This is to certify that the

thesis entitled

Experimental Non-Lethal Endotoxemia in the Pony:
Modification of the Pathophysiology by Polymixin B.

presented by

Dolores Johanna Kunze

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Large Animal Surgery
and Medicine

Major professor

Date February 13,1980

EXPERIMENTAL NON-LETHAL ENDOTOXEMIA IN THE PONY: MODIFICATION OF PATHOPHYSIOLOGY BY POLYMIXIN B SULFATE

By

Dolores Johanna Kunze

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal
Surgery and Medicine

1980

ABSTRACT

EXPERIMENTAL NON-LETHAL ENDOTOXEMIA IN THE PONY:
MODIFICATION OF THE PATHOPHYSIOLOGY BY POLYMIXIN B

By

Dolores Johanna Kunze

Two groups of five ponies were treated with very low doses of endotoxin; the first group received endotoxin only and the second received endotoxin plus polymixin B administered concurrently. Two other groups, two ponies per group, served as controls with one receiving polymixin B and the other receiving saline. Following the infusion, the ponies trembled and coughed. They also developed pyrexia, leucopenia and transient pulmonary hypertension resulting from increased pulmonary vascular resistance. In the animals receiving endotoxin plus polymixin B, the leucopenia, pulmonary hypertension and increased pulmonary vascular resistance were ameliorated. Polymixin B, which binds to the lipid A moiety of the endotoxin molecule, did not block the pyrexia, suggesting that some other part of the endotoxin molecule might be responsible for endogenous pyrogen release. An increase in circulating leucocytes, particularly lymphocytes, was observed in the ponies given polymixin B alone.

ACKNOWLEDGEMENTS

My deepest gratitude is extended to Dr. Robinson, whose patience, enthusiasm and encouragement were essential to the completion of this work. Such intelligence and empathy are rare and are very much appreciated.

Special thanks to Drs. Mather and Riley and the Large Animal Faculty for their support. In particular, I am especially grateful to Drs. Scott and Stowe for their advice and help in the preparation of this thesis.

My appreciation and thanks also belong to Ms. Bobbi Milar for not only her technical expertise and assistance, but also for her good nature, an invaluable asset.

And, finally, to my husband, Morrow, my gratitude for his calm, quiet support which was a major sustaining force throughout this sometimes hectic enterprise.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont.)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Endotoxemia is commonly recognized as a contributing factor in various pathologic states, including gastrointestinal disease, aspiration pneumonia and wounds with gram-negative sepsis. Normal intestinal microflora, largely composed of gram-negative bacteria, continuously produce and release endotoxins which are absorbed by the intestinal mucosa and are detoxified by the liver. Concurrent hepatic or gastrointestinal disease may reduce detoxification or may increase endotoxin absorption.[1,2] Since endotoxemia occurs frequently and often poses a serious threat to a patient's survival, the role of endotoxin in the pathogenesis of naturally occurring and experimental disease has been extensively investigated. Because the biologic effects of endotoxin are so varied, the endotoxemic patient may exhibit a range of signs from mild depression and pyrexia to massive, fulminating shock.[3-8] In experimental models, most researchers have used lethal doses of endotoxins and have based their conclusions on the documentation of fatal shock.[9-15] In contrast, the clinical endotoxemic patient may or may not exhibit signs of shock. Therefore, the standard experimental model depicts only one manifestation of endotoxemia. The hematologic and physiologic changes associated with clinical endotoxemia generally develop more gradually and are much more prolonged than those seen in experimental models.[13,14,16-20] Clinically, endotoxemia in the horse has been implicated or suggested as a complication in certain gastrointestinal

disorders.[19,21-23] And, as with other species, the experimental endotoxemia studies in horses produced abnormalities not entirely consistent with those observed in clinical cases.[9,13,14] Therefore, comparisons and conclusions made between experimental animals and clinical animals may not be entirely valid.

The therapeutic plan for endotoxin shock includes fluid volume replacement and correction of acid-base imbalances.[8,17,18,25] With septic shock, appropriate antimicrobial agents are often used, but the use of bactericidal drugs may actually exacerbate shock by accelerating lysis of gram-negative bacteria and release of endotoxins. This possibility is necessarily weighed against the potentially graver threat of gram-negative septicemia. While most antibiotics that are effective against gram-negative organisms will not interfere with the bioactivity of the endotoxins, the cyclic cationic polypeptides will interfere by interacting with the lipid components of the endotoxin molecule.[26-28] Polymixin B sulfate, colistin and tyrocidine are members of this group of antibiotics but are unpopular because of the accompanying nephrotoxicity.[30] Polymixin B sulfate is less nephrotoxic than others in this group and is more effective in neutralizing endotoxin when admixed before administration.[28,29] Polymixin B sulfate has been given before, during and after endotoxin administration with variable results, but some lessening of either the disease state or the mortality occurred with the use of the antibiotic concurrent with endotoxin administration.[31-43] It has even been used in a few clinical cases with limited positive results.[44] The timing of the administration of the drug appears to be critical, which has made clinical application seem

impractical. The objectives of these experiments were to investigate sublethal endotoxemia in ponies, and to determine if the subsequent pathophysiology could be blocked by polymixin B.

LITERATURE REVIEW

Origin, Composition and Preparation of Endotoxin

Endotoxin, the portion of an enteric bacterium responsible for the toxic effects, is the outer part of the cell wall. This material, a complex of lipopolysaccharide (LPS) and protein is released from cells during active growth and upon cell lysis; the molecule encompasses both the endotoxin activity and the somatic antigens of the bacterium. Three distinct regions form the molecule: the outer hydrophilic region, a heteropolysaccharide containing the repeating O-specific antigenic units; the central acid core, linking the O-antigens and the lipid portion; and the internal lipid-rich region, or lipid A.[16,25,46] Lipid A has a high affinity for cell membranes, for other lipids and for protein. It represents the biologically active part of the wild strains and the commercially prepared endotoxin, and the polysaccharide portion facilitates the solubility of the lipid in aqueous media.[16] Regardless of the bacterial species of origin, the experimental effects of purified preparations generally resemble the signs of naturally occurring gram-negative sepsis.[25] Response varies dramatically with dose, and the method of LPS extraction may modify the toxicity of the preparation. The Boivin method involves extraction with ice-cold trichloroacetic acid, resulting in a preparation consisting primarily of LPS with some protein and lipid; this preparation is slightly more active than some others because not only does it contain large amounts of lipid A, but it also has a

carbohydrate side chain which appears to enhance activity.[46,47] With the other commonly used preparation, the Westphal method, endotoxin is extracted with phenol which does not preserve some of the side chains.[16,45] Different lots obtained from a single supplier and derived from the same bacterial strain may vary in their ability to elicit a given response, particularly from platelets.[48-52] Overall, however, the major trends are consistent enough to allow comparison of experiments using varying combinations of lots, extraction methods and bacterial species. Whereas individual animals of a given species respond similarly, there are species differences in response to endotoxin.

Clinical Signs of Endotoxemia

No one set of clinical signs fits every endotoxemic patient; with varying degrees of endotoxemia, a given patient of any species, including human or equine, could appear to be normal or to be in near-terminal shock. However, the shock cases usually have similar signs: pyrexia, sweating, increased heart rate, varying degrees of depression, cold extremities, shivering, shallow respirations or some degree of dyspnea, congested or cyanotic mucous membranes, prolonged capillary refill time, and, occasionally, evidence of coagulation disturbances such as prolonged bleeding times.[8,9,17,53] Very mild cases are usually pyrexic and may have a mild secondary depression; fever is one of the most consistent signs of endotoxemia, regardless of species or degree of involvement; endotoxin is not only a very potent releaser of endogenous pyrogen, but also may have a direct cerebral effect.[5-7] The greater the degree of intoxication, the more severe the signs become.

Hematology of Endotoxemia

The familiar hematologic picture of endotoxemia, both naturally occurring and experimentally induced, is characterized by an initial neutropenia and thrombycytopenia, a somewhat later appearing lymphopenia and an even later leucocytosis.[9,15,20,25,35,44,53,61,64-66] Platelets and leucocytes predominate as the peripheral agents for endotoxin clearance; granulocytes actually remove endotoxin, and platelets appear to facilitate removal by enhancing recognition of endotoxin by phagocytes.[54] When endotoxin attaches to platelets, the combination forms the equivalent of a membrane-associated antigen. Through this interaction, both the classical and alternate complement pathways are activated with the subsequent generation of opsonins and chemotactic factors and the lysis of gram-negative bacteria.[55-57] The mechanisms of the platelet-endotoxin interactions depend critically on the presence or absence of immune adherence sites on the platelet membrane. Primate platelets lack these receptors and respond differently than the platelets of rats, rabbits, dogs, and guinea pigs.[58,59] The presence of these receptors has not been reported in the horse. In rabbits, rats, dogs, and guinea pigs, after platelet-endotoxin combination occurs, platelet responses are usually characterized by aggregation and release of platelet constituents, including ADP and vasoactive amines such as histamine and serotonin.[60] The release response of platelets may occur through lysis, in which granules and cytoplasmic constituents are liberated, or by secretion, in which the platelet membrane remains intact and only granule constituents are released. Serum complement is probably involved in the lytic response.[58] Horse platelets do respond

to endotoxin, but the exact mechanism has not been determined.[61] While little is known of the physiologic, pathophysiologic and pharmacologic characteristics of horse platelets, they respond to ADP with aggregation, and in most other species, aggregation occurs with secretion of dense granule contents.[62,63]

Endotoxin-platelet interactions may contribute to endotoxin shock through three mechanisms: through the release of vasoactive substances such as histamine and serotonin; through the formation of intravascular occlusive platelet aggregates; and through the induction of disseminated intravascular coagulation.[16,67] The release reaction has already been briefly discussed and will be discussed further in the hemodynamics section. Platelet aggregation has been extensively investigated in its contributions to endotoxin pathophysiology and shock. Platelets are activated and become sticky in response to endotoxin; clumps of platelets and leucocytes consistently form in the capillary beds of the liver, spleen and particularly of the lung.[4,10,12,25,61,68,69] The lungs are a very important site of endotoxin and host defense interactions. The aggregations that form in small vessels may contribute mechanically to the pulmonary signs observed: dyspnea with pulmonary congestion and edema and foamy mucoid material draining from the noses and mouths of moribund animals.[10,14,53,69] However, these aggregates are not directly involved in the pulmonary hemodynamic alterations, but platelet granular constituents are.[4,10,12,60,65,66,69-77] The release reaction is more important in the pathogenesis of endotoxin shock than is the aggregation reaction, but aggregation usually precedes release.[67] The extraordinary ability of endotoxins to produce tissue

injury through the initiation of coagulation changes, disseminated intravascular coagulation (DIC), has been recognized for over 50 years.[72,73] Endotoxin is a solid phase activator of Hageman factor (Factor XII) which, in turn, initiates the intrinsic coagulation pathway.[74] In addition to this, active Hageman factor can activate prekallikrein to form kallikrein which generates bradykinin, and active Hageman factor is capable of directly activating Factor VII, indicating the probable contribution of the extrinsic coagulation pathway to endotoxin initiated coagulopathies.[75-77] Endotoxin also damages endothelial cells and indirectly activates the extrinsic pathway through the release of tissue thromboplastins.[56] The diagnosis of DIC is made in the laboratory, characterized by decreased platelet numbers, prolongation of the partial thromboplastin time (intrinsic coagulation pathway), prolongation of the prothrombin time (extrinsic coagulation pathway), and elevation in fibrin degradation products. The development of coagulopathies in response to endotoxin has been reported in most species studied, including horses.[61] Subsequently, the formation of microthrombi in many organs, particularly the kidneys and lungs, contributes to mortality. An endotoxemic patient may survive the initial shock, if provided with adequate medical support, only to die from secondary complications of renal failure or pneumonitis.

The commonest hematologic event in endotoxemia is an extreme leucopenia followed by leucocytosis. Neutrophils are the most affected, probably because they not only interact directly with endotoxin, but they are involved in endotoxin-induced platelet and complement interactions as well. When endotoxin attaches to platelets, complement is

activated (alternate and classical pathways), and opsonins and chemotactic factors are generated.[55-57] Because of these, neutrophils and other granulocytes are attracted, and their enzymatic and phagocytic activity increases.[54,57] With increasing levels of endotoxin, the cellular defenses are overcome: complement factors are depleted; platelets aggregate and degranulate; coagulation factors are consumed; neutrophils marginate and migrate into tissues, decline in function, degranulate and degenerate; damaged endothelial cells exfoliate to float freely in the bloodstream; and the reticulo-endothelial system (RES) is depressed.[3,57,67,78]

In neutrophils, endotoxin directly interacts with lysosomal membranes, disrupting membrane transport and causing enzyme leakage.[54] Eventually, neutrophil chemotaxis is inhibited or overcome by humoral factors released from other cells triggered by endotoxin, but the major neutrophil functional defect stems from increased adhesiveness.[57,79] *In vivo* neutrophil adherence significantly increases and is maximal one hour after treatment with endotoxin. This increased adherence is associated with a reciprocal granulocytopenia that is followed by a progressive granulocytosis. This adhesiveness appears to be due to a heat-stable plasma component that requires a heat-labile cofactor that might be complement.[79] Granulocyte adhesiveness *in vitro* and margination *in vivo* are closely associated, complement-dependent phenomena.[80] Also, the *in vivo* granulocytopenia corresponds with histological evidence of sequestration of platelets and granulocytes in pulmonary capillary beds.[4,10,12,67]

Aside from the vascular occlusion and the resulting anoxic damage, the trapped granulocytes release their lysosomes and exacerbate the local tissue injury. Through the use of a Sanders ear chamber, the inflammatory response to endotoxin was directly observed in rabbits.[81] Within three minutes of endotoxin injection, leucocytes were sticking to venule endothelium and would momentarily stick to arteriolar walls. After ten minutes, emboli composed of platelets, leucocytes and erythrocytes appeared. By one hour, some venules and capillaries were occluded, and their endothelium was swollen. Three hours later, erythrocytes and leucocytes passed from the vessels into the surrounding connective tissue with accompanying edema formation. If the rabbit were dying, the microvascular circulation eventually stopped; this did not occur in surviving animals. In animals which recovered, the circulation did not return to normal for approximately two months when thrombi were replaced. Histopathologic examination of the same tissues from nonsurvivors revealed similar pathology: congested microvasculature with focal fibrin thrombi; swelling of endothelial cells; margination and migration of leucocytes; and extravasation of erythrocytes. In animals surviving 72 hours, the thrombotic vessels were markedly necrotic, and the supporting connective tissue was degenerative. On histological examination, the microvascular pathology of the lung was identical to that just described for the ear.

