilli. Archives of Internal Medicine 101: 184-193.

Weil, M. H.
1961 Adrenocortical steroid for therapy of acute hypotension. American Practitioner and Digest of Treatment 12: 162-168.

Whitehair, C. K.
1964 Nutritional deficiencies. In Dunne, Howard W., ed. Disease of Swine. 2nd ed. pp. 797-821. Ames, Iowa, The Iowa State University Press.

Widdowson, E. M. and McCance, R. A.
1956 The effect of development on the composition of the serum and extracellular fluids. Clinical Science 15: 361-365.

Zimmerman, D. R., Speer, V. C., Hays, V. W., and Catron, D. V.
1959 Injectable iron-dextran and several oral iron treatments for the prevention of iron-deficiency anemia of baby pigs. J. Animal Sci. 18: 1409-1415.

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VIII. APPENDIX A. TABLES

Table 13. Hematological studies of pigs in litter 1 at 1 week

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR[a] No/100 WBC
11[Fe]	38.5	12.3	4.96	9.2		2	3	56	33	6	9
4[Fe]	35.5	11.0	5.13	6.3		2	1	32	64	1	16
5[Fe]	36.0	10.3	3.75	10.2		2	1	58	28	11	6
1[Fe]	37.0	11.3	4.08	10.7				63	27	10	15
12	38.0	12.0	4.48	11.0		1	1	47	51		0
6	34.0	9.5	4.49	19.0			2	60	28	10	29
10	27.5	9.0	3.99	8.3	1	1	2	58	23	15	14
3	39.0	12.3	4.74	14.2			2	77	13	8	6
13	30.5	8.7	3.20	8.5	2		1	46	39	12	21

[a] nucleated red blood cells

[Fe] iron-injected

Table 13 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	77.6	24.8	31.9	5.7	5.8	144	145
4	69.2	21.4	31.0	*4.4	4.6	141	142
5	96.0	27.5	28.6	*5.4	5.6	137	140
1	90.7	27.7	30.5	*5.0	5.0	144	144
12	84.8	26.8	31.6	4.8	5.0	141	146
6	75.7	21.2	27.9	*5.4	5.0	144	140
10	68.9	26.6	30.1	*5.4	5.3	145	141
3	82.3	25.9	31.7	*5.5	5.5	144	144
13	95.3	27.2	28.5	4.8	4.7	140	137

* some hemolysis of the blood sample
This notation will be used in the following tables.

Table 14. Hematological studies of pigs in litter 2 at 1 week

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	40.0	11.3	3.91	10.7	3	1	2	51	38	5	8
21^{Fe}	39.0	10.8	2.85	8.7		1	1	14	78	6	3
22^{Fe}	39.0	10.8	3.85	12.3		1		53	41	5	2
23^{Fe}	41.0	11.5	3.34	6.8		1		22	73	4	1
15	25.0	6.5	2.89	7.6				34	64	2	15
16	29.0	8.5	2.84	7.5		2		40	55	3	3
17	32.0	10.0	3.73	15.5		1		50	47	2	1
18	27.0	8.0	2.96	7.9				27	72	1	16

Table 14 (Continued)

Pig. No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	102.3	28.9	28.3	5.8	5.9	144	144
21	136.8	37.9	27.7	6.3	6.3	143	143
22	101.3	28.1	27.7	5.0	5.0	134	135
23	122.8	34.4	28.0	5.9	6.0	139	143
15	86.5	22.5	26.0	6.2	6.2	137	137
16	102.1	29.9	29.3	4.4	5.0	135	141
17	85.8	26.8	31.3	5.7	5.6	146	143
18	91.2	27.0	29.6	5.0	5.0	137	137

Table 15. Hematological studies of pigs in litter 3 at 1 week

Pig. No.	PCV %	Hb mg%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
30^{Fe}	26.5	6.9	2.73	4.2			6	35	47	12	270
29^{Fe}	33.0	9.0	3.61	6.9		1	3	51	40	5	68
27^{Fe}	32.0	9.7	3.20	11.0	1	2	3	43	45	6	17
25	23.0	6.4	2.78	7.0			37	8	54	1	33
26	27.0	7.8	2.84	8.5				77	17	6	37
28	24.0	6.7	2.46	5.8				46	48	6	38

Table 15 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
30	97.1	25.3	26.0	*12.2	12.0	133	139
29	91.4	24.9	27.3	*13.6	13.4	137	140
27	100.0	30.3	30.3	*12.4	12.2	141	143
25	82.7	23.0	27.8	7.5	7.6	143	147
26	95.1	27.5	28.9	9.5	9.8	147	151
28	97.6	27.2	27.9	* 8.8	9.0	140	140

Table 16. Hematological studies of pigs in litter 4 at 1 week

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	33.0	9.3	3.12	4.3			42	22	30	6	18
34^{Fe}	27.5	8.4	2.24	3.7	1		7	71	18	3	52
31^{Fe}	34.0	9.4	3.20	4.5		2	37	24	33	4	18
32	24.0	7.4	2.52	1.7			14	8	64	14	170
33	22.0	6.7	2.74	2.2			72	4	20	4	88
36	30.0	9.0	2.69	3.5		2	42	4	44	8	80

Table 16 (Continued)

Pig. No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	105.8	29.8	28.2	*6.1	6.1	141	141
34	122.8	37.5	36.2	*6.3	6.2	148	146
31	106.3	29.4	27.6	*6.8	6.7	140	140
32	95.2	29.4	30.8	5.6	5.9	140	145
33	80.3	24.5	30.5	4.8	5.0	140	146
36	111.5	33.5	30.0	4.8	5.0	144	149

Table 17. Hematological studies of pigs in litter 1 at 2 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	43.0	13.0	5.19	7.3		4		21	65	10	5
4^{Fe}	44.0	13.5	5.32	7.9				22	76	2	8
5^{Fe}	43.0	11.5	4.01	9.2	2	2		30	63	3	1
1^{Fe}	41.0	12.2	4.40	9.6	1	1		29	65	4	5
12	28.0	8.0	4.19	5.8		3		23	70	4	13
6	34.0	8.7	4.38	12.0				51	48	1	7
10	23.0	5.6	2.95	6.0		4		23	69	4	29

Table 17 (Continued)

Pig. No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	82.9	25.0	30.2	7.0	6.8	142	137
4	82.7	25.4	30.7	6.2	6.1	135	133
5	107.2	28.7	26.7	6.1	6.1	145	146
1	93.2	27.7	29.8	*6.8	7.1	141	146
12	66.8	19.1	28.6	7.0	6.8	146	143
6	77.6	19.9	25.6	*9.3	9.7	140	135
10	78.0	19.0	24.3	5.9	6.3	142	141

Table 18. Hematological studies of pigs in litter 2 at 2 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	45.0	13.0	4.55	12.3	2	2		13	83		2
21^{Fe}	37.0	10.3	3.55	21.0		2		59	35	4	
22^{Fe}	43.0	11.8	4.11	13.5				28	68	4	
23^{Fe}	44.0	13.0	4.55	9.7		1		35	64		3
15	25.0	6.2	3.58	9.9				18	76	6	14
16	25.0	6.7	3.14	9.0			2	12	85	2	10
17	29.0	8.8	4.76	12.0		2	2	30	66		2
18	24.0	7.0	4.08	7.1	2		8	8	82		58

Table 18 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	98.9	28.6	28.9	6.4	6.4	140	144
21	104.2	29.0	27.8	*5.7	5.8	140	145
22	104.6	28.7	27.4	*6.4	6.4	135	135
23	96.7	28.6	29.5	5.2	5.3	141	144
15	69.8	17.3	24.8	5.9	5.9	139	139
16	79.6	21.3	26.8	*6.1	6.1	139	140
17	60.9	18.5	30.3	5.9	5.8	143	143
18	58.8	17.2	29.2	5.3	5.1	143	140

Table 19. Hematological studies of pigs in litter 3 at 2 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
30^{Fe}	35.0	10.8	3.08	5.0	1	1	2	58	29	9	35
29^{Fe}	34.5	10.8	3.27	9.0		3	2	42	35	18	6
27^{Fe}	38.0	12.3	3.64	9.5	1	1	2	50	40	6	6
25	18.0	5.0	2.07	9.7			3	76	18	3	23
26	20.0	5.9	1.89	6.8			3	46	39	12	38
28	16.5	4.6	2.11	5.2	1		3	22	61	13	39

Table 19 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
30	113.6	35.1	30.9	6.0	5.9	146	144
29	105.5	33.0	31.3	*7.0	7.0	150	152
27	104.4	33.8	32.4	*5.5	5.4	142	143
25	87.0	24.2	27.8	6.6	6.4	145	146
26	105.8	31.2	33.9	7.0	7.3	138	142
28	78.2	21.8	27.9	5.0	5.0	135	133

Table 20. Hematological studies of pigs in litter 4 at 2 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	36.5	10.3	3.32	13.3	1		3	61	32	3	6
34^{Fe}	32.0	9.4	3.04	9.8	1		1	71	26	1	4
31^{Fe}	31.0	9.2	3.27	9.2		1	1	84	14	1	2
32	21.0	5.4	2.51	5.1				82	17	1	38
33	19.0	5.4	2.00	7.5				84	15	1	14
36	26.0	7.3	2.63	10.4				83	17		11

Table 20 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	109.9	31.0	38.2	*7.0	7.0	139	140
34	105.3	30.9	29.4	*5.7	5.9	145	146
31	94.8	28.1	29.7	*5.6	5.7	135	136
32	83.7	21.5	25.7	5.7	5.4	144	138
33	95.0	27.0	28.4	5.4	5.7	140	141
36	98.9	27.8	28.1	*6.0	5.9	140	141

Table 21. Hematological studies of pigs in litter 1 at 3 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	41.0	12.5	5.57	5.9		2		17	79	2	
4^{Fe}	43.0	12.3	4.83	7.8		1		28	69	2	
5^{Fe}	40.0	11.5	4.44	8.0		3		36	61		1
1^{Fe}	38.5	10.8	4.43	6.6				28	68	4	1
12	25.0	6.3	3.58	6.5				20	77	3	15
6	27.0	7.3	3.83	8.7			1	54	36	9	13
10	25.0	5.5	3.95	4.7		3		30	64	3	65

Table 21 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	73.6	22.4	30.5	8.6	8.2	127	125
4	89.0	25.5	28.6	9.6	9.8	146	148
5	90.1	25.9	28.8	7.8	7.6	120	120
1	86.9	24.4	28.1	8.7	8.2	136	134
12	69.8	17.6	25.2	7.6	6.9	136	133
6	70.5	19.1	27.0	7.6	7.6	138	138
10	63.3	13.9	22.0	7.3	7.3	136	136

Table 22. Hematological studies of pigs in litter 2 at 3 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	45.0	11.5	3.45	12.6		1	1	61	35	2	
21^{Fe}	35.5	10.5	3.77	7.2		2		20	76	2	
22^{Fe}	39.0	12.8	4.47	8.3				33	65	2	
23^{Fe}	40.0	14.5	3.97	7.2	1	2		43	49	5	
15	26.0	6.3	2.55	6.5			3	21	74	2	10
16	28.0	6.8	3.09	6.0		1		20	78	1	29
17	36.0	9.3	3.80	9.6		3		21	74	2	
18	27.0	5.5	2.33	4.6		1		19	80		61

Table 22 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	130.4	33.3	25.6	*8.6	8.7	143	145
21	94.2	27.9	29.6	8.1	8.4	149	154
22	87.2	28.6	32.8	*7.9	8.5	141	142
23	100.8	36.5	36.3	*8.6	8.8	140	136
15	102.0	24.7	24.2	*8.1	7.6	155	149
16	90.6	22.0	24.3	7.1	6.9	149	146
17	94.7	24.5	25.8	*8.0	*8.0	140	142
18	115.9	23.6	20.4	6.8	6.6	148	145

Table 23. Hematological studies of pigs in litter 3 at 3 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
30^{Fe}	32.0	8.8	3.10	3.6		3	1	50	40	6	6
29^{Fe}	32.0	9.7	3.16	7.2				53	47		1
27^{Fe}	38.0	11.3	3.93	9.9				52	42	6	
25	16.0	3.9	2.00	6.3			4	25	69	2	18
26	17.0	4.5	2.28	5.1		2	7	56	32	3	13
28	16.0	4.1	1.87	6.4		3	2	25	65	5	16

Table 23 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
30	103.2	28.4	27.5	*8.9	8.8	156	156
29	101.3	30.7	30.3	*8.1	8.4	140	144
27	96.7	28.8	29.7	*8.9	8.5	138	136
25	80.0	19.5	24.4	*8.4	8.0	148	146
26	74.6	19.7	26.5	*7.5	7.6	140	143
28	85.6	21.9	25.6	7.1	6.7	147	142

Table 24. Hematological studies of pigs in litter 4 at 3 weeks

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	39.0	10.8	3.85	12.1	1		1	66	31	2	3
34^{Fe}	35.5	10.3	3.53	8.5		1	2	66	29	2	5
31^{Fe}	32.0	8.8	3.28	10.7			8	70	22		
32	17.0	3.8	2.10	5.9			9	42	47	2	30
33	18.0	4.5	2.14	3.5			3	71	25	1	25
36	20.5	5.6	2.32	8.3			1	69	29	1	19

Table 24 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	101.3	28.1	27.7	*9.9	9.9	144	146
34	100.6	29.2	29.0	*8.1	8.2	143	145
31	97.6	26.8	27.5	*8.6	8.4	145	144
32	81.0	18.1	22.4	*7.8	8.0	145	150
33	84.1	21.0	25.0	7.8	7.8	148	153
36	88.4	24.1	27.3	7.2	7.0	140	141

Table 25. Hematological studies of pigs in litter 1 at 15 minutes postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	39.0	12.8	3.62	7.4		2	3	29	61	4	8
4^{Fe}	37.0	11.8	2.23								
5^{Fe}	37.0	11.3	2.90	1.2		1		25	74		24
1^{Fe}	36.0	10.3	3.09	2.5				4	93	3	21
12	25.0	6.3	2.90	5.3		1		24	71	4	10
6	23.0	6.3	2.44	1.3	2		2	2	92	2	396
10	21.0	5.5	2.44	1.3		2	3	5	90		383

Table 25 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	107.7	35.4	32.8	8.1	8.3	142	143
4	114.6	36.5	31.9	8.6	8.3	137	131
5	127.6	39.0	30.5	*9.0	8.7	142	139
1	116.5	33.3	28.6	*8.4	8.0	145	142
12	86.2	21.7	25.2	8.6	8.6	150	150
6	94.3	25.8	27.4	6.0	5.8	138	135
10	86.1	22.5	26.2	6.8	6.5	143	143

Table 26. Hematological studies of pigs in litter 2 at 15 minutes postinjection

Pig No.	PCV %	HB gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	37.00	11.5	3.23	11.3				48	51	1	
21^{Fe}	36.00	10.5	3.09	2.9	2			28	69	1	13
22^{Fe}	37.00	12.8	3.24	3.0					100		4
23^{Fe}	42.00	14.0	3.43	2.0				12	88		8
15	25.00	6.3	2.70	5.7			2	25	71	2	17
16	31.00	6.5	2.55	2.0		1		10	89		97
17	32.00	8.8	2.93	3.4				32	66	2	2
18	30.00	5.3	2.39	1.3				1	98	1	262

Table 26 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	114.6	35.6	31.1	*8.2	8.0	150	147
21	116.5	34.0	29.2	*7.8	7.6	141	139
22	114.2	39.5	34.6	*9.5	9.2	139	138
23	122.4	40.8	33.3	*9.0	8.6	140	135
15	92.6	23.3	25.2	*7.6	7.6	145	147
16	121.6	25.5	21.0	*6.4	6.4	147	151
17	109.2	30.0	27.5	*7.3	7.0	140	136
18	125.5	22.2	17.7	*6.1	6.4	140	145

Table 27. Hematological studies of pigs in litter 3 at 15 minutes postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso &	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
30^{Fe}	30.0	8.8	2.49	2.5			8	44	44	4	30
29^{Fe}	32.0	8.5	2.92	2.4				2	92	6	55
27^{Fe}	35.0	10.3	3.03	3.5			1	4	91	4	25
25	14.0	3.5	2.33	4.0				15	79	6	60
28	16.0	4.3	1.60	3.0					98	2	114

Table 27 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
30	120.5	35.3	29.3	*8.8	8.2	158	154
29	109.6	29.1	26.6	*8.0	8.5	144	145
27	115.5	34.0	29.4	8.6	8.5	137	137
25	60.1	15.0	25.0	8.3	8.3	146	149
26				7.7	7.8	147	149
28	100.0	26.9	26.9	6.5	6.5	136	136

Table 28. Hematological studies of pigs in litter 4 at 15 minutes postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	35.5	10.6	3.45	10.5				56	42	2	2
34^{Fe}	35.0	10.8	3.18	1.9				8	92		36
31^{Fe}	30.5	8.8	2.85	1.5					96	4	40
33	17.0	4.4	2.12	.4				10	90		600
36	20.0	5.2	2.26	1.3			2		98		150

Table 28 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	102.9	30.7	29.9	*10.0	9.5	145	143
34	110.1	34.0	30.9	* 8.9	8.9	135	134
31	107.0	30.9	28.9	* 8.1	7.9	140	136
32				* 8.6	8.3	151	151
33	80.2	20.8	25.9	7.5	7.2	144	147
36	88.5	23.0	26.0	6.0	6.7	143	147

Table 29. Hematological studies of pigs in litter 1 at 1 hour postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	39.0	12.8	3.62	7.4		2	3	29	61	4	8
4^{Fe}	37.0	11.8	3.23								
5^{Fe}	37.0	11.3	2.90	1.2		1		25	74		24
1^{Fe}	36.0	10.3	3.09	2.5				4	93	3	21
12	25.0	6.3	2.90	5.3		1		24	71	4	10
6	23.0	6.3	2.44	1.3	2		2	2	92	2	396
10	21.0	5.5	2.44	1.3		2	3	5	90		383

Table 29 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	107.7	35.4	32.8	10.3	9.5	153	155
4	114.6	36.5	31.9	7.5	7.9	147	152
5	127.6	39.0	30.5	7.6	7.6	144	144
1	116.5	33.3	28.6	7.7	7.6	154	157
12	86.2	21.7	25.2	6.8	7.0	148	147
6	94.3	25.8	27.4	7.0	7.1	145	141
10	86.1	22.5	26.2	6.7	6.7	145	148

Table 30. Hematological studies of pigs in litter 2 at 1 hour postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	37.0	11.5	3.23	11.3				48	51	1	
21^{Fe}	36.0	10.5	3.09	2.9	2			28	69	1	13
22^{Fe}	37.0	12.8	3.24	3.0					100		4
23^{Fe}	42.0	14.0	3.43	2.0				12	88		8
15	25.0	6.3	2.70	5.7			2	25	71	2	17
16	31.0	6.5	2.55	2.0		1		10	89		97
17	32.0	8.8	2.93	3.4				32	66	2	2
18	30.0	5.3	2.39	1.3				1	98	1	262

Table 30 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	114.6	35.6	31.1	*8.0	7.8	145	146
21	116.5	34.0	29.2	*7.1	6.8	143	140
22	114.2	39.5	34.6	*6.4	6.3	137	137
23	122.4	40.8	33.3	*7.5	7.4	144	145
15	92.6	23.3	25.2	*6.8	7.2	142	142
16	121.6	25.5	21.0	*5.3	5.4	141	140
17	109.2	30.0	27.5	6.7	6.6	146	145
18	125.5	22.2	17.7	6.0	6.0	145	149

Table 31. Hematological studies of pigs in litter 3 at 1 hour postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
30^{Fe}	30.0	8.8	2.49	2.5			8	44	44	4	20
29^{Fe}	32.0	8.5	2.92	2.4				2	92	6	55
27^{Fe}	35.0	10.3	3.03	3.5			1	4	91	4	25
25	14.0	3.5	2.33	4.0				15	79	6	60
28	16.0	4.3	1.60	3.0					98	2	114

Table 31 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
30	120.5	35.3	29.3	*7.6	7.5	148	145
29	109.6	29.1	26.6	*7.5	7.1	155	151
27	115.5	34.0	29.4	7.2	7.0	147	143
25	60.1	15.0	25.0	7.6	7.6	150	152
28	100.0	26.9	26.9	6.2	6.0	148	145