Concurrently, in these same animals, circulating leucocyte numbers decreased dramatically in ten minutes and remained low for six hours, suggesting leucocyte destruction or migration into tissues. In other experiments, when circulating leucocyte counts were followed over 24 hours,

the decrease in total leucocytes was mainly due to a drop in neutrophils which was maximal at about one hour.[9,10,14,15,37,61,64,67,80,82] Monocytes decreased at the same time, lymphocytes decreased later, and at about 24 hours, the leucocytes, particularly neutrophils, increased to normal levels or higher with an increase in immature forms. The total blood granulocyte pool is composed of the circulating granulocyte pool and the marginated granulocyte pool. Radioisotope labeling shows that the transient granulocytopenia caused by endotoxin is due to a shift of cells from the circulating pool to the marginated pool without an increase in total granulocytes. Later, in the granulocytosis phase, all three pools increase without altering the ratio between circulating and marginated cells.[82] These events correlate well with the increased adhesiveness induced by endotoxin.[79] Augmentation of adherence was maximal one hour after endotoxin administration and inhibition of adherence was marked by 24 hours.

These findings explain the shifts between peripheral compartments but don't explain the overall number changes or the appearance of immature forms. This leucocytosis does not appear to be due to a direct effect on blood flow through the marrow, but rather due to a leucocytosis-inducing factor released in response to granulocytopenia.[56] Electron microscopy of bone marrow events showed broad gaps in the normally closed sinus endothelium through which immature cells entered the sinus lumina five minutes after endotoxin. By 15 minutes, the sinus hyperemia had increased, and by 60 minutes, sinus destruction was so extensive that, in some areas, sinus walls were no longer recognizable. The overall result was an increase in immature forms of all cell lines

in the general circulation.[83] Thus, the granulocytosis is the sum of shifting of mature cells back from the marginated pool, and the early release of mature and immature cells from the bone marrow.

The effect of endotoxin on lymphocytes is not well understood, but lymphopenia follows the early granulocytopenia by a few hours. This lymphopenia may be an indirect rather than a direct effect, for serum cortisol is known to increase in many species following endotoxin, and corticosteroids can directly and indirectly affect lymphocytes.[25,26,70,84] In some species, corticosteroids lyse lymphocytes (a dose-related phenomenon), in others initiate sequestration, probably in the bone marrow, and in yet others the reaction is probably mixed.[26,84] Also, migratory function appears impaired, lymphokine production is antagonized, and recruitment of additional lymphoid cells is inhibited.[84,85] Endotoxin appears to interact with receptors on lymphocytes and macrophages; it is mitogenic for B cells but not T cells, transforms B lymphocytes, induces a macrophage chemotactic factor from B cells, activates macrophages, and causes them to release lysosomal enzymes.[85] In the horse, specific interactions of endotoxin and lymphocytes have not been defined, but the lymphopenia is believed to be a secondary effect of endogenous cortisol release.[61] The major effects of endotoxin seem to occur mainly through induced hormones and humoral factors.

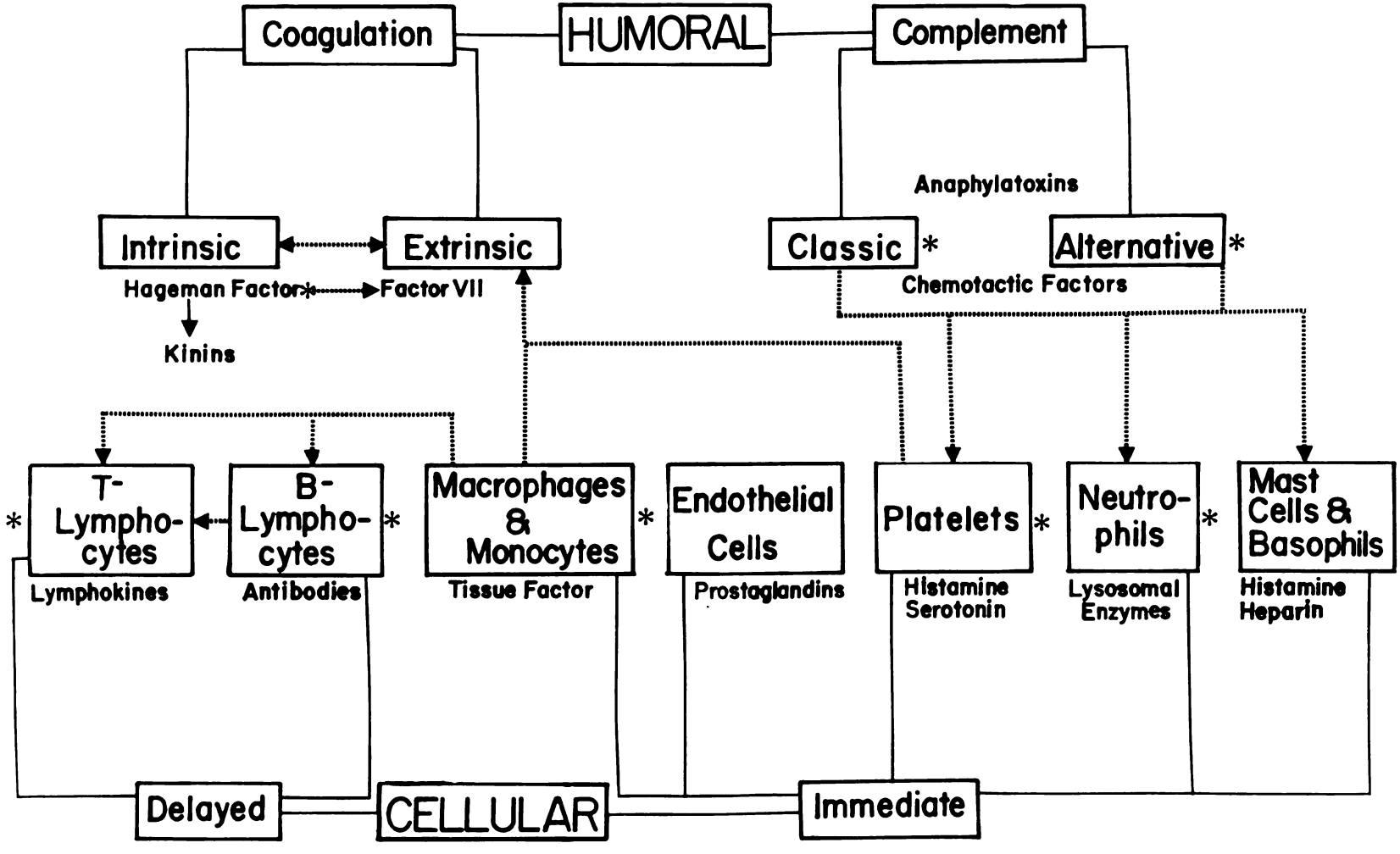
One hematologic hallmark of endotoxin remains to be discussed: the marked elevation of packed cell volume (PCV) observed in endotoxin shock.[14,25,61,64] An endotoxin receptor on erythrocytes has been

isolated, but this is more important in complement activation.[28,43] Epinephrine released during sympathicoadrenal activity in response to arterial hypotension is a potent initiator of splenic contraction, and increases in circulating levels of epinephrine are reported in endotoxin shock.[70,87-90] In species such as the horse with splenic capsule contractility, endotoxin has a marked effect on PCV, and when the spleen contracts, erythrocyte reserves are ejected into the general circulation.[61,64,86,87] A secondary rise in blood viscosity follows the PCV elevation.[87] Other contributions to the PCV increase are losses in plasma volume secondary to increased capillary permeability and increased lymph production.[91] Increased blood viscosity may contribute to the coagulopathies and, therefore, may exacerbate the tissue hypoxia of shock. Figure 1 shows a schematic overview of the interplay of humoral and cellular components.

Hemodynamics of Endotoxemia

From the preceding discussion, it should be obvious that no endotoxin induced event stands alone; the interactions are circuitous and interdependent. Similarly, the hemodynamic alterations and the initiating events of the patient in endotoxin shock are complex and well interrelated. Classically, the hemodynamic response to endotoxin can be divided into three categories on the basis of species differences. The first is characterized by early splanchnic venous pooling and is seen in the dog, rat, mouse, coyote, and bear; the second category is characterized by pulmonary venous pooling, and this group includes the cat, horse, sheep, calf, and rabbit; and lastly, in primates and presumably in man, multiple sites of venous pooling are

FIGURE 1. OVERVIEW OF HOST DEFENSES AND ENDOTOXIN INTERACTIONS
(Sites at which endotoxin is known to exert a direct effect are indicated by *)



suspected.[10-13,17,25,36,66,70,87,91] Each category will be discussed individually using the responses of the dog, horse and monkey as typical of the species in each group. In most species, the vascular reaction to endotoxin is systemic arterial hypotension which develops as a result of decreasing cardiac output due to low venous return, secondary to vascular pooling. In experimental endotoxemia, initially the vasculature is constricted and vascular resistance increases, but later vascular resistance decreases and vessels dilate.[70]

The early reactions in the dog are transitory; portal vein pressure rises with a concurrent fall in central venous pressure. An increase in small intestine weight suggests a further contribution to the splanchnic venous pooling (the mechanisms involved are poorly understood). Also, pulmonary arterial pressure increases due to a rise in both pulmonary arterial and pulmonary venous resistances.[10,92] Pulmonary vascular changes occur rapidly and are over by 30 minutes. Total peripheral resistance rises only slightly, but the cardiac output drops precipitously with a resulting systemic hypotension.[93] Hence, the fall in blood pressure is due to decreased total blood flow, the result of diminished venous return from blood pooling in the liver, and not due to peripheral dilation or myocardial weakness. This initial hypotension later yields to a return to normal pressure levels, reflecting the release of pooled blood from the liver. The final, prolonged hypotension leads to fatal shock, but the exact site (or sites) of the causative venous pooling is unknown; it does not appear to be the liver.[94] The progressive, late-occurring fall in total peripheral resistance, coupled with low cardiac output, results in irreversible shock.[93]

Following slow intravenous injection of endotoxin in the horse, early changes include an immediate increase in pulmonary arterial pressure, a marked decrease in systemic arterial pressure, and only a slight rise in central venous pressure. Mesenteric pooling does not appear to occur, but there is a striking early rise in pulmonary vascular resistance and subsequent pulmonary venous pooling. The systemic arterial pressure returns to normal levels and remains there for several hours. With the later occuring preterminal hypotension, again venous pooling probably occurs, but the exact site remains unknown. The total peripheral resistance may be low at this time.[13,14]

In monkeys, endotoxin shock is characterized by decreased cardiac output, decreased central arterial blood pressure, and decreased total peripheral resistance. In contrast to other species, the arterial blood pressure declines gradually and after relatively stable spells, drifts into shock levels. Portal venous pressure rises only slightly, but there is no evidence of hepatic or splanchnic pooling; mesenteric blood flow does not change at any time, but mesenteric vascular resistance declines at about 45 minutes after endotoxin injection and remains low for the remainder of the experiment. A probable deficient venous return would account for a decreased cardiac output and the hypotension.[70,95]

The patterns differ, but the hemodynamic events of all species have similar features, as do the hematologic events of endotoxemia, and not surprisingly, the two areas are interrelated; white blood cell and platelet numbers decrease concurrently with the early pressure changes. The relationship is direct; a component of whole blood is responsible for the pressor effects of endotoxin, and more specifically, platelets

are essential for the hemodynamic pathophysiology.[10,69,71] Concurrently with the thrombocytopenia characteristic of endotoxemia, serum concentrations of several vasoactive substances, particularly pressors, increase. Plasma serotonin reaches a maximum level 15 seconds after endotoxin injection and rapidly disappears.[16,65,66,96,97] Platelet aggregation is not essential for the pressor response, but release is; concurrent administration of a serotonin antagonist will not block the thrombocytopenia, i.e., will not block clumping but will block release.[69] As previously mentioned, bradykinin increases through Hageman factor activation and may account for some of the hypotension.[74,88,89] Histamine and the catecholamines also occur in higher than normal levels with endotoxin shock.[69,70,88,89] Histamine is found in the platelets of most species and in the leucocytes of almost all species.[69] There is not only an immediate histamine increase, but there is also a later rise, apparently "induced" histamine, synthesized in or near vascular endothelial cells following endotoxin.[98]

The release of biogenic amines in endotoxemia is slower and more gradual than that in anaphylaxis, but similarities do exist: profound hemoconcentration, leucopenia, thrombocytopenia, and pulmonary arterial hypertension with decreased central venous pressure. In acute systemic anaphylaxis in the horse, histamine and serotonin concentrations appeared coupled with white blood cell and platelet numbers; very early in anaphylactic shock, whole blood histamine concentrations rise sharply, but later a much lower histamine level coincides with the most profound period of leucopenia and thrombocytopenia.[99] Horse leucocytes are particularly rich in histamine and serotonin, and while levels of biogenic

amines have not been measured in the horse, on the basis of the induced anaphylaxis data and the known equine platelet response data, it is not unreasonable to assume that the hemodynamic changes in horses in endotoxin shock are attributable to vasoactive amines such as histamine and serotonin.[62,63,99-101] Histamine is a constrictor of smooth muscle of the trachea and pulmonary arteries and veins of horses.[99,101] Histamine also produces hepatic vein constriction in the dog and is a potent systemic arteriolar dilator. Catecholamines increase in many species in endotoxin shock, possibly accounting for the transient increase in blood pressure following the initial decrease.[12,88] The effects are probably dose related, and the wide variability in vasoactive amine responses among species may explain the varied species responses to endotoxin.

Structure and Molecular Interactions of Polymixin B

As previously mentioned, polymixin B is a cyclic, cationic polypeptide; it has a molecular weight of approximately 1250 daltons and contains threonine, α, γ-diaminobutyric acid, and a nine carbon saturated fatty acid.[29,30] This family of drugs is nephrotoxic, and a relationship between intramolecular structure and toxicity has been suggested. While polymixin B is considerably less nephrotoxic than some of the other polymixins, the toxicity of these peptides may be partly due to their D-amino acid content.[30] In levels approximating the usual therapeutic dose, polymixin B does not produce clinical signs of depression of renal function in normal dogs.[102] Also, the polymixin antibiotics are inactivated *in vitro* by tissues because they bind to the phospholipids of cell membranes; polymixin B persists in liver, kidney, brain, heart, muscle, and lung for as long as 72 hours.[103] The binding

is through electrostatic attraction to the negatively charged phospholipids of membranes.[37,103] Another interesting example of the binding of the drug is *in vitro* degranulation of mast cells by polymixin B.[104] While the exact significance of this is unknown, in one study the control animals given polymixin B sulfate alone developed a leucocytosis, possibly secondary to *in vivo* mast cell degranulation, and the release of chemotactic factors.[37] Overall, the tendency of the polymixins to bind the mammalian membranes may be an important feature of their toxicity, but it does not appear to account for their beneficial effects.

Polymixin B is bactericidal and is generally more active against gram-negative than gram-positive organisms. Susceptible bacteria absorb considerable amounts of the drug, which is a surface active agent, much like a cationic detergent. Detergents disorganize cell membranes and denature certain proteins, and similarly, polymixin combines with and disorganizes cell structures that are responsible for osmotic equilibrium; combination of polymixin B and sensitive bacteria results in leakage of low molecular weight cell constituents and thereby kills the cells. More specifically, polymixin B combines with the inner layer or membrane of the bacterial cell wall, the region of the cell responsible for endotoxin activity.[30] The bacterial action of the polymixins is probably due to complexing of the drug to bacterial lipids; very significantly, polymixin B binds to the lipid A portion of endotoxin.[27,29] The stoichiometric relationship appears to be one to one with the formation of complexes with higher molecular weights.[29] These complexes are reversible, and because the bond forms at the lipid A region, all types of endotoxin should respond similarly to polymixin B.[29,30]

Modification of Endotoxin Pathophysiology by Polymixin B

The cyclic cationic polypeptides, especially polymixin B, directly neutralized endotoxin in a series of experiments in which the antibiotic was admixed with endotoxin prior to administration.[28,31] In various other experiments, primarily in laboratory animals, polymixin B decreased the lethality, neutralized the coagulation derangements, and abrogated the hematologic and hemodynamic aberrations induced by endotoxin.[31,32,34-43,105] In most of these experiments, the polymixin B and the endotoxin were combined before injection. However, in one experiment, the antibiotic was given before and after endotoxin, and survival rate still improved significantly.[43] In another trial, polymixin B admininstered after endotoxin injection ameliorated the endotoxin-induced coagulopathy.[41] And, during a clinical trial, polymixin B did not improve the survival rate in patients with coagulopathies secondary to liver cirrhosis, but it did partially ease the coagulation disorders.[44]

An ideal endotoxin-blocking dose of polymixin B has not been derived in any species, but most investigators have used a dose of 2.5 mg/kg of body weight. To be effective, polymixin B has to combine with the lipid A moiety of endotoxin before it becomes fixed in tissues. With some bacteria, the parent bacterial susceptibility and the susceptibility of the derived endotoxin to polymixin B are related; however, these two qualities can also be quite divergent.[106] Whereas sensitive bacteria absorb much larger amounts of the antibiotic than do resistant strains, and polymixin B usually neutralizes endotoxin derived from sensitive microorganisms more efficiently than that derived from resistant bacteria, it is still effective against the endotoxins of nonsusceptible

bacteria.[28,33] The beneficial mechanism of action of polymixin B on endotoxin-induced pathophysiology occurs through combination of the drug with the lipid A portion of the molecule, i.e., the portion of the molecule that is constant between bacterial species and responsible for the bioactivity of endotoxin.

Experimental Rationale

The spectrum of clinical endotoxin syndromes ranges from clinically normal animals with pyrexia and/or hematologic alterations (absolute leucopenias) to massive, lethal shock. But the majority of experimental studies, particularly in horses, has been done with lethal doses of endotoxin.[9-15] Thus, the pathophysiology of nonlethal endotoxemia is not well defined. Similarly, in the treatment of endotoxemia and endotoxin shock, most of the agents or techniques used are directed at various manifestations of endotoxin pathology and not specifically at the endotoxin molecule.[8,17,18,25] There is a direct interaction between polymixin B and the lipid A portion of the endotoxin molecule.[28,29,31,33,106] Lipid A is the bioactive portion of the endotoxin molecule and is present in all commercially prepared and naturally occurring forms of endotoxin.[16,25,46] With these factors in mind, this study was designed to investigate sublethal endotoxemia, and to attempt to block or modify the resulting pathophysiology. Specifically, the febrile, hematologic and hemodynamic responses were measured and evaluated in ponies given very low doses of endotoxin, and comparisons of these parameters with and without polymixin B were made in an attempt to evaluate the potential of this antibiotic as a therapeutic adjunct in the treatment of clinical endotoxemia.

MATERIALS AND METHODS

Experimental Animals

Mature, clinically normal Shetland ponies (half were females and half were castrated males), weighing 130 to 240 kg, were brought indoors and allowed to acclimate to their surroundings for at least 24 hours before being readied as experimental preparations. All animals were examined and were found to be free of clinically detectable disease. A total of 14 experiments was performed.

Surgical Procedure

Twenty-four hours prior to the experimental period, the ponies were anesthetized with 0.2% thiamylal sodium in a 5% glyceryl guaiacolate plus 5% dextrose solution. Following intubation, anesthesia was maintained with halothane and oxygen. The right side of the neck was clipped and surgically prepared, the skin and cutaneous colli muscle were incised, and with blunt dissection, the external jugular vein and the carotid artery were exposed. After freeing these structures from the surrounding tissues, the carotid artery was cannulated with a No. 7 French teflon end-hole catheter (United States Catheter and Instruments Corporation, Glen Falls, New York) which was filled with heparinized saline (0.05% sodium heparin in physiological saline, beef lung extraction, Upjohn Company, Kalamazoo, Michigan) and secured with catgut ligatures (American Cyanamid Company, Pearl River, New York). This catheter was used to measure systemic arterial pressure. Two catheters

were introduced into the jugular vein; a No. 7 French balloon-tipped Swan-Ganz triple lumen catheter (Columbus Instruments International Corporation, Columbus, Ohio) was positioned in the pulmonary artery, and a second No. 7 French end-hole teflon catheter was positioned in the right atrium. Both were filled with heparinized saline prior to placement. The Swan-Ganz catheter, which has a thermistor near the tip, was used to measure body temperature, pulmonary arterial pressure and the changes in blood temperature during cardiac output determination. The second teflon catheter was used to record right atrial pressure and served as the injection port for the cold saline bolus used in the cardiac output determination by thermodilution. Both of these catheters were secured with catgut ligatures, and the skin was closed with a nonabsorbable suture material (Suprylon, Pfrimmer, West Germany) in a simple continuous pattern. All catheters were connected to pressure transducers (Statham P_{23} Db, Statham, Hato Rey, Puerto Rico), and all catheter placements were verified by pressure measurements (PDV-22 pressure preamplifier, Electronics for Medicine, Incorporated, White Plains, New York) and by the shape of pressure tracings recorded on a two-channel recorder (Model 2M-SB, MFE Recorders, Salem, New Hampshire). Transducers were calibrated prior to each experiment against a mercury manometer. After catheter placement was verified, all catheters were secured by a bandage, and the ponies were allowed to recover from anesthesia.

Following recovery, each pony was returned to a stall and was given free choice of water and feed. The next day, the pony was placed in a specially designed restraining box and was allowed to become accustomed

to the box and equipment for 60 to 90 minutes before control values were taken. Catheter patency was maintained with periodic flushes of heparinized saline, and the pressure transducers were placed at the level of the base of the heart (approximately the point of the shoulder).

Experimental Protocol

Control measurements were made of the following variables: systemic arterial blood pressure (systolic, diastolic and mean), mean right atrial pressure, pulmonary arterial pressure (systolic, diastolic and mean), body temperature, cardiac output, packed cell volume, total white blood cell count, and differential count of white blood cells.

After control measurements were taken, each pony was given one of the following solutions at the following level: 1 μg/kg body weight Escherichia coli 026:B6 Boivin extracted endotoxin, lot 639746 (Difco Laboratories, Detroit, Michigan) in physiological saline at a concentration of 1 μg/ml; 2.5 mg/kg body weight polymixin B sulfate U.S.P. (Pfizer, Incorporated, New York) prepared by dissolving the micronized powder in 5% dextrose solution and sterilizing by microfiltration (Nalgene Labware Division, Sybron Corporation, Rochester, New York); 2.5 mg/kg body weight polymixin B sulfate and 1 μg/kg body weight endotoxin; or sterile physiological saline. All solutions were given by slow infusion (average infusion time approximately five minutes) through the right atrial catheter, with the exception of those experiments in which both endotoxin and polymixin B were given. In those, the two agents were given through separate catheters simultaneously: the polymixin B through the right atrial catheter and the endotoxin through the

proximal hole of the Swan-Ganz catheter. A total of 14 experiments was performed: 5 endotoxin only, 5 endotoxin plus polymixin B, 2 polymixin B only, and 2 physiological saline only.

Measurement of Blood Pressure

Blood pressure was measured directly using the heparinized saline filled indwelling catheters connected to the transducers, amplifier and recorder. Catheters were assumed to be accurately measuring blood pressure when blood could easily be aspirated through them, and they could readily be flushed. With the electronic damping of the recorder, the mean pressure values were recorded.

Measurement of Cardiac Function

Cardiac output was measured by thermodilution;[107-109] a ten ml volume of cold physiologic saline (0-2°C) was injected manually as rapidly as possible into the right atrium through the teflon catheter. The blood temperature change in the pulmonary artery was sensed by the thermistor at the tip of the Swan-Ganz catheter, the other end of which was connected to the cardiac output computer (Cardiotherm -500, Columbus Instruments International Corp., Columbus, Ohio). The temperature of the injectate was detected by a second thermistor connected to the computer. At least five cardiac output determinations were made, and an average of these was recorded for each time period.

Cardiac output and blood pressure readings were taken prior to infusion, at 15 minutes, at 30 minutes, and hourly from one to four hours following infusion. Times were measured from the finish of the infusion. Additionally, in the endotoxin experiments, the first noticeable blood pressure rise was 10 minutes after injection, and in the

endotoxin-polymixin B experiments, the first noticeable pressure increase was at 50 minutes. At these times, additional recordings of cardiac output and pressures were made.

Hematologic Parameters

All blood samples were collected in evacuated glass tubes containing EDTA for anticoagulation (EDTA vacutainer; Becton, Dickinson and Co., Rutherford, N.J.). The blood samples were drawn from the carotid artery catheter immediately prior to infusion, at 15 minutes, at 30 minutes, at 1 hour, and at hourly intervals from 2 hours to 10 hours, and at 24 hours after infusion. Again, times were measured from the finish of the infusion. The packed cell volumes were determined by the microhematocrit method using a microcapillary centrifuge (International Equipment Co.; Boston, Massachusetts), and plasma solids were determined by refractometer (American Optical Corporation; Buffalo, New York).

White blood cell counts were determined manually by using a hemocytometer (Spencer Bright-Line, American Optical Co., Buffalo, New York) charged to contain a 0.9 mm^3 volume. The hemocytometer was filled from a Unopette dilution vial (Unopette Test 5855; Becton, Dickinson and Co., Rutherford, New Jersey) which has a buffered ammonium oxalate solution that hemolyzes mature red blood cells and preserves the platelets, leucocytes and reticulocytes. The Unopette system has a capillary pipette that fills automatically to a 20 µl volume which, when mixed with the vial's contents, becomes a 1:100 dilution. After allowing 10 minutes for the red cells to hemolyze, the hemocytometer was charged, and the number of white blood cells in its block of nine squares was

counted. Ten percent of the count was added to the number of cells counted and the sum multiplied by 100 to arrive at the number of leucocytes/mm^3. The differential leucocyte count was determined by microscopic examination of 100 cells of a Wright's stained blood smear. All smears were made immediately after the collection of blood.

Statistical Analysis

Data were analyzed in a two-way analysis of variance, each animal as its own control, in a completely randomized block design. Mean differences were compared by the Student-Newman-Keuls' test. The Student's t test was used to detect any significant difference between means in the endotoxin experiments and the endotoxin and polymixin B experiments when a significant change occurred in one of these sets of experiments. The coefficient of variability was used to determine the relative variability of the sets of experiments. All comparisons were made at the $p=0.05$ level. The use of the coefficient of variability allows comparison of variability, thereby indicating the presence or lack of uniformity within the data for a given set of experiments.[110,111]

RESULTS

Clinical Observations

In all of the experiments, the ponies remained bright and alert, and their oral mucous membrane color and capillary refill time remained normal. At about 10 or 15 minutes after endotoxin injection, the ponies' pulse and respiration rates increased moderately, and they began to tremble. The trembling decreased gradually and stopped after about 2 hours. Also, at about 15 to 30 minutes after injection, the ponies "rattled" as they breathed, as if they had fluid in their tracheas. This lasted only a few minutes, and during this time, several of the ponies coughed a few times. No exudate was ever seen at the noses or mouths of these ponies.

Of the group given endotoxin plus polymixin B, three also began trembling, but in these the trembling began about 50 to 60 minutes after injection. The trembling stopped within 20 minutes in all but one pony which continued to tremble for about 1 hour. Also, one of these trembling ponies coughed several times after the trembling began.

The body temperatures of both the endotoxin group and the endotoxin plus polymixin B group were significantly elevated 1 hour after infusion (Figure 2, Table 1). The time courses of the temperature rises of these two groups were parallel out to the 3 hour sampling time. The mean maximum temperature of the endotoxin group was 38.8^{o}C at 4 hours post-infusion.

By 24 hours, the body temperature returned to normal. In the endotoxin plus polymixin B group, the mean maximum temperature was 39°C, which was reached at 2 hours.

FIGURE 2

Changes in body temperature from pre-infusion values in ^{o}C. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * = statistically significant change (p=0.05) from pre-infusion value, using randomized complete block analysis of variance and Student-Newman-Keuls' tests. (*) = both means represented by these points are significantly different from respective pre-infusion means.