Table 32. Hematological studies of pigs in litter 4 at 1 hour postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	35.5	10.6	3.45	10.5				56	42	2	2
34^{Fe}	35.0	10.8	3.18	1.9				8	92		36
31^{Fe}	30.5	8.8	2.85	1.5					96	4	40
33	17.0	4.4	2.12	.4				10	90		600
36	20.0	5.2	2.26	1.3			2		98		150

Table 32 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	102.9	30.7	29.9	*9.3	9.5	138	143
34	110.1	34.0	30.9	*8.2	8.4	141	146
31	107.0	30.9	28.9	*6.5	6.4	142	141
32				*7.1	7.3	140	142
33	80.2	20.8	25.9	5.8	5.6	143	140
36	88.5	23.0	26.0	5.8	5.4	145	141

Table 33. Hematological studies of pigs in litter 1 at 5 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	36.0	11.5	3.40	6.4		2		48	46	4	2
4^{Fe}	40.5	12.3	3.89	2.5			20	36	44		32
5^{Fe}	40.5	11.4	3.17	1.5			7	31	62		60
1^{Fe}	38.0	11.8	3.11	1.7			5	14	81		46
12	22.0	6.0	2.73	6.8			1	12	87		15
6	24.0	6.7	2.80	1.4			1	53	41	5	265
10	21.5	5.0	2.94	.8		1	1	14	82	2	692

Table 33 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	105.9	33.8	31.9	7.9	7.7	140	140
4	104.1	31.6	30.4	*9.7	9.7	142	140
5	127.8	36.0	28.1	*10.2	10.4	153	154
1	122.2	37.9	31.1	8.7	8.7	143	141
12	80.6	22.0	27.3	7.4	7.5	137	139
6	85.7	23.9	27.9	8.7	8.8	148	150
10	73.1	17.0	23.3	8.1	8.0	135	132

Table 34. Hematological studies of pigs in litter 2 at 5 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	39.0	11.8	3.63	9.2				56	43	1	2
21^{Fe}	43.0	12.8	4.13	1.3				30	70		50
22^{Fe}	40.0	12.3	3.37	1.4				24	68	8	76
23^{Fe}	45.0	14.5	4.20	1.8		1		20	79		40
15	24.0	6.3	3.09	5.8		2	1	41	52	4	9

Table 34 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	107.4	32.5	30.3	*8.3	8.3	141	141
21	104.1	31.0	29.8	*8.7	8.6	147	146
22	118.7	36.5	30.8	*7.9	8.0	139	137
23	107.1	34.5	32.2	*8.6	8.6	140	140
15	77.7	20.4	26.3	8.3	8.0	148	144
16				13.0	12.8	152	149
17				11.6	11.4	147	146

Table 35. Hematological studies of pigs in litter 4 at 5 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
35^{Fe}	33.0	9.7	3.15	11.3			3	47	48	2	
34^{Fe}	37.0	10.8	2.85	1.4					96	4	50
31^{Fe}	34.0	9.7	3.29	2.2			6	22	66	6	10
32	14.0	3.5	1.69	3.7				80	16	4	60
33	18.0	4.5	2.22	.7					100		290
36	20.0	4.9	2.35	1.2				4	96		200

Table 35 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
35	104.8	30.8	29.4	*9.2	9.2	138	138
34	129.8	37.9	29.2	*9.8	9.4	152	149
31	103.3	29.5	28.5	*7.8	8.0	138	142
32	82.8	20.7	25.0	*7.5	7.3	148	145
33	81.1	20.3	25.0	*6.8	6.7	139	138
36	85.1	20.9	24.5	6.6	6.5	137	137

Table 36. Serum electrolytes of pigs in litter 1 at 8 hours postinjection

Pig No.	K mEq/L		Na mEq/L	
$_{11}Fe$	*8.4	8.5	142	143
$_{4}Fe$	*10.0	9.8	143	141
$_{5}Fe$	*15.4	15.2	147	150
$_{1}Fe$	*10.4	10.3	150	146
12	7.2	7.2	145	145
6	10.1	10.4	143	144
10	9.8	9.5	152	146

Table 37. Serum electrolytes of pigs in litter 2 at 8 hours postinjection

Pig No.	K mEq/L		Na mEq/L	
20^{Fe}	*7.9	8.1	137	139
21^{Fe}	*8.6	8.9	133	138
22^{Fe}	*9.9	9.6	141	136
23^{Fe}	*9.9	9.9	134	135
15	*7.1	7.3	136	138

Table 38. Serum electrolytes of pigs in litter 4 at 8 hours postinjection

Pig No.	K mEq/L		Na mEq/L	
35^{Fe}	*9.4	9.0	143	139
34^{Fe}	12.0	12.4	142	144
31^{Fe}	*8.5	8.6	131	135
32	*7.3	7.2	146	145
36	7.0	6.8	138	136

Table 39. Hematological studies of pigs in litter 1 at 24 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	37.0	11.8	4.88	8.1	1	1	2	46	49	1	1
4^{Fe}	37.0	12.3	3.97	18.5			41	28	31		12
12	23.0	6.3	3.26	6.0			4	35	61		38

Table 39 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	75.8	24.2	31.9	7.1	7.2	150	151
4	93.2	31.0	33.2	*7.0	7.0	152	150
12	70.6	19.3	27.4	5.5	5.5	143	143

Table 40. Hematological studies of pigs in litter 2 at 24 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	37.0	11.3	3.90	9.4	1	1	2	61	28	7	5
21^{Fe}	26.0	6.9	3.14	6.9			12	22	66		38
22^{Fe}	38.0	10.5	3.58	9.9			9	53	34	4	15
23^{Fe}	41.0	12.3	3.99	18.9			27	56	13	4	6
15	30.0	5.3	2.84	6.5				35	56	9	11

Table 40 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	94.9	29.0	30.5	*4.7	4.8	137	138
21	82.8	22.0	26.5	*10.3	10.2	138	138
22	106.1	29.3	27.6	*5.0	5.2	146	149
23	102.8	30.8	30.0	*6.3	6.3	143	144
15	105.6	18.7	17.7	6.1	6.0	147	146

Table 41. Hematological studies of pigs in litter 1 at 120 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
11^{Fe}	33.0	9.7	3.92	5.7		1	2	46	50	1	3
4^{Fe}	33.0	10.0	3.57	6.4	1	2	3	62	32		5
12	24.0	6.0	2.57	2.8		1		35	60	4	87

Table 41 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
11	84.2	24.7	29.4	*10.4	10.5	137	140
4	92.4	28.0	30.3	* 9.2	9.4	127	129
12	93.4	23.3	25.0	*10.2	10.4	141	143

Table 42. Hematological studies of pigs in litter 2 at 120 hours postinjection

Pig No.	PCV %	Hb gm%	RBC 10^6/cmm	WBC 10^3/cmm	Baso %	Eos %	Band %	Seg %	Lymph %	Mono %	NR No/100 WBC
20^{Fe}	30.0	9.3	3.52	9.8				20	80		4
22^{Fe}	29.5	9.0	2.97	8.0				28	69	3	2
23^{Fe}	32.5	10.5	3.13	7.3				62	32	6	
15	20.0	5.5	3.59	8.1		1		38	49	12	6

Table 42 (Continued)

Pig No.	MCV u^3	MCH uug	MCHC %	K mEq/L		Na mEq/L	
20	85.2	26.4	31.0	7.9	8.1	147	149
22	99.3	30.3	30.5	*7.9	7.8	140	140
23	103.8	33.5	32.3	*6.9	7.1	149	148
15	55.7	15.3	27.5	*8.0	8.0	146	149

IX. APPENDIX B. FIGURES

Figure 1. Means of red blood cell counts per cu mm of porcine blood

In this figure and Figures 1 - 12 the following legend will be used:

significance due to iron injection (1.0% level)

significance due to iron injection (0.5% level)

significance due to endotoxin injection (1.0% level)

significance due to endotoxin injection (0.5% level)