FIGURE 2

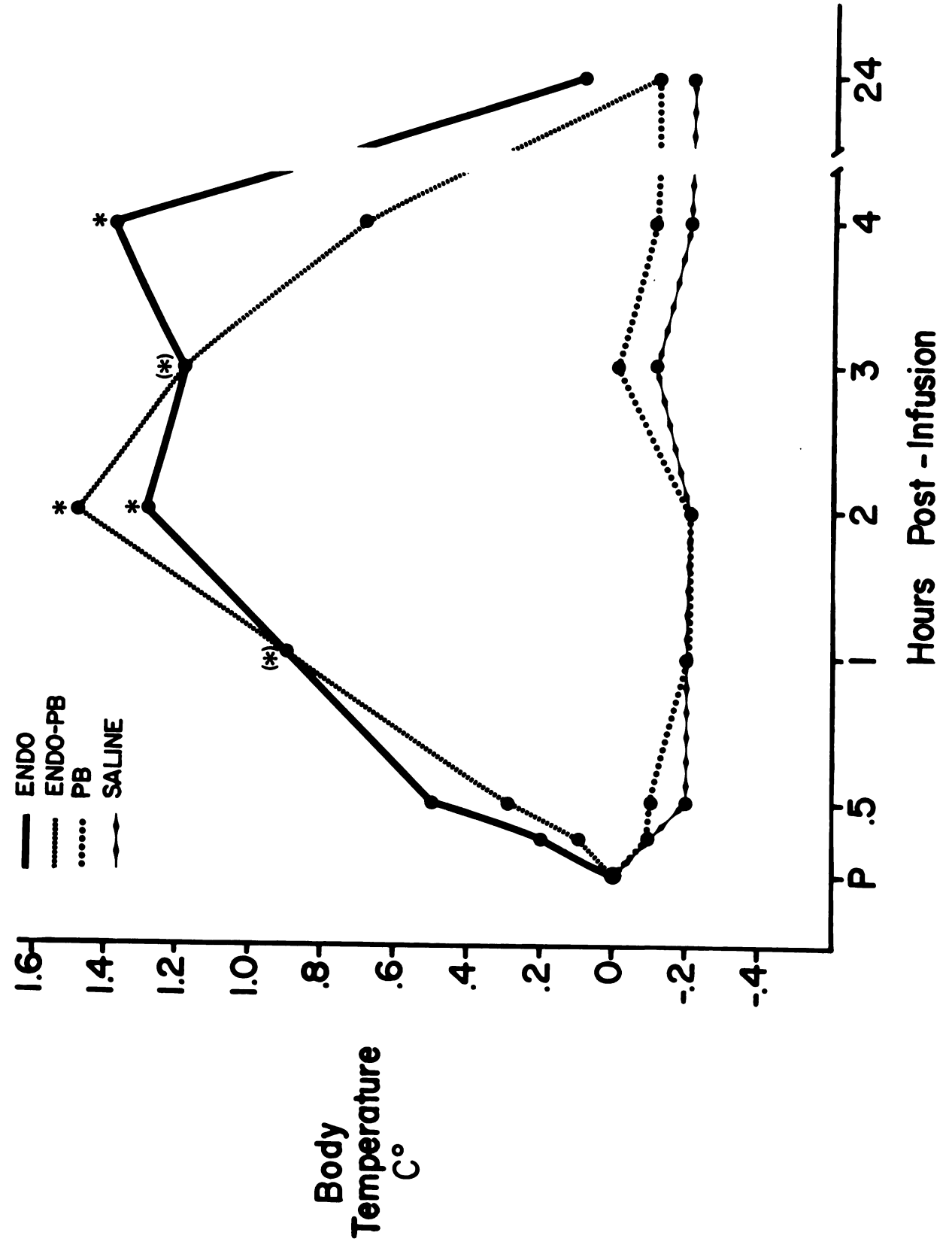

ENDO
ENDO-PB
PB
SALINE
Body Temperature C°
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
-.2
-.4
P
.5
1
2
3
4
24
Hours Post-Infusion
*
(*)

TABLE 1. Statistical comparisons between means of body temperature (^{o}C).

Endotoxin

pre-inf.	24 hr.	15 min.	30 min.	1 hr.	3 hr.	2 hr.	4 hr.
37.4	37.5	37.7	37.9	38.3	38.6	38.7	38.8

Endotoxin plus Polymixin B

pre-inf.	24 hr.	15 min.	30 min.	4 hr.	1 hr.	3 hr.	2 hr.
37.5	37.5	37.6	37.8	38.2	38.4	38.7	39

(Means underscored by the same line are not significantly different at the 0.05 level.)

Coefficients of variability - body temperature

$CV_{ENDO} = 0.86\%$
$CV_{ENDO+PB} = 1.21\%$
$CV_{PB} = 0.40\%$
$CV_{SAL} = 0.40\%$

In this group, the mean body temperature at 4 hours approached the pre-infusion temperature mean. In the polymixin B treated and the saline treated groups, no significant differences in temperature were seen. Student's t test performed on pre-treatment, 1 hour, and 24 hour means showed no significant difference between the body temperatures of the ponies given endotoxin and those given endotoxin and polymixin B.

Hematologic Effects

Following endotoxin infusion, the total white count began to decline sharply; by 15 minutes, a significant decrease in circulating white blood cell count appeared (Figure 3, Table 2). This decline continued until it reached its lowest point at 1 hour. From there, the mean count began to rise and approached the pre-infusion level at 8 hours. A significant increase had developed by 24 hours. Similarly, in the endotoxin plus polymixin B group, the total white count was also lowest at 1 hour but remained within the normal range for horses. Unlike the endotoxin group, there was a significant increase by 8 hours that persisted to 10 hours. At 24 hours, the total white count had returned to pre-infusion levels. In the polymixin B treated group, total white counts fluctuated only slightly with a significant increase 4 hours after the infusion of the antibiotic. That increase did not persist. The saline treated group showed even less variation in white count over the experimental period, and at no time did a significant difference from the pre-injection mean appear. Student's t test performed on the pre-treatment means, the 1 hour means and the 24 hour means of the ponies treated with endotoxin and the ponies treated with endotoxin plus polymixin showed a significant difference between the two treatment groups only at the 1 hour comparison.

FIGURE 3

Changes in circulating white blood cell counts from pre-infusion values, P, in 10^3 cells/mm^3. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance.

FIGURE 3

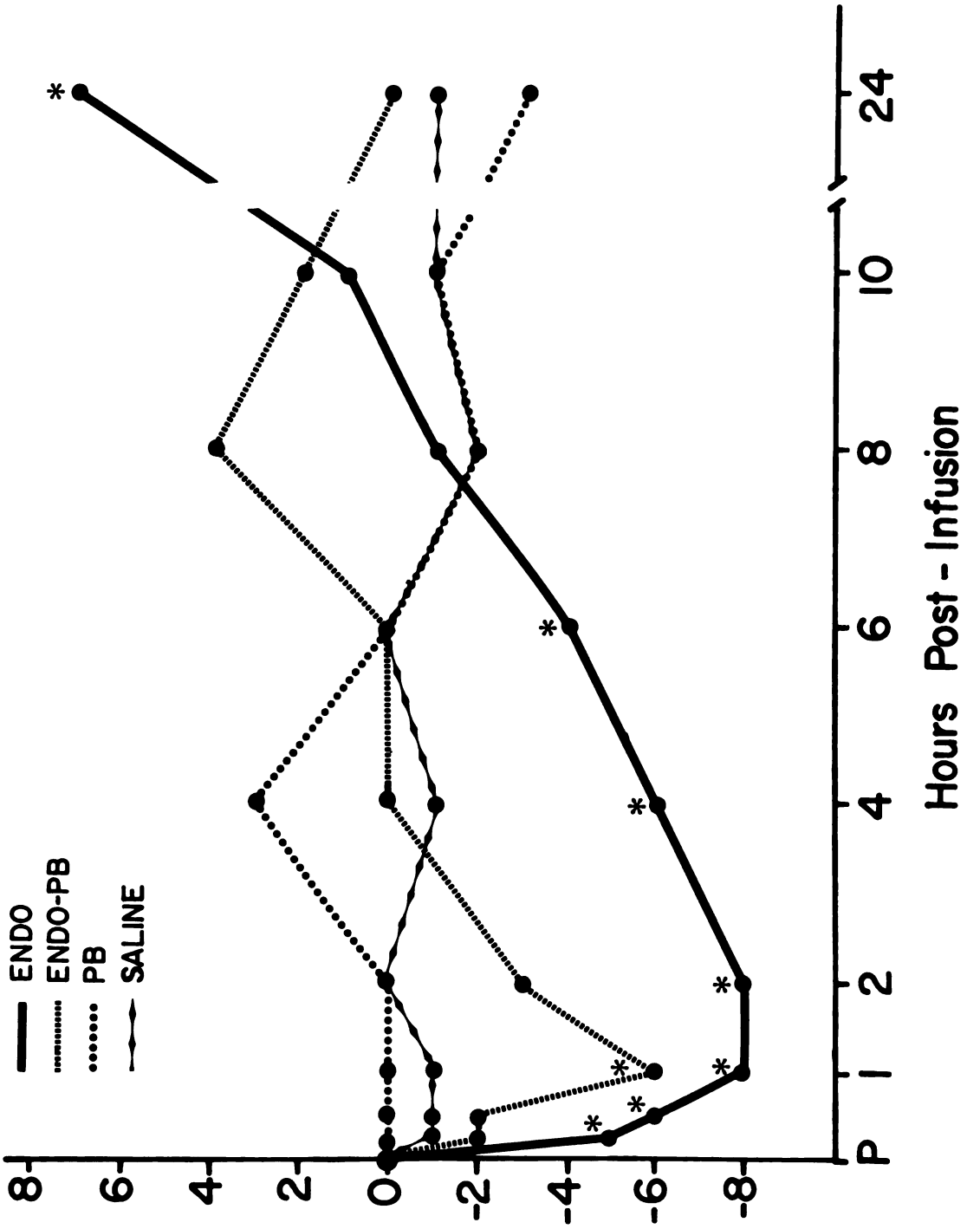

ENDO
ENDO-PB
PB
SALINE
Circulating WBC's
8
6
4
2
0
-2
-4
-6
-8
P
1
2
4
6
8
10
24
Hours Post - Infusion

TABLE 2. Statistical comparisons between means of circulating white blood cell counts (cells/mm^3).

Endotoxin

1 hr.	2 hr.	30 min.	4 hr.	15 min.	6 hr.	8 hr.	pre-inf.	10 hr.	24 hr.
2,840	3,360	4,730	5,260	6,500	7,120	10,200	11,060	12,160	18,420

Endotoxin and Polymixin B

1 hr.	2 hr.	30 min.	4 hr.	15 min.	6 hr.	24 hr.	pre-inf.	10 hr.	8 hr.
8,240	10,540	11,560	11,720	13,380	13,580	13,660	13,940	16,480	17,960

Polymixin B

24 hr.	8 hr.	10 hr.	30 min.	1 hr.	6 hr.	2 hr.	15 min.	pre-inf.	4 hr.
11,350	12,100	12,900	13,500	13,700	13,800	13,850	13,900	14,400	16,700

(Means underscored by the same line are not significantly different at the 0.05 level.)

There was no significant difference in the white blood cell counts throughout the experiments for the saline treated ponies. The mean was 12,790 cells/mm^3.

Coefficients of variability - white blood cells

CV_{ENDO} = 21.3%
$CV_{ENDO+PB}$ = 19.4%
CV_{PB} = 9.5%
CV_{SAL} = 10%

Most of the endotoxin-induced changes in the total leucocyte counts were attributable mainly to the changes in segmented neutrophils (Figure 4, Table 3). Again, the greatest decrease in cell numbers occurred 1 hour post-infusion. This decrease was profound with a pre-treatment mean of 6,801 cells/mm^3 going to a 1 hour mean of of 202 cells/mm^3, a 97% reduction. Mature neutrophils began returning to circulation after 1 hour but did not approach the pre-infusion level until 8 hours. An absolute increase of 11,510 cells/mm^3 was present at 24 hours, representing an increase of 69% over the pre-treatment level. In contrast, the 1 hour mean for the endotoxin plus polymixin B group was 5,139 cells/mm^3, and was 56% of the pre-treatment count. By 4 hours post-infusion, the mean count approached the pre-treatment mean and continued to increase to a maximum of 12,030 cells/mm^3 at 8 hours, a 31% increase over the pre-treatment level. There was no significant difference in the segmented neutrophil means throughout the experiments for either the polymixin B-treated or the saline-treated ponies. The coefficients of variability show mild to moderate heterogeneity of the data. Student's t tests performed on pre-infusion, 1 hour and 24 hour means between pony groups treated with endotoxin and those treated with endotoxin plus polymixin B showed a significant difference only at the 1 hour comparison.

Non-segmented neutrophil counts basically followed the patterns of the segmented neutrophil counts (Figure 5, Table 4). In those animals given endotoxin, the few non-segmented neutrophils initially present disappeared by 1 hour. At 4 hours, the non-segmented neutrophils were increased over pre-infusion levels, and at 10 hours, a mean of 1,605 cells/mm^3 represented 13% of the total white count. At 24 hours,

FIGURE 4

Changes in circulating segmented neutrophil counts from pre-infusion values, P, in 10^3 cells/mm^3. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance.