F x E interaction

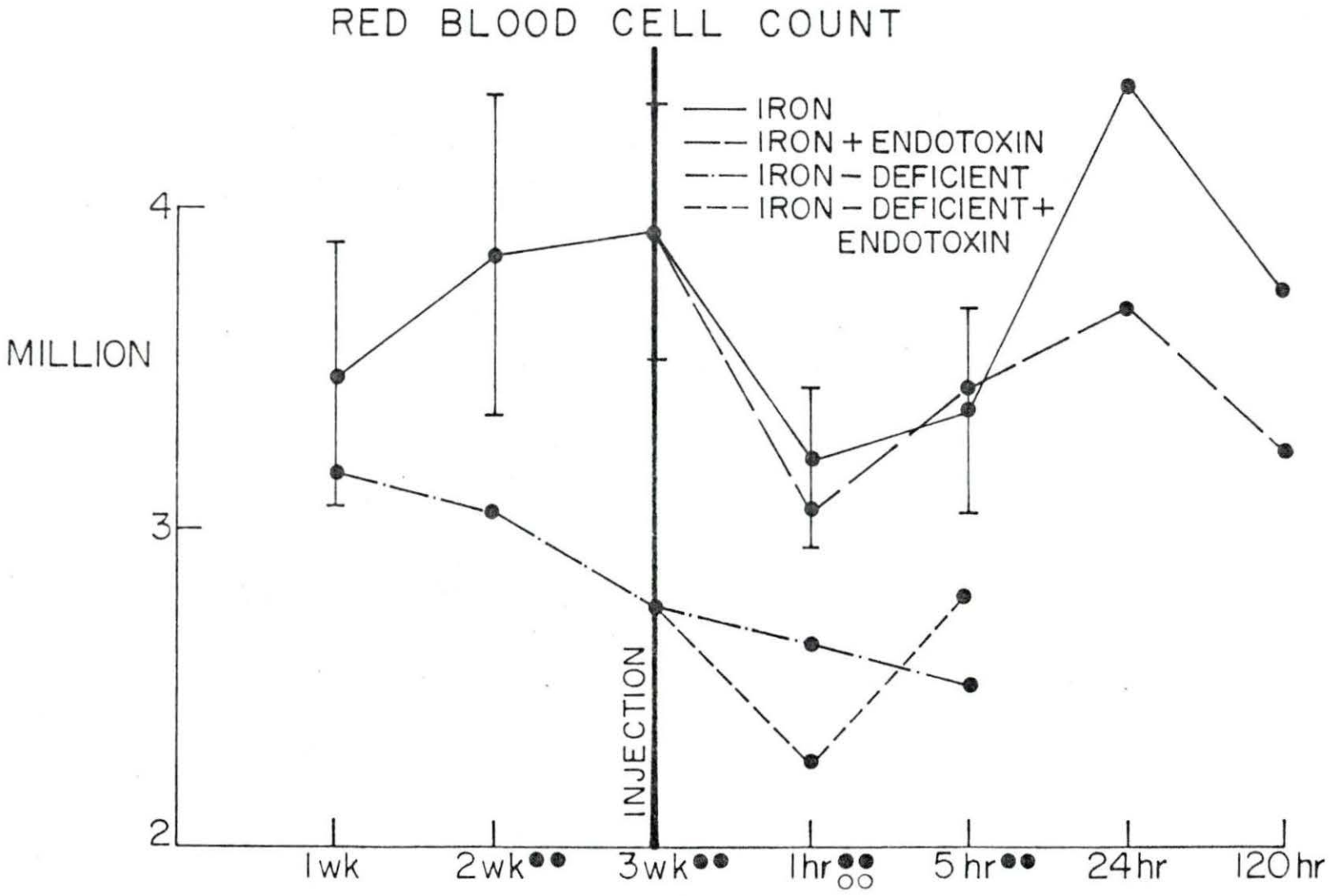

RED BLOOD CELL COUNT
IRON
IRON + ENDOTOXIN
IRON – DEFICIENT
IRON – DEFICIENT + ENDOTOXIN
MILLION
4
3
2
INJECTION
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 2. Means of hemoglobin values of porcine blood

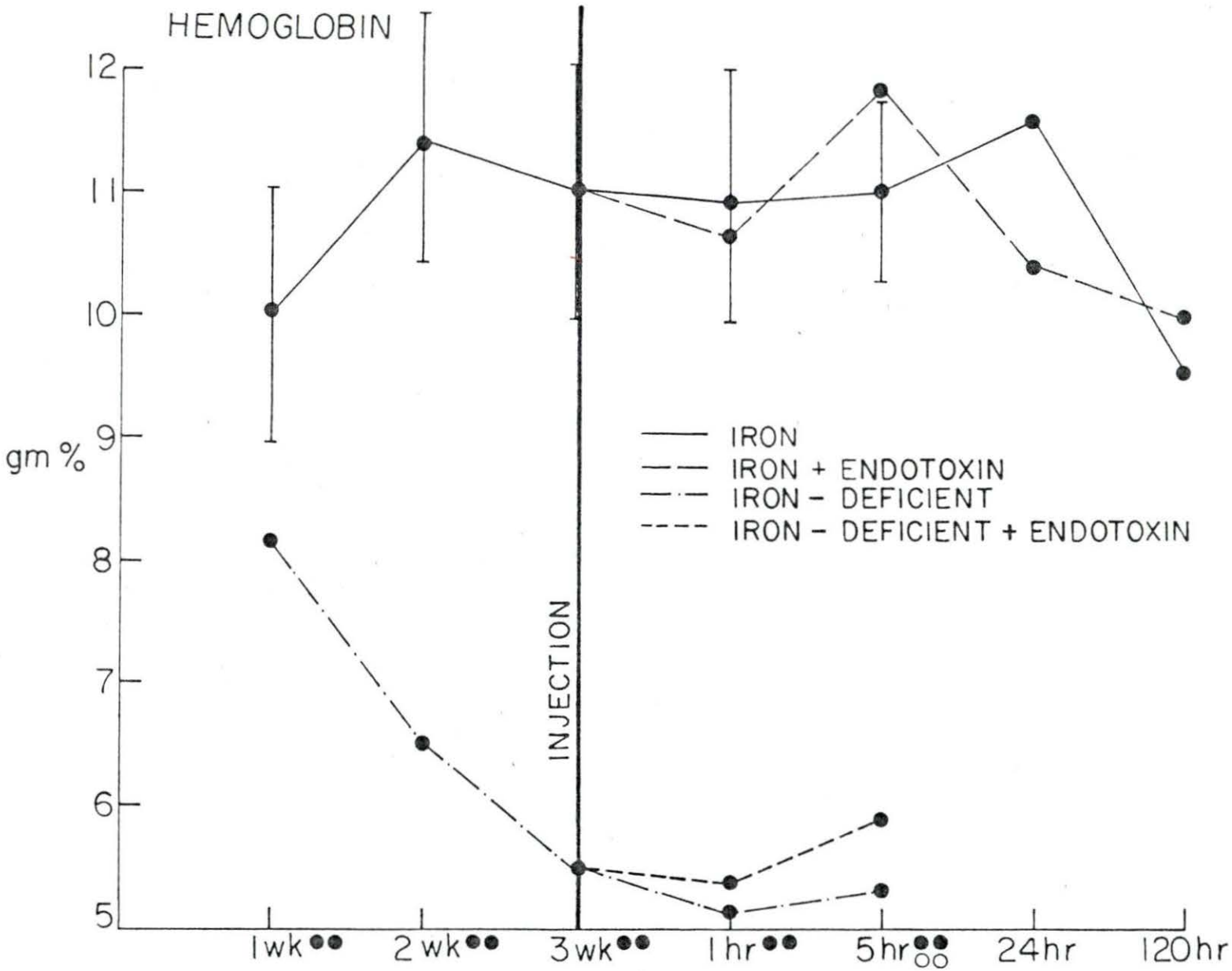

HEMOGLOBIN
12
11
10
9
8
7
6
5
gm %
INJECTION
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 3. Means of packed cell volumes of porcine blood

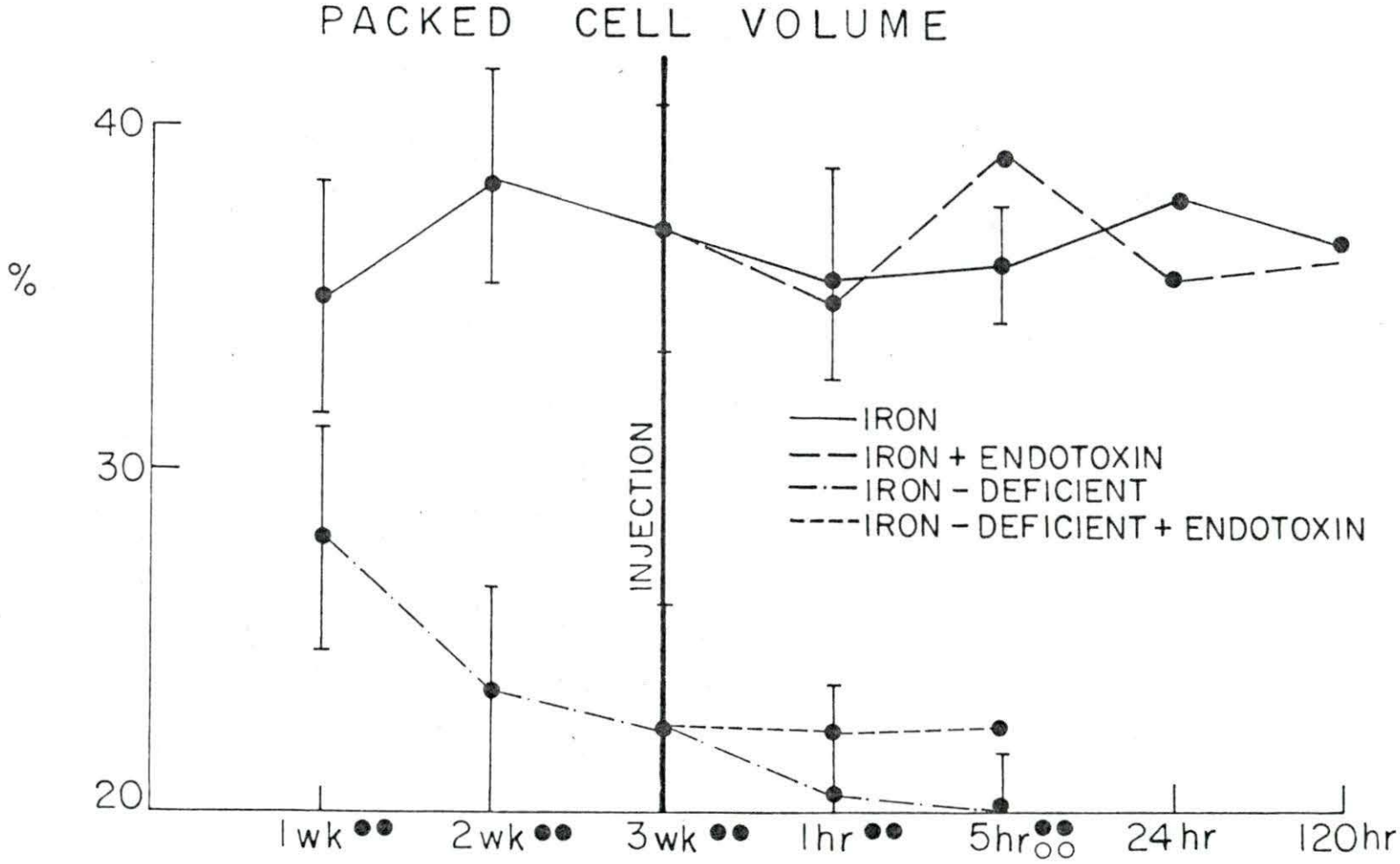

PACKED CELL VOLUME
%
40
30
20
INJECTION
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 4. Means of the mean corpuscular volumes of porcine blood

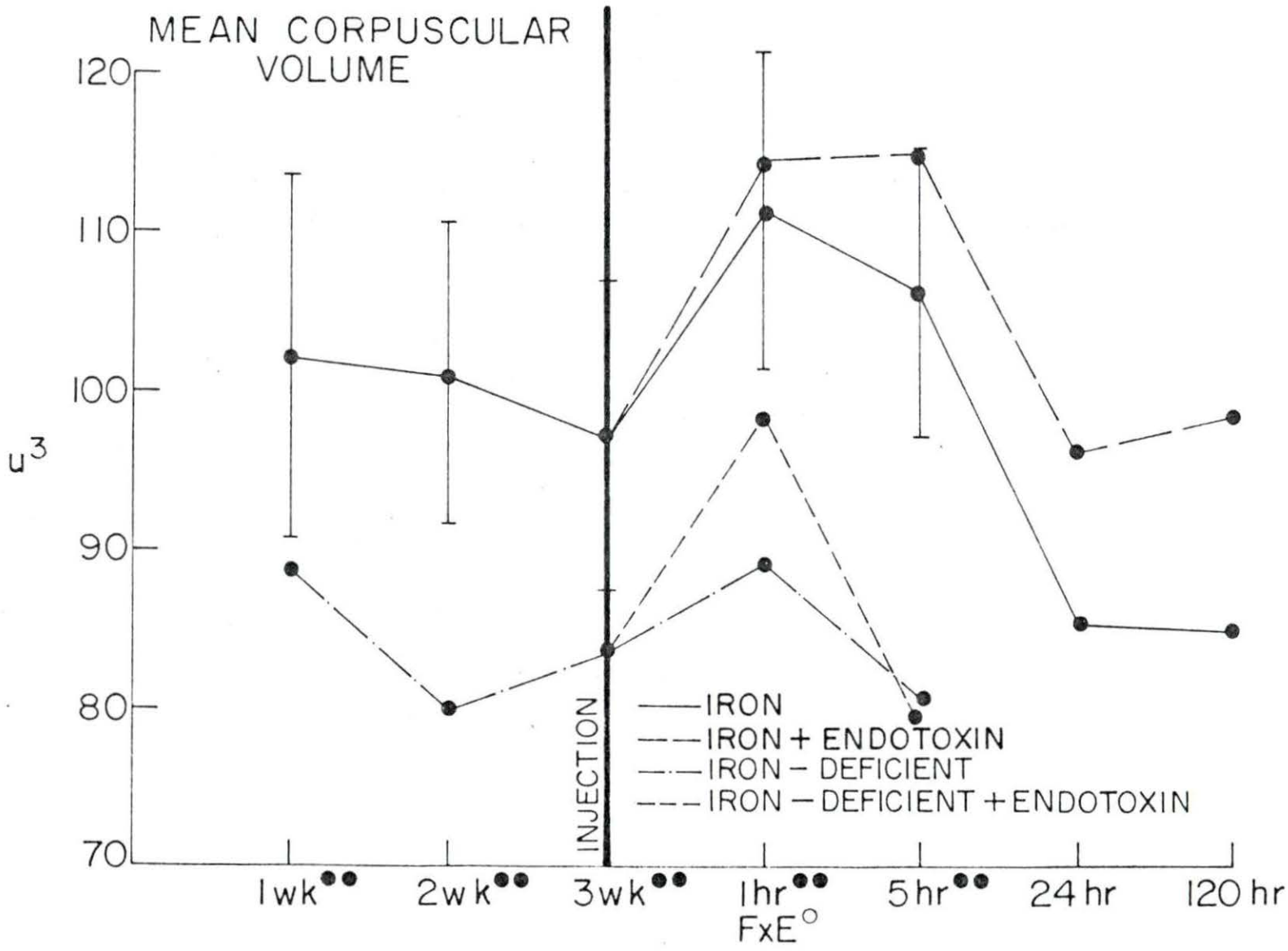

MEAN CORPUSCULAR VOLUME
120
110
100
90
80
70
μ3
INJECTION
IRON
IRON + ENDOTOXIN
IRON – DEFICIENT
IRON – DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
1hr
5hr
24hr
120hr
FxE°

Figure 5. Means of the mean corpuscular hemoglobin values of porcine blood

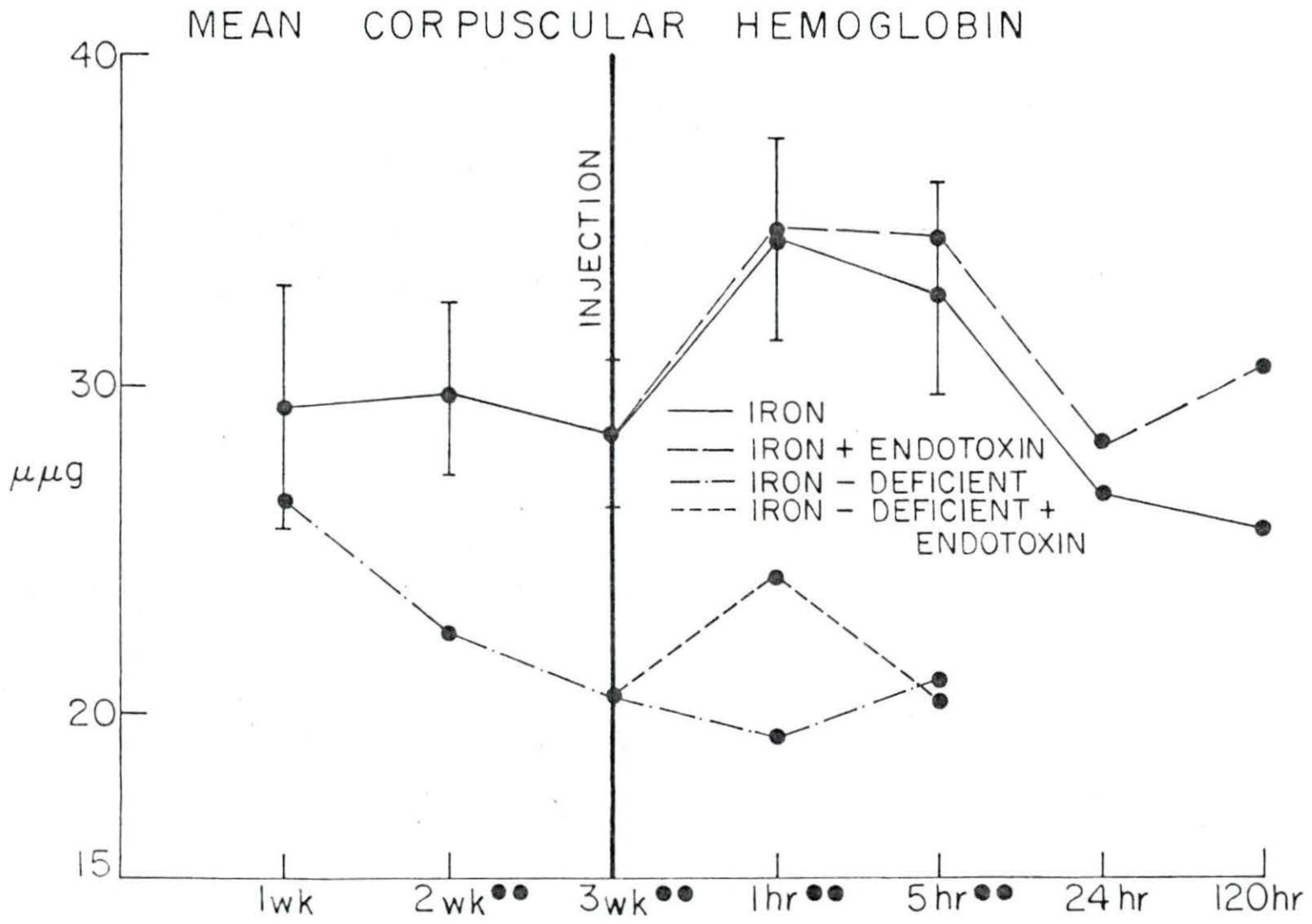

MEAN CORPUSCULAR HEMOGLOBIN
40
30
20
15
μμg
INJECTION
IRON
IRON + ENDOTOXIN
IRON – DEFICIENT
IRON – DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
1hr
5hr
24 hr
120hr

Figure 6. Means of the mean corpuscular hemoglobin content value of porcine blood