FIGURE 4

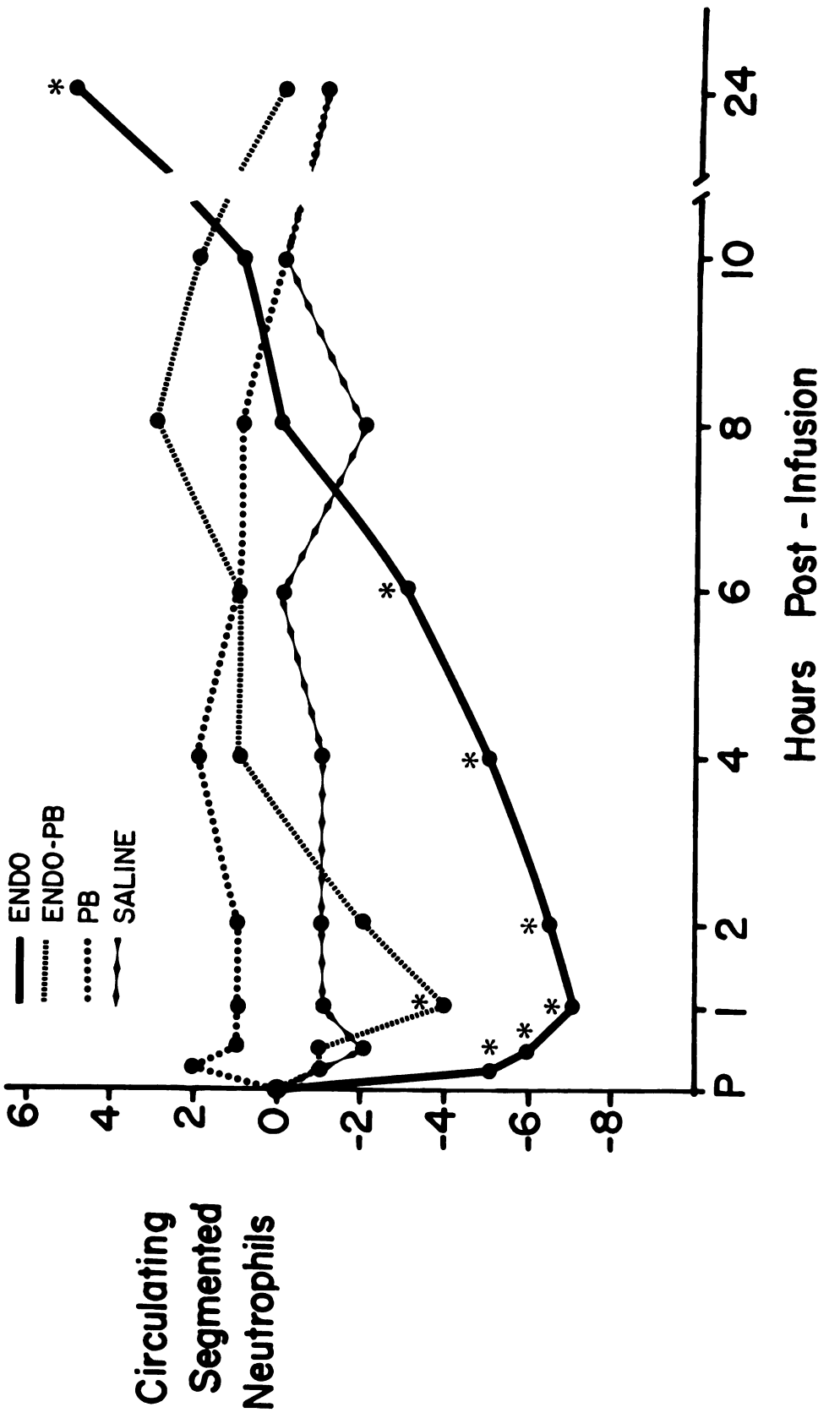

ENDO
ENDO-PB
PB
SALINE
Circulating Segmented Neutrophils
6
4
2
0
-2
-4
-6
-8
P
1
2
4
6
8
10
24
Hours Post - Infusion

TABLE 3. Statistical comparisons between means of circulating segmented neutrophil counts (cells/mm^3).

Endotoxin

1 hr.	2 hr.	30 min.	15 min.	4 hr.	6 hr.	8 hr.	pre-inf.	10 hr.	24 hr.
202	448	955	2,228	2,327	4,103	6,658	6,801	7,995	11,510

Endotoxin plus Polymixin B

1 hr.	2 hr.	15 min.	30 min.	24 hr.	pre-inf.	4 hr.	6 hr.	10 hr.	8 hr.
5,139	7,147	8,071	8,311	8,506	9,117	9,827	10,120	10,790	12,030

(Means underscored by the same line are not significantly different at the 0.05 level.)

There was no significant difference in the segmented neutrophil counts throughout the experiments for either the polymixin B treated or the saline treated ponies. The means were 7,763 and 5,997 cells/mm^3 respectively.

Coefficients of variability - segmented neutrophils:

CV_{ENDO} = 31%
$CV_{ENDO+PB}$ = 23%
CV_{PB} = 11%
CV_{SAL} = 20%

FIGURE 5

Changes in circulating non-segmented neutrophil counts from pre-infusion values, P, in 10^3 cells/mm^3. ENDO indicates infusion with 1 µg/kg BW, n=5. ENDO-PB indicates infusion with 1 µg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance.

FIGURE 5

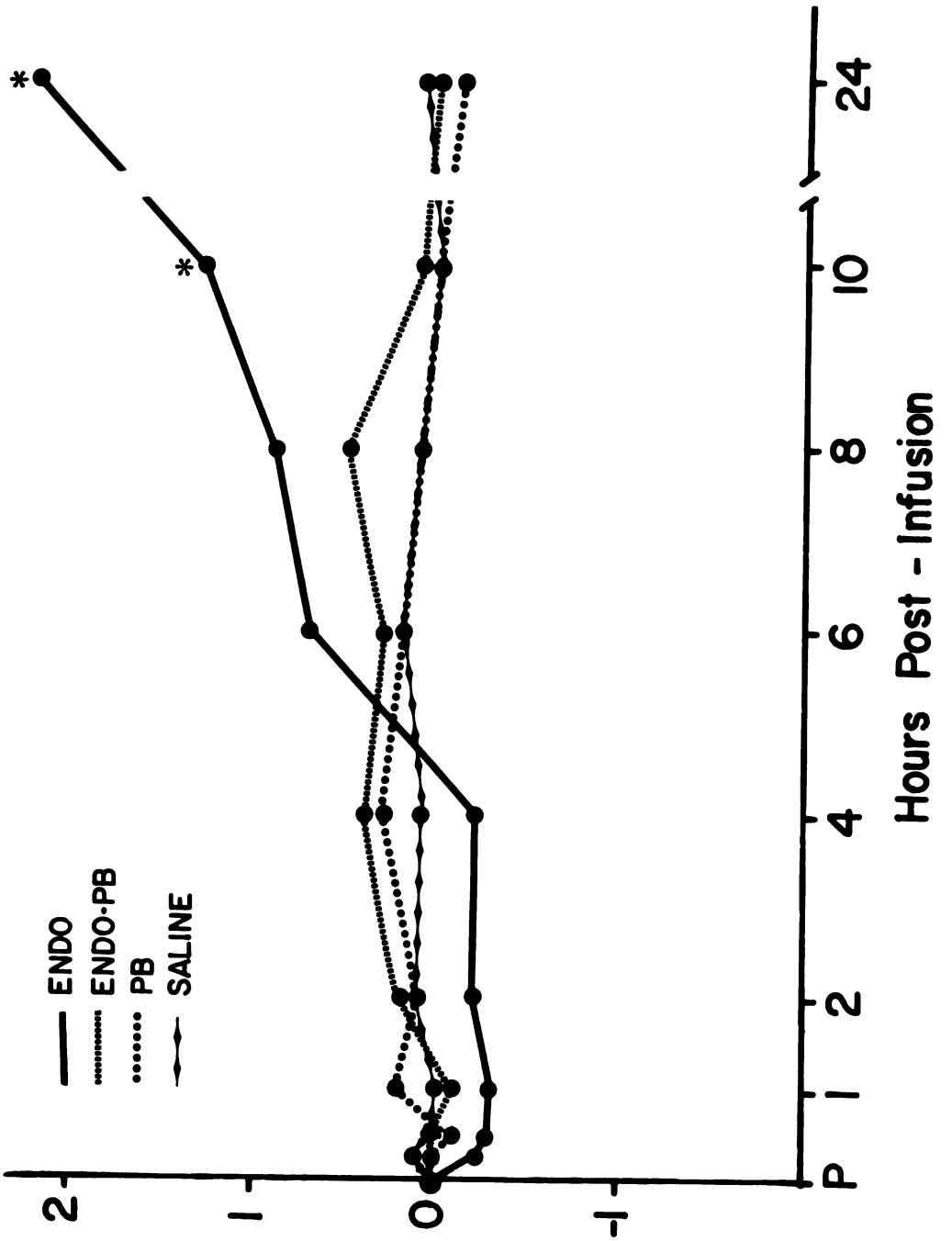

ENDO
ENDO-PB
PB
SALINE
Circulating
Non-segmented
Neutrophils
2
1
0
-1
P
1
2
4
6
8
10
24
Hours Post - Infusion
*
*

TABLE 4. Statistical comparisons between means of circulating non-segmented neutrophil counts (cells/mm^3).

Endotoxin

1 hr.	30 min.	2 hr.	15 min.	pre-inf.	4 hr.	6 hr.	8 hr.	10 hr.	24 hr.
0	28	53	92	256	524	846	1,241	1,605	2,528

(Means underscored by the same line are not significantly different at the 0.05 level.)

There were no significant differences in the non-segmented neutrophil counts throughout the experiments for the endotoxin plus polymixin B treated ponies, the polymixin B treated ponies or the saline treated ponies. The means were 333, 117 and 70 cells/mm^3 respectively.

Coefficients of variability - non-segmented neutrophils

CV_{ENDO} = 99%
$CV_{ENDO+PB}$ = 103%
CV_{PB} = 83%
CV_{SAL} = 180%

non-segmented neutrophils made up 14% of the total white blood cell count. The 10 hour and the 24 hour means for the endotoxin treated ponies were significantly greater than the pre-treatment means, but on Student's t test comparisons between this group and the endotoxin plus polymixin B treated animals, there was no significant difference between the pre-infusion, 1 hour and 24 hour means. The coefficients of variability for these experiments were extremely large, reflecting the tremendous variability in the non-segmented neutrophil numbers (range within the endotoxin experiments: 0 to 2,528 cells/mm^3).

The changes in circulating lymphocytes were not as dramatic as were those in the neutrophils (Figure 6, Table 5). In the endotoxin group, a decreasing trend was maximal at 6 hours with a mean of 1,797 cells/mm^3, which represented 52% of the pre-infusion mean. At 24 hours, the mean of 3,372 cells/mm^3 was only slightly above the pre-infusion mean of 3,435 cells/mm^3. Over the course of the endotoxin plus polymixin B experiments, lymphocytes declined to 2,469 cells/mm^3 at 4 hours, a 37% decrease. At 24 hours, they rose to 4,993 cells/mm^3, a 27% increase. There were no significant differences in lymphocyte means in the polymixin B or saline experiments, but at 4 hours a sharp increase in lymphocyte numbers was noted in the polymixin B treated animals. The means were 5,420 and 7,010 cells/mm^3 respectively. Student's t test comparisons between means of endotoxin and endotoxin plus polymixin B experiments on pre-infusion, 6 hour and 24 hour means indicated no significant difference between these experiments at the 0.05 level.

There were no statistically significant differences in the monocyte counts in any of the four treatment categories. The means for the

FIGURE 6

Changes in circulating lymphocyte counts from pre-infusion values, P, in 10^3 cells/mm^3. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2.

FIGURE 6

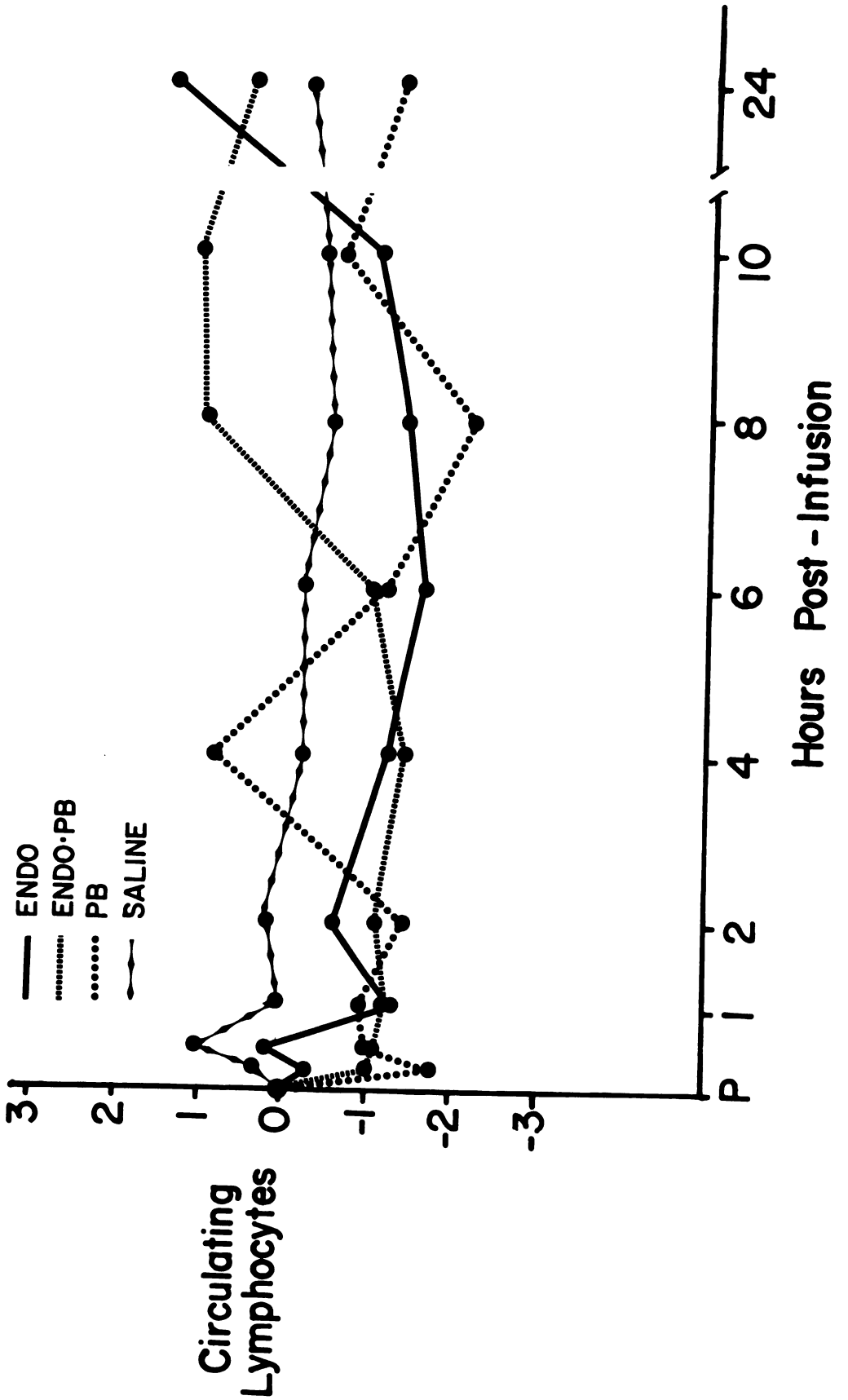

ENDO
ENDO·PB
PB
SALINE
Circulating Lymphocytes
3
2
1
0
-1
-2
-3
P
1
2
4
6
8
10
24
Hours Post-Infusion

TABLE 5. Statistical comparisons between means of circulating lymphocyte counts (cells/mm^3).