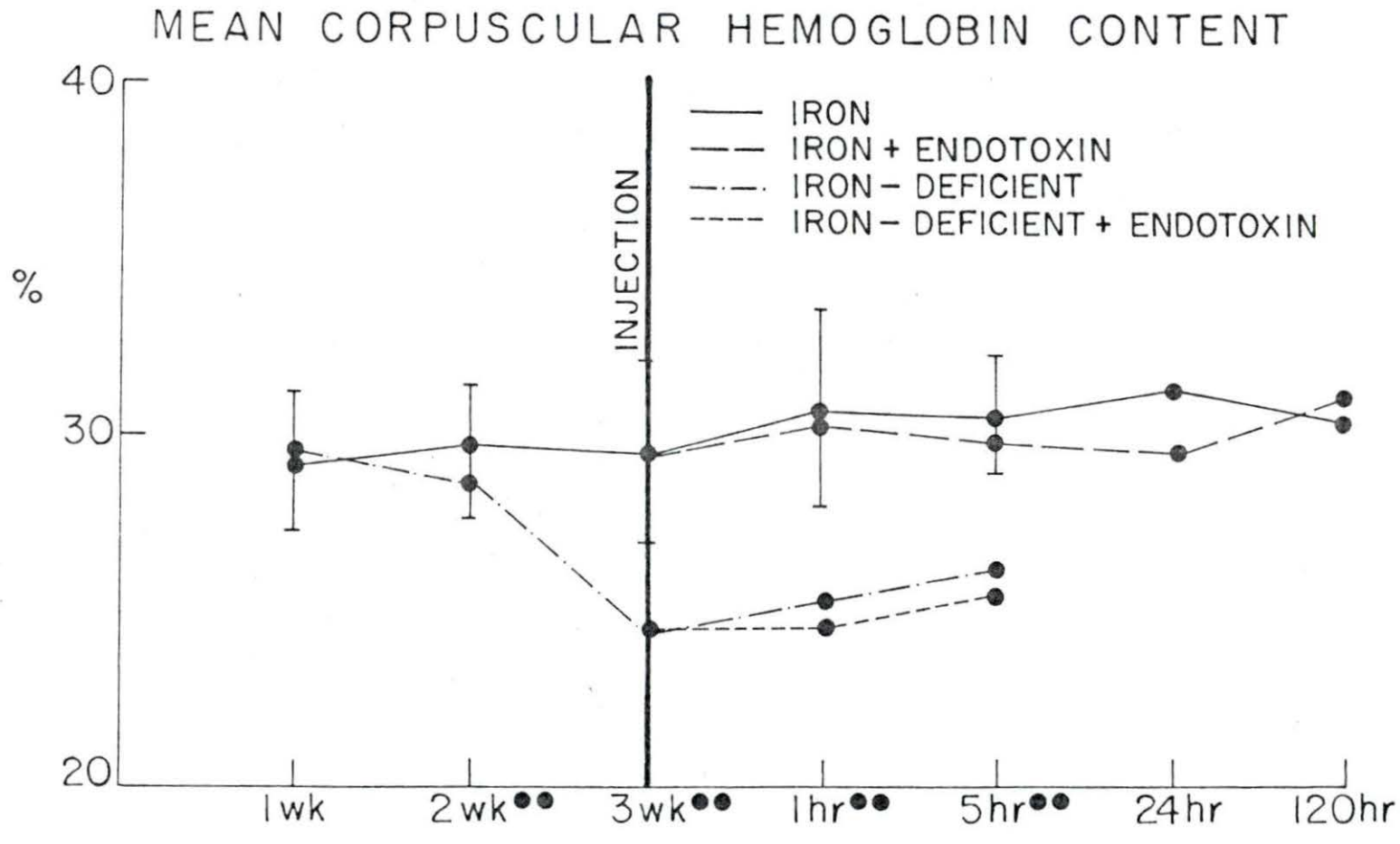

MEAN CORPUSCULAR HEMOGLOBIN CONTENT
40
30
20
%
INJECTION
IRON
IRON + ENDOTOXIN
IRON – DEFICIENT
IRON – DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 7. Means of the total leukocytes per cu mm of porcine blood

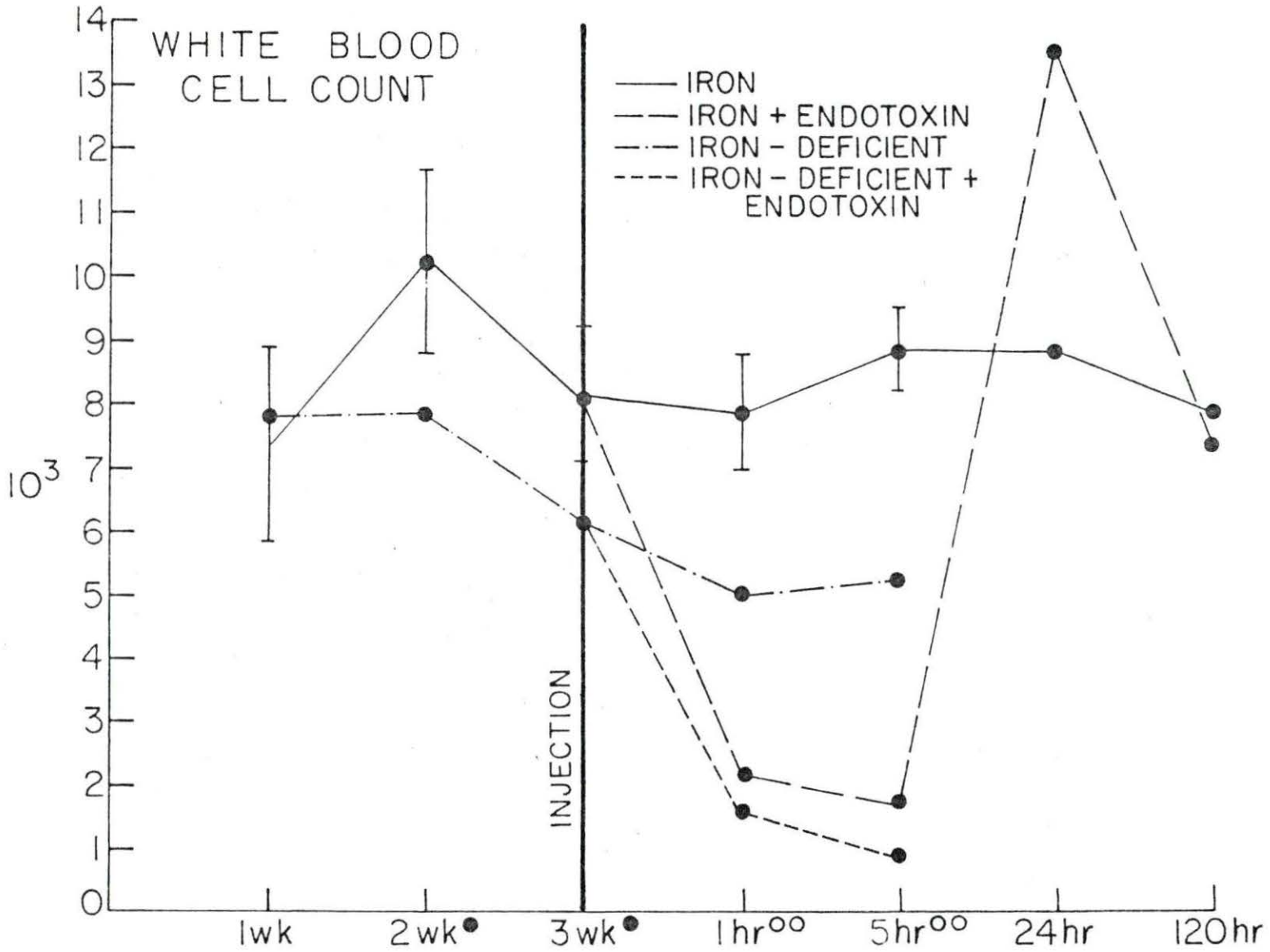

WHITE BLOOD CELL COUNT
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
10^3
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
INJECTION
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 8. Means of neutrophil and lymphocyte percent of the total leukocyte count in porcine blood

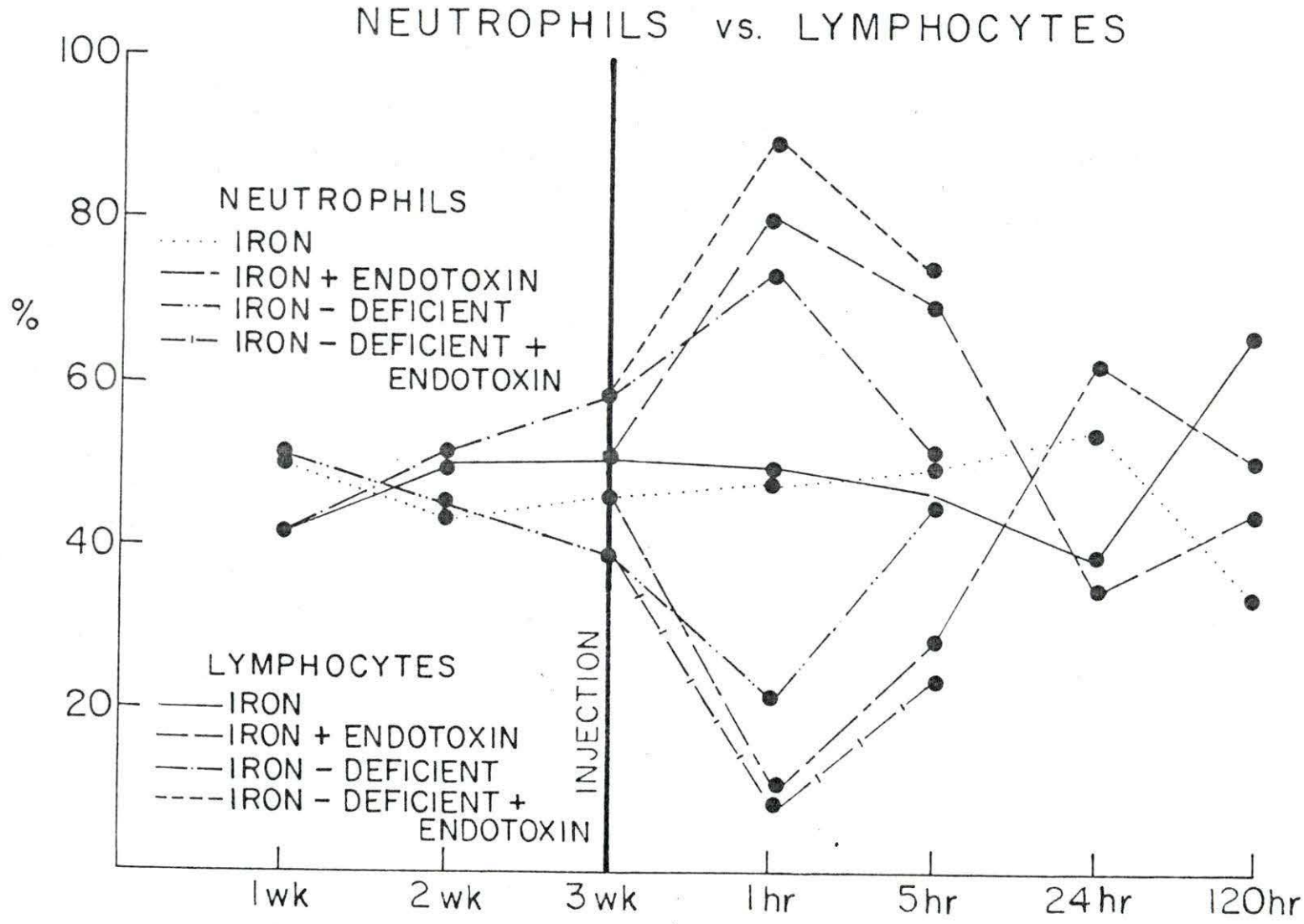

NEUTROPHILS vs. LYMPHOCYTES
100
80
60
40
20
%
NEUTROPHILS
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
LYMPHOCYTES
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
INJECTION
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 9. Means of nucleated erythrocytes per 100 leukocytes in porcine blood

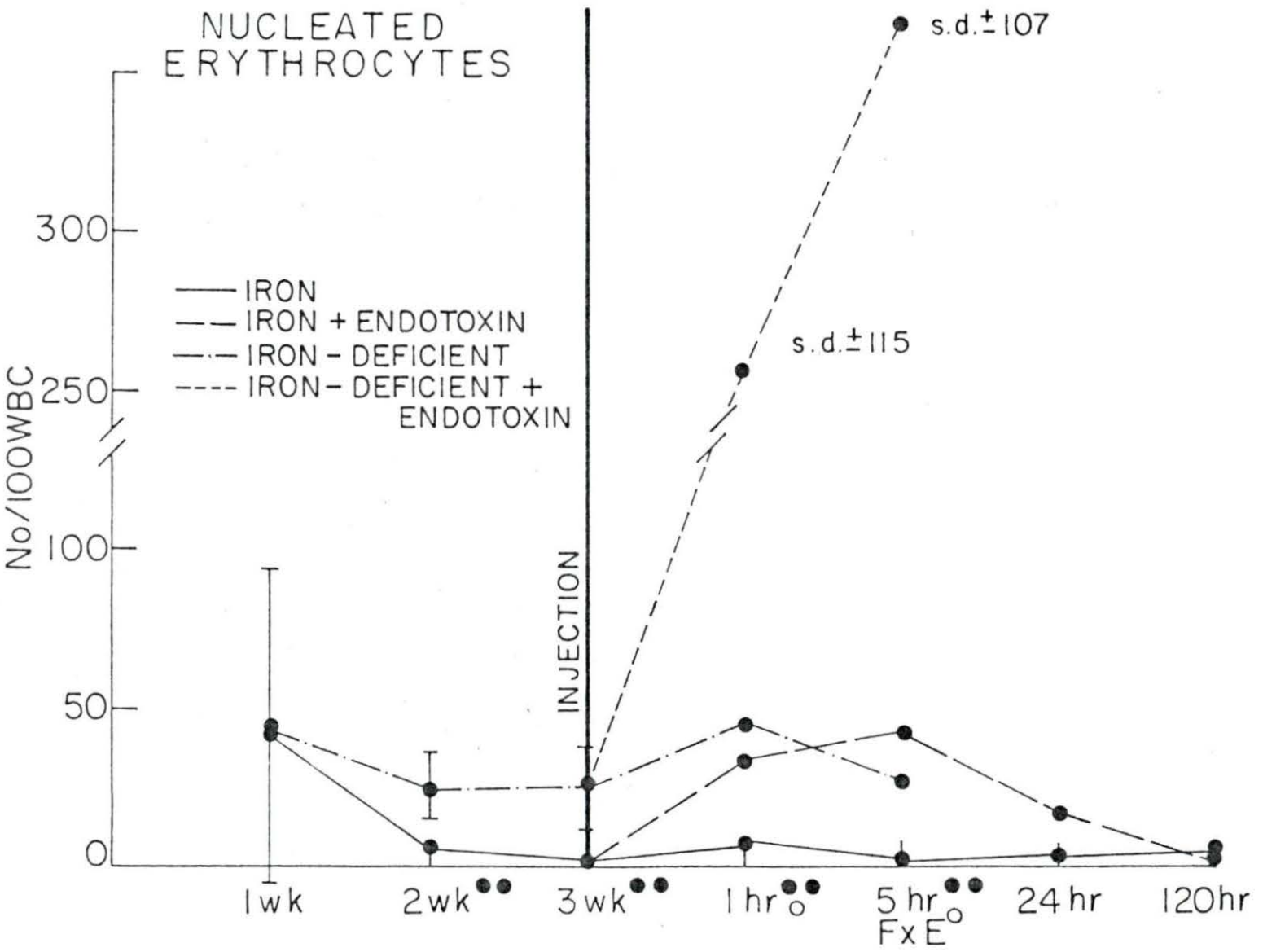

NUCLEATED
ERYTHROCYTES
No/100WBC
300
250
100
50
0
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
INJECTION
s.d.±107
s.d.±115
1wk
2wk
3wk
1hr
5hr
FxE
24hr
120hr

Figure 10. Means of erythrocyte sedimentation rates per hour in porcine blood

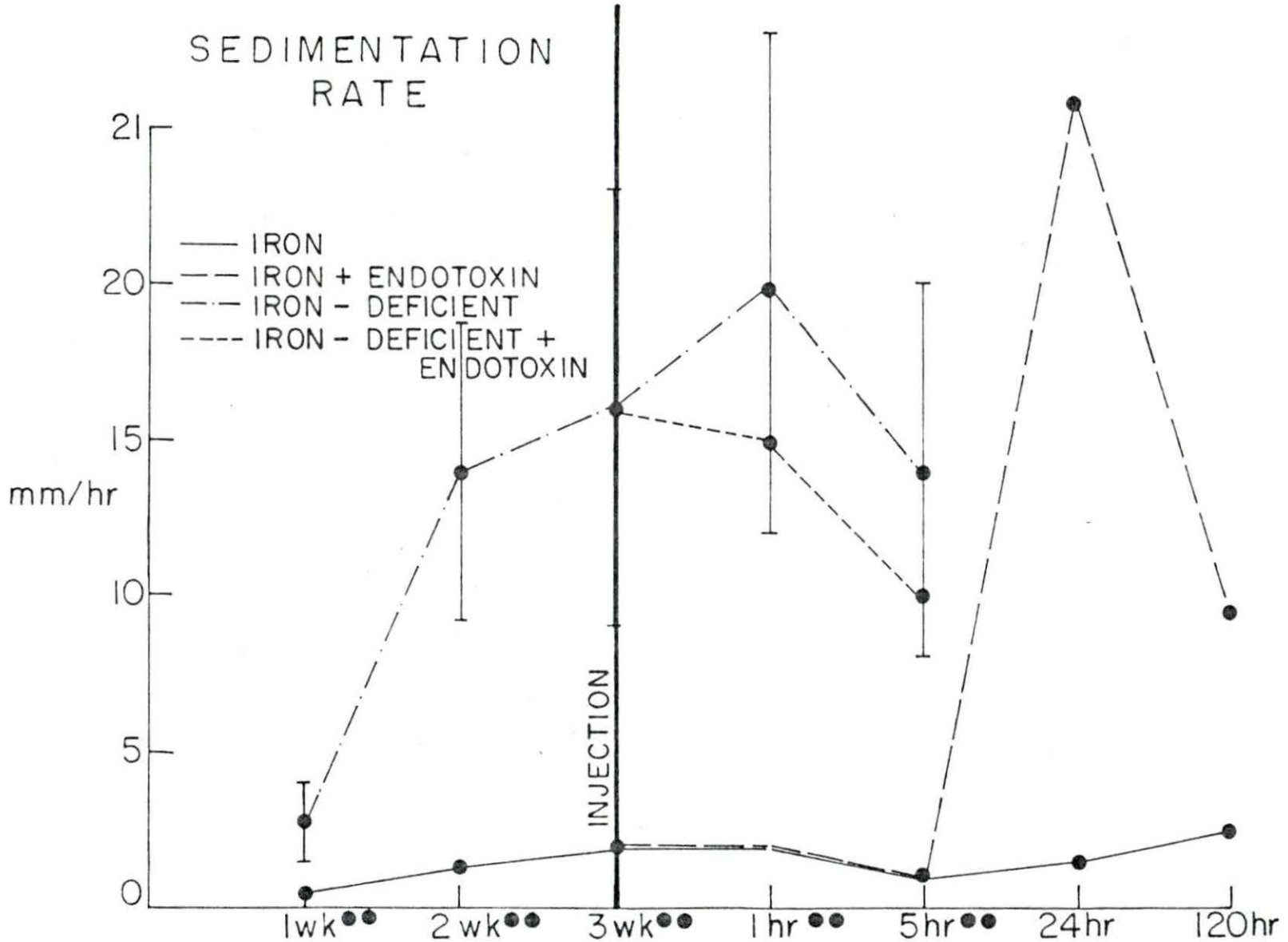

SEDIMENTATION RATE
21
20
15
10
5
0
mm/hr
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
INJECTION
1wk
2wk
3wk
1hr
5hr
24hr
120hr