Endotoxin

6 hr.	8 hr.	4 hr.	10 hr.	1 hr.	2 hr.	15 min.	pre-inf.	30 min.	24 hr.
1,797	2,033	2,194	2,420	2,532	2,789	3,057	3,435	3,651	3,732

Endotoxin plus Polymixin B

4 hr.	1 hr.	30 min.	2 hr.	6 hr.	15 min.	pre-inf.	24 hr.	8 hr.	10 hr.
2,469	2,764	2,828	2,847	2,872	2,896	3,925	4,405	4,936	4,993

(Means underscored by the same line are not significantly different at the 0.05 level.)

There were no significant differences in the lymphocyte means throughout the experiments for the polymixin B treated or the saline treated ponies. The means were 5,460 and 7,010 cells/mm^3 respectively.

Coefficients of variability - lymphocytes

$CV_{ENDO} = 31\%$
$CV_{ENDO+PB} = 26\%$
$CV_{PB} = 15\%$
$CV_{SAL} = 14\%$

endotoxin, the endotoxin plus polymixin B, the polymixin B, and the saline groups were 239, 176, 205, and 228 cells/mm^3 respectively. The variability of this data was enormous; the coefficients of variability for the endotoxin, the endotoxin plus polymixin B, the polymixin B, and the saline groups were 118%, 90%, 83%, and 50% respectively.

A slight change was noted in the packed cell volume in the animals treated with endotoxin without any accompanying increase in total plasma solids. The mean PCV started to increase immediately after infusion, and at one hour was 34%, an increase of 31% over the pre-treatment mean of 26% (Figure 7, Table 6). The endotoxin plus polymixin B group had no significant change in PCV until 24 hours when it increased. The Student's t test comparison showed a significant difference between the 1 hour means but not the pre-infusion or the 24 hour means of the endotoxin and the endotoxin plus polymixin B experiments. In the polymixin B groups, one pony had an unexplained increase in PCV at 24 hours, and in the saline group, one pony had an unexplained decrease in PCV at 24 hours. The coefficients of variability show mild heterogeneity of the PCV data.

Hemodynamic Effects

Systemic arterial blood pressure did not change significantly in any of the four experiments (Figure 8). However, some trends appeared; at 10 minutes in the endotoxin treated animals, systemic arterial pressure increased sharply, at 1 hour fell to its lowest point and at 4 hours increased again. In the endotoxin plus polymixin B animals, systemic arterial pressure decreased very slightly, then rose above pre-infusion levels at 1 hour and returned to pre-infusion levels at

FIGURE 7

Changes in packed cell volume from pre-infusion values, P, in %. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance.

FIGURE 7

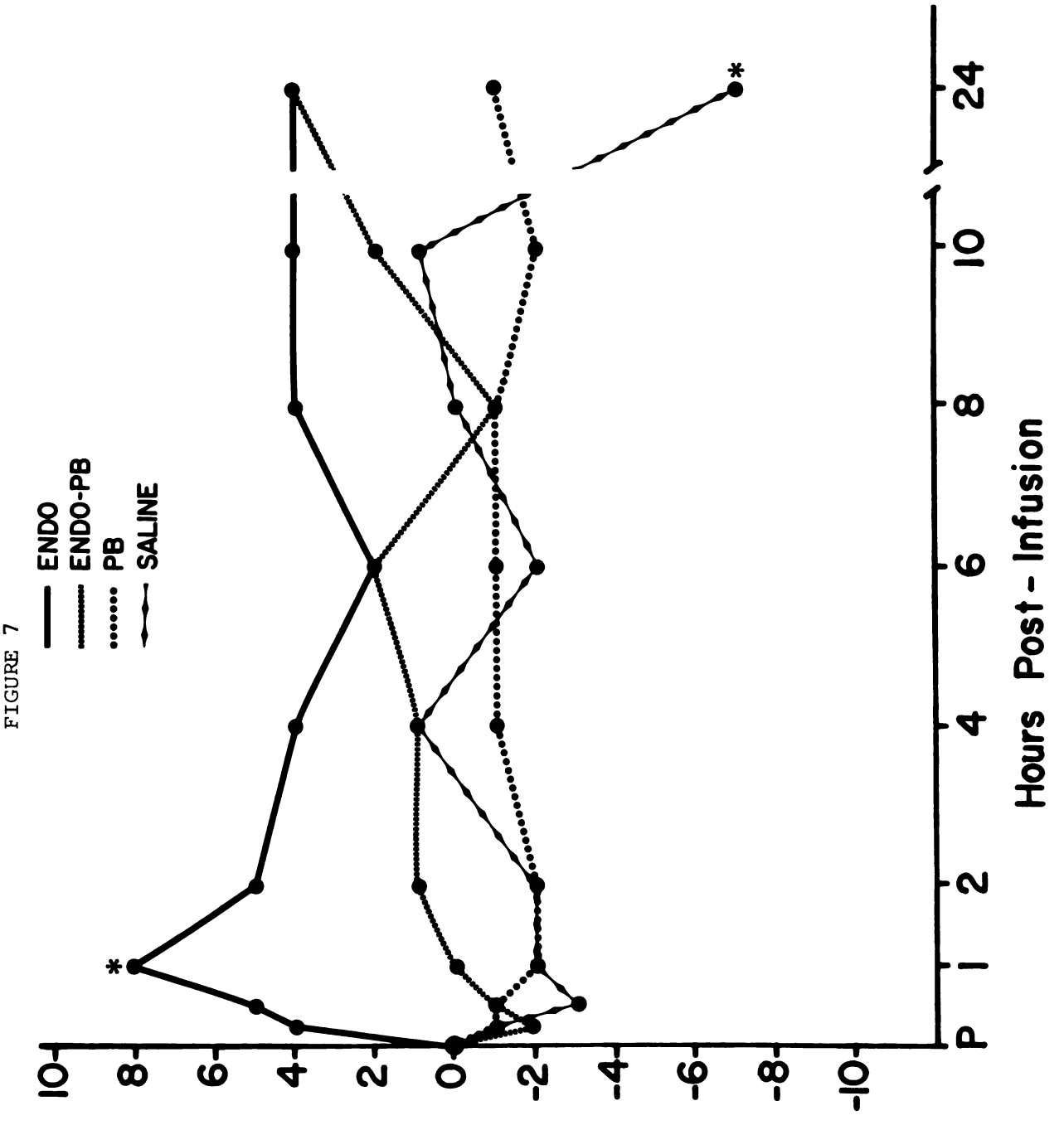

ENDO
ENDO-PB
PB
SALINE
Packed Cell Volume %
10
8
6
4
2
0
-2
-4
-6
-8
-10
P
1
2
4
6
8
10
24
Hours Post- Infusion
*
*

TABLE 6. Statistical comparisons between means of packed cell volumes (%).

Endotoxin

pre-inf.	6 hr.	10 hr.	15 min.	8 hr.	4 hr.	24 hr.	30 min.	2 hr.	1 hr.
26	28	28	28	30	30	30	31	31	34

Endotoxin plus Polymixin B

15 min.	8 hr.	pre-inf.	30 min.	1 hr.	4 hr.	2 hr.	6 hr.	10 hr.	24 hr.
23	24	25	25	25	26	26	27	27	29

Saline

24 hr.	30 min.	2 hr.	1 hr.	6 hr.	15 min.	pre-inf.	8 hr.	4 hr.	10 hr.
22	26	27	27	28	28	29	29	30	30

(Means underscored by the same line are not significantly different at the 0.05 level.)

There was no significant difference in the packed cell volumes throughout the experiments for the polymixin B treated ponies. In this group, the average mean PCV was 25%.

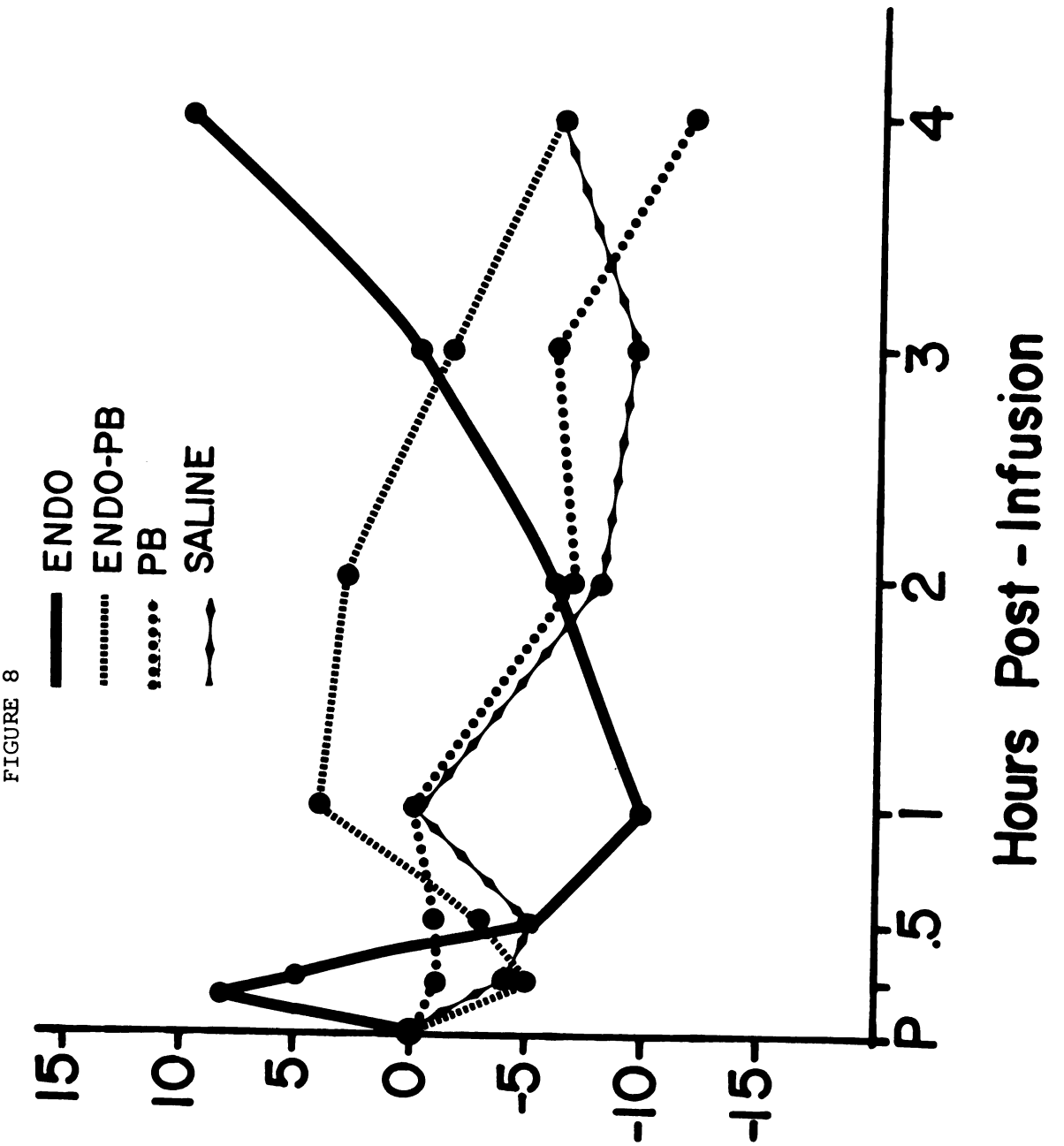
Coefficients of variability - packed cell volume

CV_{ENDO} = 11%
$CV_{ENDO+PB}$ = 9%
CV_{PB} = 7%
CV_{SAL} = 3%

FIGURE 8

Changes in systemic arterial blood pressure from pre-infusion values, P, in mm Hg. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2.

FIGURE 8


ENDO
ENDO-PB
PB
SALINE
Systemic Arterial Pressure mmHg
15
10
5
0
-5
-10
-15
P
.5
1
2
3
4
Hours Post-Infusion

3 hours. In the polymixin B and saline treated animals, systemic arterial pressure declined gradually over the experiments. The coefficients of variability for the endotoxin, endotoxin plus polymixin B, polymixin B, and saline groups were 12%, 7%, 3%, and 3% respectively, revealing mild heterogeneity of the data.

The changes in mean pulmonary arterial blood pressure were abrupt and transient (Figure 9, Table 7). Ten minutes after injection with endotoxin, the mean pulmonary arterial pressure increased to 55 mm Hg, 136% over the pre-infusion mean of 25 mm Hg, started to decrease immediately and was not significantly different from pre-infusion levels at 30 minutes. In the endotoxin plus polymixin B group, the mean pulmonary arterial pressure at 50 minutes was 33 mm Hg, an 83% increase over the pre-infusion level of 18 mm Hg. It returned to the pre-infusion level by 2 hours. There was no significant change in mean pulmonary arterial pressure in either the polymixin B or the saline groups, each with a mean of 21 mm Hg. At 10 minutes, there was a significant difference between the endotoxin treated ponies and the endotoxin plus polymixin B treated ponies when compared by the Student's t test at the 0.05 level. There was no significant difference by t test between these 2 groups at pre-infusion, 50 minutes and 4 hours.

There was no significant change in mean right atrial pressure during any of the experiments. The mean of the endotoxin group of 7 mm Hg was somewhat higher than the endotoxin plus polymixin B, the polymixin B and the saline means, all at 3 mm Hg.

There was no significant change in cardiac output during any of the four experiments (Table 8). The mean cardiac output was 15 L/min for the endotoxin group, 16 L/min for the endotoxin plus polymixin B group,

FIGURE 9

Changes in pulmonary arterial blood pressure from pre-infusion values, P, in mm Hg. ENDO indicates infusion with 1 μg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 μg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance. Of the means marked (*), only the endotoxin plus polymixin B value at 30 min. is significantly different from its pre-infusion value by two-way analysis of variance.