Figure 11. Means of serum sodium in porcine serum

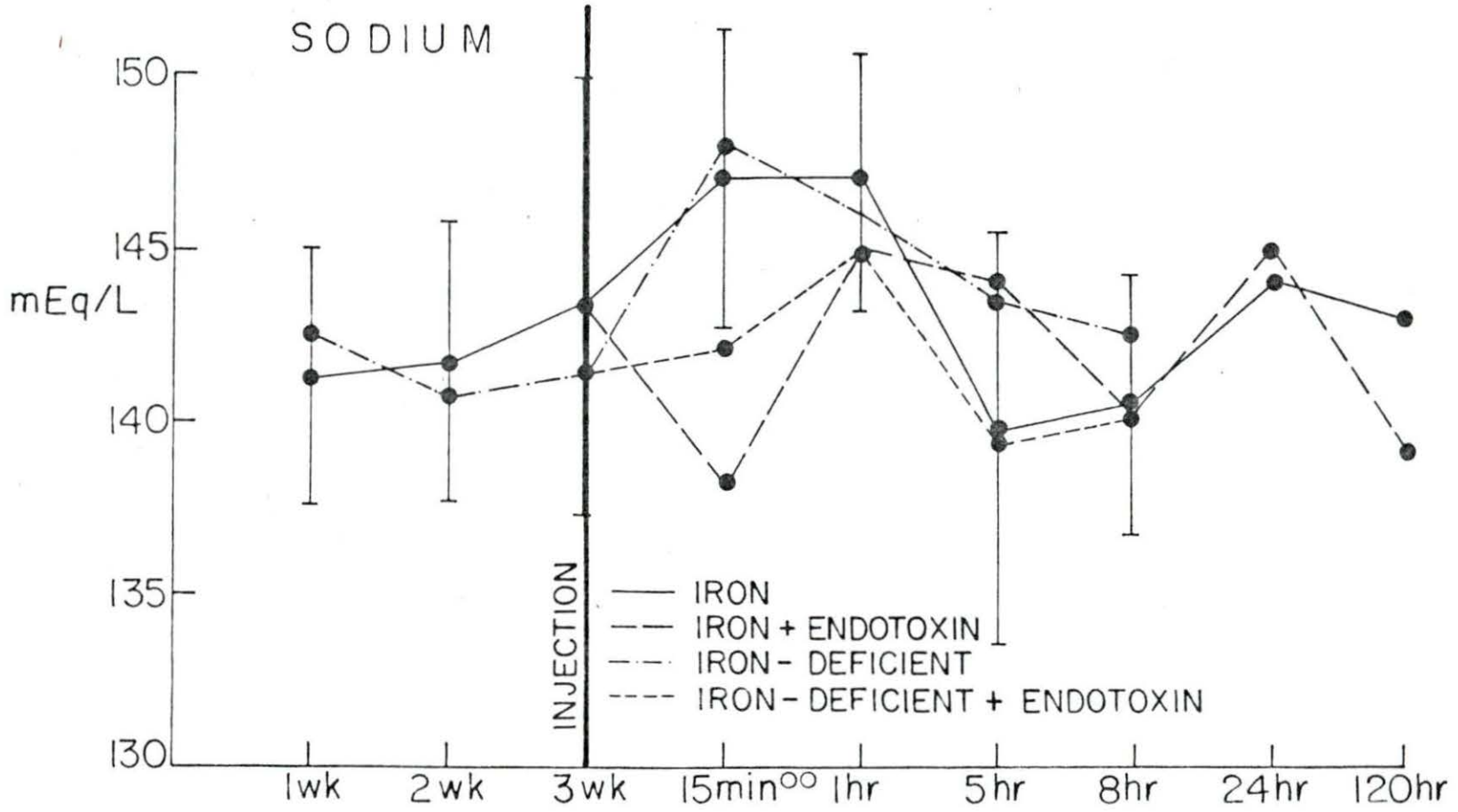

SODIUM
150
145
140
135
130
mEq/L
INJECTION
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
15min
00
1hr
5hr
8hr
24hr
120hr

Figure 12. Means of serum potassium in porcine serum

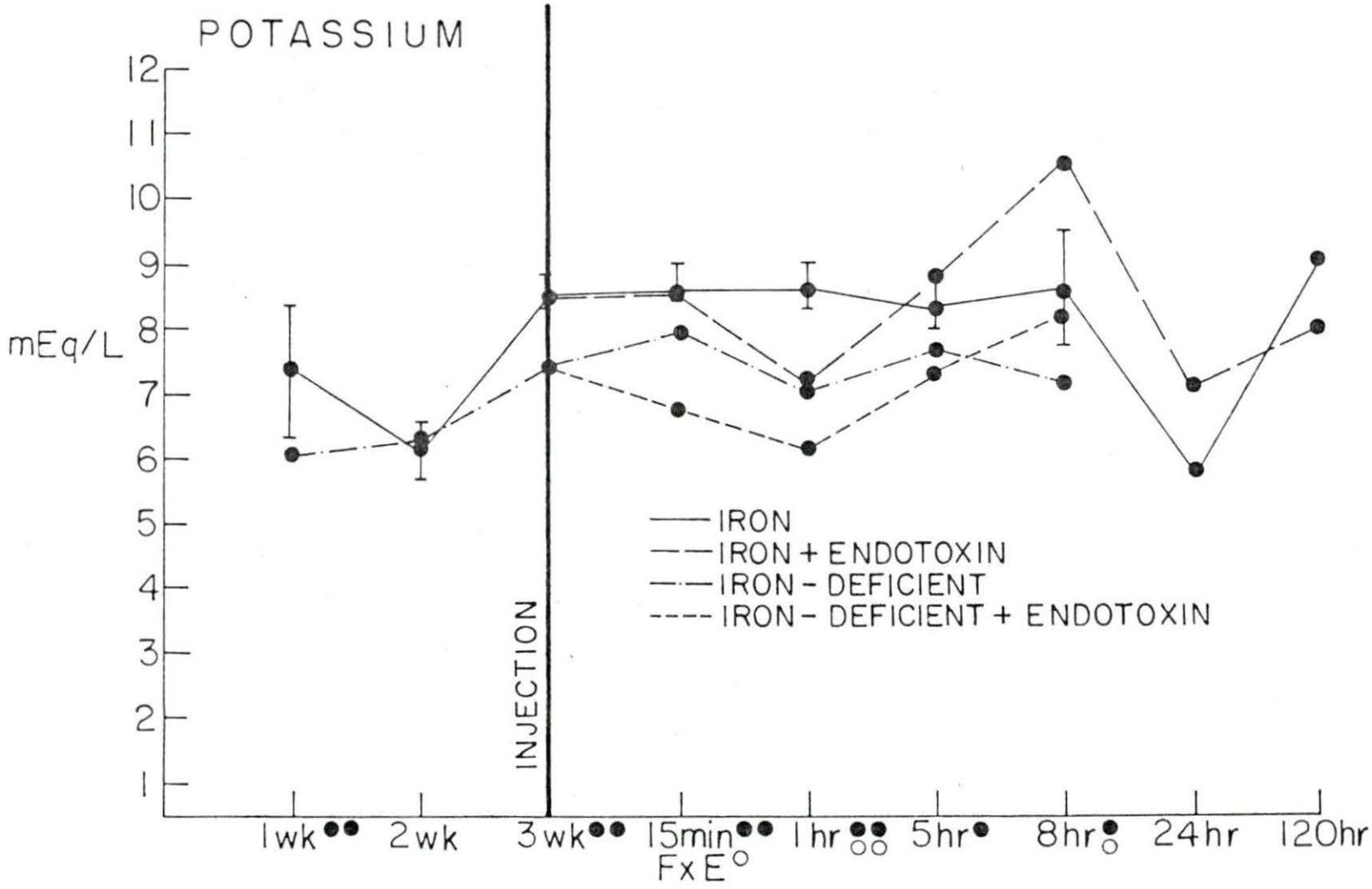
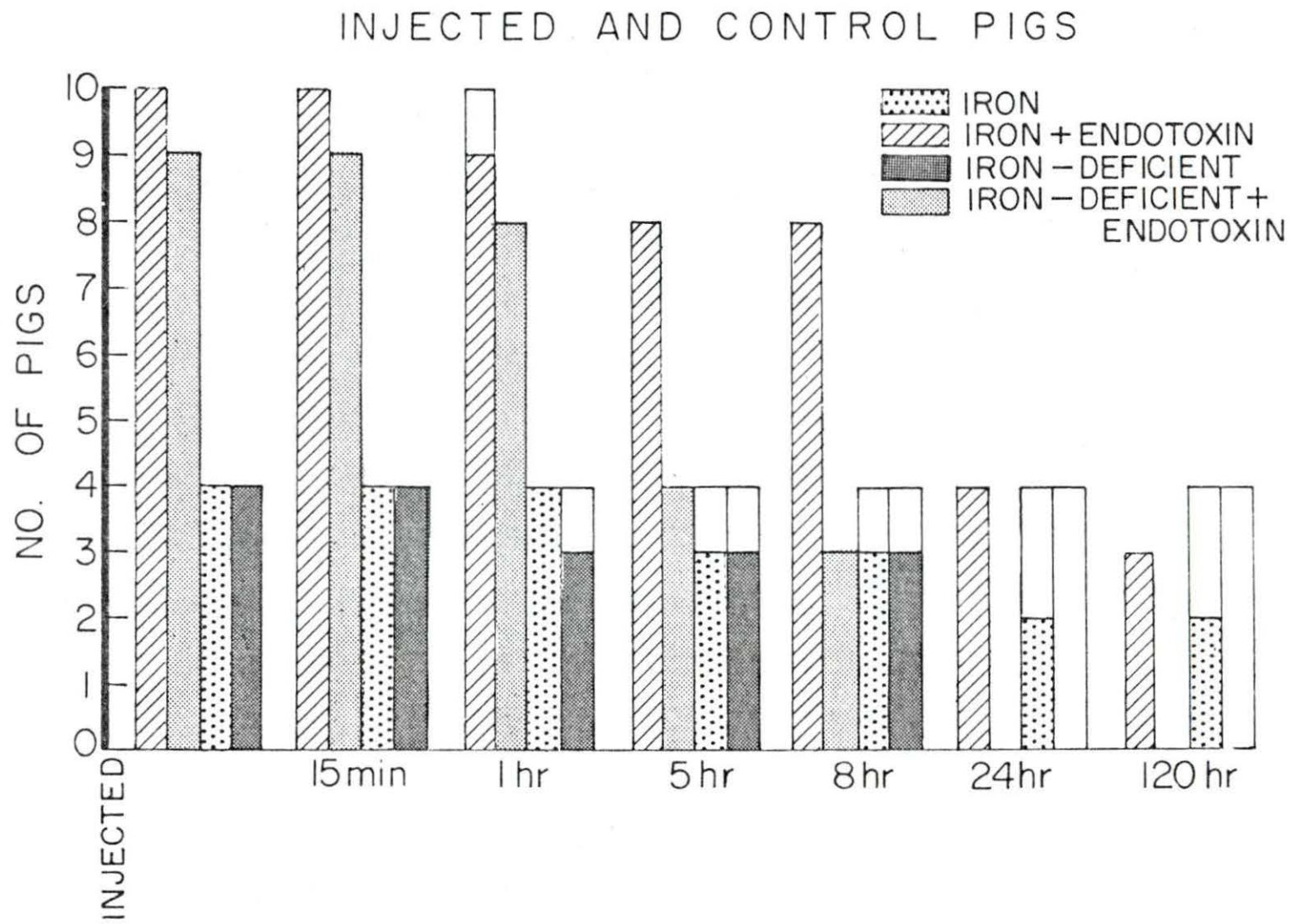

POTASSIUM
mEq/L
12
11
10
9
8
7
6
5
4
3
2
1
INJECTION
IRON
IRON + ENDOTOXIN
IRON - DEFICIENT
IRON - DEFICIENT + ENDOTOXIN
1wk
2wk
3wk
15min
1hr
5hr
8hr
24hr
120hr
FxE°

Figure 13. Number of pigs surviving at different postinjection times

The blank portion of the bar indicates live pigs which did not contribute a blood sample.


INJECTED AND CONTROL PIGS
NO. OF PIGS
0
1
2
3
4
5
6
7
8
9
10
IRON
IRON + ENDOTOXIN
IRON – DEFICIENT
IRON – DEFICIENT + ENDOTOXIN
INJECTED
15 min
1 hr
5 hr
8 hr
24 hr
120 hr