FIGURE 9

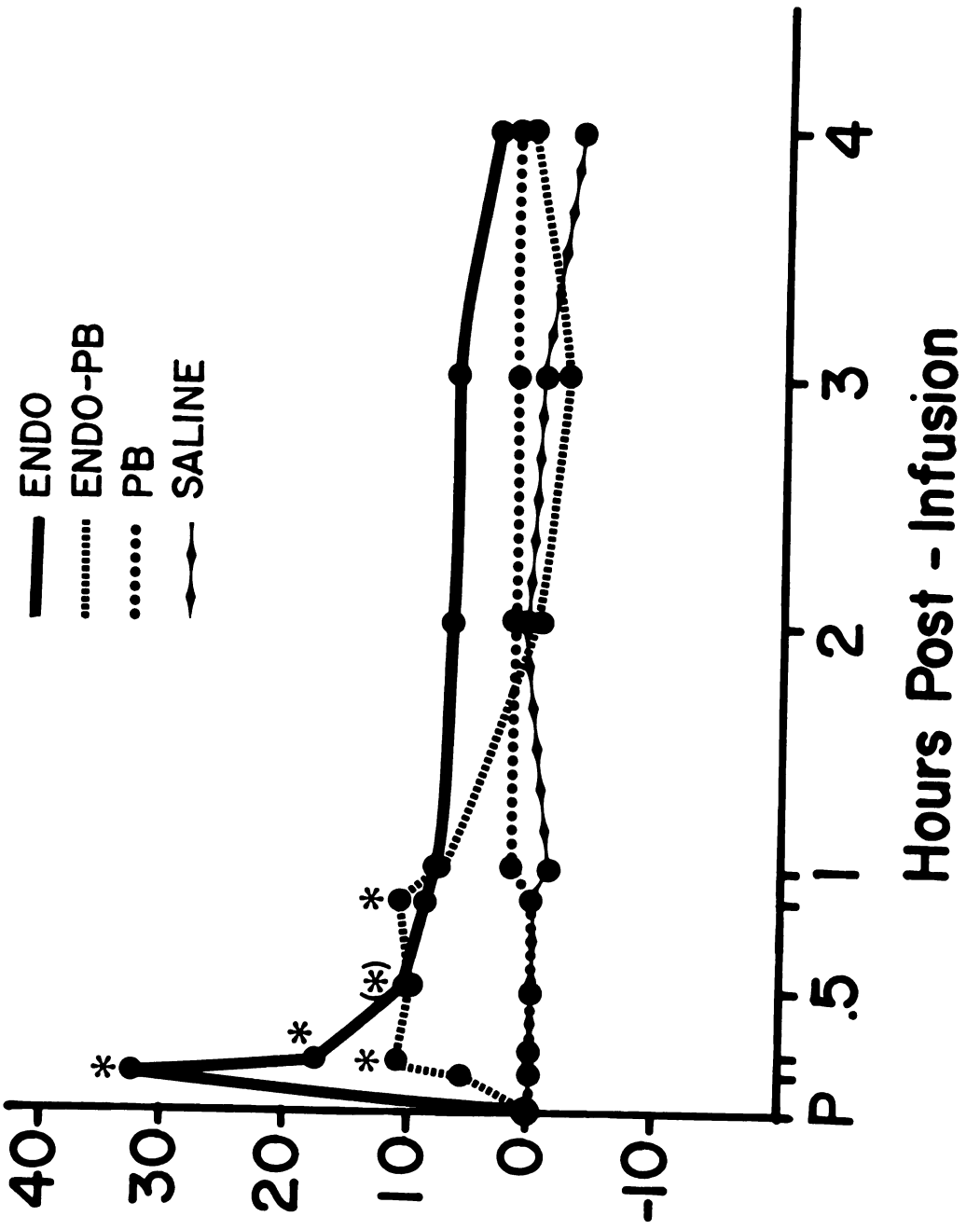

ENDO
ENDO-PB
PB
SALINE
Pulmonary Arterial Pressure mmHg
40
30
20
10
0
-10
P
.5
1
2
3
4
Hours Post - Infusion
*
*
*
(*)
*

TABLE 7. Statistical comparisons between means of pulmonary arterial blood pressure (mm Hg).

Endotoxin

pre-inf.	4 hr.	2 hr.	3 hr.	30 min.	1 hr.	15 min.	10 min.
25	30	33	33	35	35	42	58

Endotoxin plus Polymixin B

3 hr.	4 hr.	pre-inf.	2 hr.	1 hr.	30 min.	15 min.	50 min.
18	19	20	20	30	33	33	33

(Means underscored by the same line are not significantly different at the 0.05 level.)

There was no significant difference in mean pulmonary arterial blood pressure throughout the experiments for the polymixin B treated or the saline treated ponies. The mean pressures were both 21 mm Hg.

Coefficients of variability - mean pulmonary arterial blood pressure

$CV_{ENDO} = 19\%$
$CV_{ENDO+PB} = 24\%$
$CV_{PB} = 8\%$
$CV_{SAL} = 10\%$

TABLE 8. Cardiac outputs.

(L/min)

	pre-inf.	10 min.	15 min.	30 min.	1 hr.	2 hr.	3 hr.	4 hr.
Endotoxin	15.4	14.8	15.5	15	16.1	14.4	14.1	14.0
Endotoxin + PB	15.7		16	16.3	16.1	16.1	15.6	15.5
Polymixin B	16		17.1	16.9	16.9	17.2	16.9	16.4
Saline	16.8		17.8	17.6	18.7	17.8	17.2	17.8

17 L/min for the polymixin B group, and 18 L/min for the saline group. The coefficients of variability show only mild heterogeneity: CV_{ENDO} = 10%, $CV_{ENDO+PB}$ = 7%, CV_{PB} = 5%, CV_{SAL} = 3%.

The total peripheral vascular resistance of all of the endotoxin treated ponies abruptly increased 10 minutes after infusion (Figure 10, Table 9). This 10 minute mean was not significantly different from the pre-treatment mean, but it was significantly different from the 1 hour mean. There were no significant differences throughout the other three experiments. Their means were 7.4 mm Hg/L/min for the endotoxin plus polymixin B group, 7.2 mm Hg/L/min for the polymixin B group and 6.8 mm Hg/L/min for the saline group. Student's t test comparisons between the endotoxin-treated group revealed no significant difference at any time.

At 10 minutes, the pulmonary vascular resistance of the endotoxin-treated ponies abruptly increased and was significantly different from the mean of any other sampling time for this experiment (Figure 10, Table 10). Pulmonary vascular resistance also increased slightly in the endotoxin plus polymixin B-treated ponies, but not enough to make the 15 minute mean significantly different from the pre-treatment mean. Student's t test comparisons of the means for endotoxin and the endotoxin plus polymixin B groups were different at the 0.05 level for the pre-infusion, 15 minute and 4 hour means.

FIGURE 10

Total peripheral resistance and pulmonary vascular resistance in mm Hg/L/min. ENDO indicates infusion with 1 µg/kg BW endotoxin, n=5. ENDO-PB indicates infusion with 1 µg/kg BW endotoxin simultaneously with 2.5 mg/kg BW polymixin B, n=5. PB indicates infusion with 2.5 mg/kg BW polymixin B, n=2. SALINE indicates infusion with 1 ml/kg BW normal saline, n=2. * represents significant difference from pre-infusion values by two-way analysis of variance.

FIGURE 10

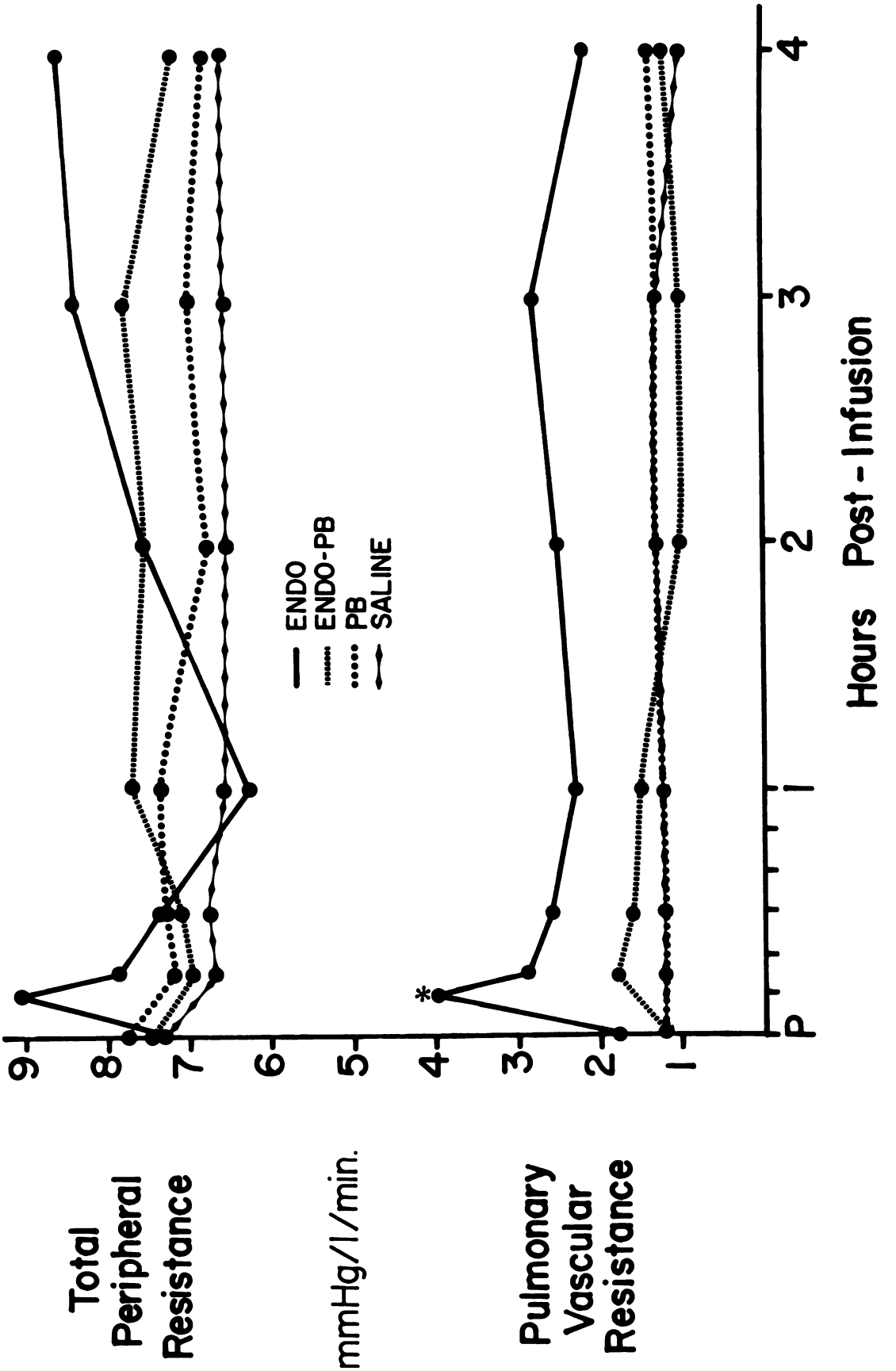

Total Peripheral Resistance
mmHg/l/min.
Pulmonary Vascular Resistance
9
8
7
6
5
4
3
2
1
*
ENDO
ENDO-PB
PB
SALINE
P
1
2
3
4
Hours Post-Infusion

TABLE 9. Statistical comparisons between means of total peripheral resistance (mm Hg/L/min).

Endotoxin

1 hr.	30 min.	pre-inf.	2 hr.	15 min.	3 hr.	4 hr.	10 min.
6.3	7.4	7.4	7.6	7.9	8.4	8.6	9.1

(Means underscored by the same line are not significantly different at the 0.05 level.)

There were no significant differences in total peripheral vascular resistance throughout the experiments for the endotoxin plus polymixin B, the polymixin B or the saline groups. The mean resistances were 7.4, 7.2 and 6.8 mm Hg/L/min respectively.

Coefficients of variability - total peripheral resistance

$CV_{ENDO} = 16\%$
$CV_{ENDO+PB} = 13\%$
$CV_{PB} = 5\%$
$CV_{SAL} = 3\%$

TABLE 10. Statistical comparisons between means of pulmonary vascular resistance (mm Hg/L/min).

Endotoxin

pre-inf.	4 hr.	1 hr.	3 hr.	2 hr.	30 min.	15 min.	10 min.
1.9	2.2	2.3	2.5	2.8	2.6	2.9	4.1

Endotoxin plus Polymixin B

3 hr.	2 hr.	pre-inf.	4 hr.	1 hr.	30 min.	15 min.
1.0	1.0	1.2	1.2	1.5	1.6	1.8

(Means underscored by the same line are not significantly different at the 0.05 level.)

There were no significant differences in pulmonary vascular resistance throughout the experiments for either the polymixin B treated or the saline treated ponies. The mean pulmonary vascular resistances were 1.28 and 1.21 mm Hg/L/min respectively.

Coefficients of variability - pulmonary vascular resistance

CV_{ENDO} = 22%
$CV_{ENDO+PB}$ = 28%
CV_{PB} = 8%
CV_{SAL} = 12%

DISCUSSION

The effects of the very low endotoxin dose used in this study were extremely mild. The only clinical signs of endotoxemia observed in these experiments were pyrexia with trembling and the tracheal "rattle" with an occasional cough. Endotoxin is a well-known releaser of endogenous pyrogen which in turn interacts with the preoptic region of the anterior hypothalamus, the thermoregulatory center of the brain. The result of this interaction appears to be an upward displacement of the normal temperature set-point, and the animal actively attempts to raise its temperature by heat-seeking and by vasoconstriction and shivering.[5,6,7] In these experiments, the endotoxin treated animals began to tremble about 10 or 15 minutes after infusion, and by 1 hour after infusion had succeeded in raising their body temperatures significantly. The trembling stopped when the temperatures peaked at 2 hours, indicating that the new set-point had probably been achieved. The temperatures were decreased at 3 hours but had turned upward again at 4 hours without any trembling. By 24 hours, the temperatures were back to normal.

The tracheal rattle and the accompanying cough of the ponies of these experiments are presumptive evidence of the formation of pulmonary edema as has been reported by other investigators of endotoxemia in horses and several other species.[9,13,14,53,69,70] The increased respiratory effort may be the cumulative effect of this edema mechanically obstructing the airways and the effects of the endotoxin-released

vasoactive amines (particularly histamine) on respiratory smooth muscle.[4,69] In these experiments, these signs were mild and transient, but perhaps a naturally occurring sustained endotoxemia could predispose an animal to a secondary pulmonary invader.

The leucocyte responses of the ponies of these experiments were similar to those reported by other investigators. The profound decrease in white blood cell numbers at 1 hour was due to the loss of neutrophils from circulation. The decrease in lymphocytes noted at 6 hours was not of the same magnitude and did not influence the total white count greatly. In vitro and in vivo studies of neutrophil kinetics in people and several animal species show that neutrophil adhesiveness is maximal 1 hour after endotoxin exposure.[57,79,80] The data of the present experiments correspond well with the reported adhesiveness changes, and if the horse is similar to other species in its response to endotoxin, the neutrophils probably were marginated in the pulmonary capillary bed. With the subsequent inhibition of adhesion, which is maximal at 24 hours, a leucocytosis develops.[79,82] The significant increase in the non-segmented neutrophils observed in these endotoxin experiments suggests that endotoxin also accelerates bone marrow release of immature cells in horses as has been documented in laboratory animals.[83] The non-segmented neutrophils began appearing in higher than normal levels at 6 hours, and the increase became significant at 10 hours. This suggests that, while some of the initial elevation in non-segmented neutrophils may come from their release from pulmonary vascular sequestration, the bone marrow is making a significant contribution by 10 hours.

The characteristic elevation in PCV following endotoxin administration in the horse was seen in the present experiments as well.[9,14,51,64,114] Total plasma solids did not change in the animals of the present experiments, suggesting that splenic contraction initiated by endotoxin-induced catecholamine release resulted in the increase in PCV. The mechanism of possible catecholamine release in the present experiments is unexplained because at the time at which the PCV began to increase, there was no initiating systemic hypotension. The increase in PCV of the present experiments was significant only at the 1 hour sampling time and did not appear to persist as did the PCV increases reported by other investigators of endotoxemia in the horse; it may be a dose-related phenomenon.[9,13]

In contrast to other reports of experimental endotoxemia in ponies, there was no immediate decrease in systemic arterial blood pressure in the present experiments.[13,14] In all ponies of the present experiments receiving endotoxin only, an upward trend in systemic arterial blood pressure was observed at 10 minutes, but this trend did not represent a significant change. The variability of this data might account for the lack of significance. The later occurring decrease in systemic arterial blood pressure in the present experiments was maximal at one hour. This and the 10 minute increase were identical to the changes seen in other horse endotoxemia experiments.[13,14]

With regard to pulmonary arterial pressure, the results of the present experiments were slightly different than previous endotoxin experiments in ponies.[13,14] In the present experiments, the pulmonary arterial blood pressure increased significantly at 10 minutes, but a significant increase was not noted in the other endotoxin experiments

in ponies. In previous reports, no mention was made of changes in vascular resistance. Since cardiac output did not change in the present experiments, the observed changes in vascular pressures must have been due to increased vascular resistance. Systemic vascular resistance showed an upward trend at 10 minutes and a downward trend at 1 hour, but neither was large enough to be significant. With pulmonary vascular resistance, there was a significant increase at 10 minutes, a finding noted in several other species.[10,92] In many species, cardiac output decreases following endotoxin administration and results in systemic hypotension.[93] In the present experiments, no significant decrease in cardiac output occurred, and the hypotensive trend noted at 1 hour was not significant.

The pulmonary vascular resistance increase noted in other species has been attributed to the release of vasocactive substances (e.g., histamine, serotonin) from the platelet aggregates in the pulmonary vascular bed.[4,10,12,60,65,66,67,71] In a pilot study conducted prior to the experiments discussed in this thesis, no significant change in platelet numbers was observed in ponies treated with the same low endotoxin dose. Consequently, platelet counts were not included in the present experimental protocol. In people, many drugs, including halothane, acetylpromazine and barbiturates, are known to inhibit secondary platelet aggregation, and this effect can be recognized by *in vitro* testing with an aggregometer.[113] In this study, *in vitro* aggregation assessed by aggregometer was blocked, presumably by the anesthetic agent. Therefore, whether or not the platelets of these ponies aggregated and released could not be determined. There is no available literature to confirm that this does occur in horses. However, the

ponies in these experiments were tested before and after the anesthetic episode required for catheterization and exhibited normal platelet aggregation prior to anesthesia but had abnormal aggregation 24 hours after anesthesia. While secondary aggregation is blocked by these drugs, release is not, and from what is known about horse platelets, it is reasonable to speculate that horse platelets also release their constituents after endotoxin exposure.[60,61] To carry this speculation further, these platelet constituents are probably responsible for the pulmonary vasoconstriction. The most likely vasopressor would be histamine, which in induced systemic anaphylaxis is a known constrictor of vascular and respiratory smooth muscle in the horse.[99] In induced and systemic anaphylaxis in the horse, histamine and serotonin concentrations appear to correlate with severe decreases in numbers of leucocytes and platelets.

The overall effect of polymixin B when administered concurrently with endotoxin was an amelioration of the hematologic and hemodynamic effects induced by endotoxin. All of the cellular effects were modified, and the effects that were noted were of much shorter duration than those seen with endotoxin alone. The hemodynamic effects of endotoxin were lessened by the polymixin B, e.g. the increase in pulmonary arterial pressure, or delayed, e.g. the trend in systemic arterial pressure. Polymixin B blocks the biologic activity of endotoxin by binding to the lipid A portion of the molecule, but the complex is assumed to dissociate over time.[26-28,31-43] The systemic hemodynamic alterations in the animals treated with endotoxin and polymixin B, since they were later appearing (at 50 minutes), may represent the activity of the endotoxin released from the complexes.

The only pathologic effect of endotoxin not affected by concurrent treatment with polymixin B was body temperature. The fevers of both the endotoxin group and the endotoxin plus polymixin B group started at the same time, peaked similarly and started to decline at 3 hours. There was little difference in the magnitude of the fevers of the two groups. Yet, the other endotoxin pathophysiology was modified by polymixin B. This would suggest that the part of the endotoxin molecule responsible for the induction of endogenous pyrogen is not the lipid A moiety or that the receptors on the cells responsible for endogenous pyrogen production recognize another portion of the endotoxin molecule. This concept, that the pyrogenic and other pathologic responses might be separable, has also been suggested by experiments in which endotoxin tolerance was induced in horses and dogs.[112,114] After repeated doses of endotoxin, some of the cellular reactions were lessened, but the fever was not modified.

Since the stoichiometric relationship between endotoxin and polymixin B appears to be one to one, increasing the dose of polymixin B with the 1 μg/kg body weight endotoxin dose would probably further modify the pathophysiology. This would suggest that in a naturally occurring case in which higher levels of endotoxin are involved, a higher dose of polymixin B would probably be necessary to realize any benefit. Polymixin B may also have a direct effect on leucocytes. While not statistically significant, leucocytes did increase at 4 hours in those ponies given polymixin B only. A small part of the increase came from the segmented neutrophils, but most of the increase came from

the lymphocytes. The mechanism is unknown, but polymixin B is known to degranulate mast cells, and a similar leucocytosis has been observed in rabbits.[37,104]

Polymixin B pre-treatment has had limited success in laboratory animal endotoxin experiments.[41,43] And, in a clinical trial, polymixin B partially improved the coagulopathy secondary to liver cirrhosis but did not improve the overall survival rate.[44] It is likely that if this drug is going to be able to improve the outcome of a naturally occurring endotoxemia, it should be used as early in the course of the condition as possible.

SUMMARY

This study shows that in ponies, a minute amount of endotoxin produces significant decreases in leucocyte numbers and increases in pulmonary vascular resistance and body temperature. At these degrees of pathology, these changes may not be very dangerous in their own right, but a natural, sustained, low-grade endotoxemia may prove enough of a breach of body defenses to allow further infection or complication.

Polymixin B will block many of the pathologic effects of endotoxin in ponies when given concurrently with endotoxin, but whether or not this benefit can be realized clinically remains to be proven. Endotoxemia is often not recognized until after the animal is showing severe clinical signs. By that time, many pathologic events have taken place. However, in those situations in which there is a potential for endotoxemia to develop, such as immediately after correction of a large bowel displacement or shortly after a known aspiration of ingesta, perhaps polymixin B could serve as a useful adjunct in the medical management of these cases.

LIST OF REFERENCES

1. Nolan, J.P., Ali, M.V.: Endotoxemia in liver disease. Lancet. 1:999, 1974.

2. Jacob, A.J., Goldberg, P.K., Bloom, N., Degenshein, G.A., Kozinn, P.J.: Endotoxin and bacteria in portal blood. Gastroenterology. 72:1268-1270, 1977.

3. Young, L.S., Martin, W.J., Meyer, R.D., Weinstein, R.J., Anderson, E.T.: Gram-negative rod bacteremia: Microbiologic, immunmologic, and therapeutic considerations. Annals of Int. Med. 86:456-471, 1977.

4. Clowes, G.H.A.: The pulmonary response to sepsis and endotoxin: Clinical and experimental observations, in Urbaschek, B., Urbaschek, R., Neter, E. (eds): Gram-Negative Bacterial Infections and Mode of Endotoxin Actions. New York, Springer-Verlag, 1975, pp. 419-428.

5. Bennett, I.L., Petersdorf, R.J., Keene, W.R.: Pathogenesis of fever: Evidence of direct cerebral action of bacterial endotoxins. Assoc. Am. Physicians. 70:64-73, 1957.

6. Bernheim, H.A., Block, L.H., Atkins, E.: Fever: Pathogenesis, pathophysiology, and purpose. Annals of Int. Med. 91:261-270, 1979.

7. Dinarello, C.A., Wolff, S.M.: Pathogenesis of fever in man. New Eng. J. Med. 289:607-612, 1978.

8. Wilson, R.F., Thal, A.P., Kindling, P.H, Grifka, T., Ackerman, E.: Hemodynamic measurements in septic shock. Arch. Surg. 91:121-129, 1965.

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

10. Hymann, A.L., Pennington, D.G., Kadowitz, P.J., Jaques, W.E.: Studies of the mechanisms of the pulmonary vasopressor response to endotoxin: Effects of corticosteroids and catecholamines, in Glenn, T.M. (ed): Steroids and Shock. Baltimore, Univ. Park Press, 1974, pp. 129-149.

11. Rhoden, C.H., Leyser, D.B., Smith, I.M., Evans, T.C., Duling, B.R.: Blood pressure changes in mice after lethal staphylococcal infection and endotoxin challenge. Proc. Soc. Exp. Bio. and Med. 149:622-625, 1975.

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

13. Burrows, G.E.: Hemodynamic alterations in the anesthetized pony produced by slow intravenous administration of Escherichia coli endotoxin. Am. J. Vet. Res. 31:1975-1982, 1970.

14. Burrows, G.E.: Endotoxemia induced by rapid intravenous injection of Escherichia coli in anesthetized ponies. Am. J. Vet. Res. 31:1967-1973, 1970.

15. Corrigan, J.J., Kiernat, J.F.: Effect of heparin in experimental gram-negative septicemia. J. Inf. Dis. 131:138-143, 1975.

16. Bradley, S.G.: Cellular and molecular mechanisms of action of bacterial endotoxins. Ann. Rev. Microbiol. 33:67-94, 1979.

17. McClure, J.J.: Endotoxin shock. Vet. Clin. N. Am. 6:193-202, 1979.

18. Shubin, H., Weil, M., Nishijima, H.: Clinical features in shock associated with gram-negative bacteremia. See ref. 4. pp. 411-418.

19. Nelson, A.W., Collier J.R., Griner, L.A.: Acute surgical colonic infarction in the horse. Am. J. Vet. Res. 29:315-327, 1968.

20. Datt, S.C., Usenik, E.A.: Intestinal obstruction in the horse. Cornell Vet. 65:152-172, 1975.

21. Littlejohn, A.: The surgical relief of intestinal obstruction in the horse: A review. I. Mortality, anaesthesia, and laparotomy. Br. Vet. J. 121:497-508, 1965.

22. Tennant, B., Wheat, J.D., Meagher, D.N.: Observations on the causes and incidence of acute intestinal obstruction in the horse. Proc. Am. Assoc. Equine Pract. 18:251-257, 1972.

23. Nelson, A.W., Adams, O.R.: Intestinal infarction in the horse: Acute colic arterial occlusion. Am. J. Vet. Res. 27:707-710, 1966.

24. Beadle, R.E., Huber, T.L.: Blood chemistry changes associated with rapid intravenous administration of Escherichia coli endotoxin in anesthetized ponies. J. Eq. Med. and Surg. 1:371-375, 1977.

25. Sibbald, W.J.: Bacteremia and endotoxemia: A discussion of their roles in the pathophysiology of gram-negative sepsis. Heart and Lung. 5:765-771, 1976.

26. Zurier, R.B., Weismann, G.: Anti-immunologic and anti-inflammatory effects of steroid therapy, in Azarnoff, D.L. (ed): Steroid Therapy. Philadelphia, W. B. Saunders Co., 1975, pp. 226-228.

27. Few, A.V.: The interaction of polymixin E with bacterial and other lipids. Biochimica et Biophysica Acta. 16:137-145, 1955.

28. Rifkind, D., Palmer, J.D.: Neutralization of endotoxin toxicity in chick embryos by antibiotics. J. Bact. 92:815-819, 1966.

29. Morrison, D.C., Jacobs, D.M.: Binding of polymixin B to the lipid A portion of bacterial lipopolysaccharides. Immunochemistry. 13:813-818, 1976.

30. Newton, B.A.: The properties and mode of action of the polymixins. Bact. Rev. 20:14-27, 1956.

31. Rifkind, D.: Prevention by polymixin B of endotoxin lethality in mice. J. Bact. 93:1463-1464, 1967.

32. Rifkind, D., Hill, R.B.: Neutralization of the Shwartzman reactions by polymixin B. J. of Immunology. 3:564-569, 1967.

33. Rifkind, D.J.: Studies on the interaction between endotoxin and polymixin B. J. Inf. Dis. 117:433-438, 1967.

34. Corrigan, J.J.: Prevention of the generalized Schwartzmann reaction with polymixin B. Blood. 34:835, 1969.

35. Corrigan, J.J., Bell, B.M.: Endotoxin-induced intravascular coagulation: Prevention with polymixin B sulfate. J. Lab. Clin. Med. 77:802-810, 1971.

36. Palmer, J.D., Rifkind, D.: Neutralization of the hemodynamic effects of endotoxin by polymixin B. Surg. Gyn & Obst. 138:755-759, 1974.

37. Corrigan, J.J., Sieber, O.F., Ratajozak, H., Bennett, B.B.: Modification of human neutrophil response to endotoxin with polymixin B sulfate. J. Inf. Dis. 130:384-387, 1974.

38. Morrison, D.C., Jacobs, D.M.: Inhibition of liposaccharide-initiated activation of serum complement by polymixin B. Infect. Immun. 13:298-301, 1976.

39. Butler, T., Moller, G.: Mitogenic response of mouse spleen cells and gelation of limulus lysate by lipopolysaccharide of Yersinia pestis and evidence for neutralization of the lipopolysaccharide by polymixin B. Infect. Immun. 18:400-404, 1977.

40. Corrigan, J.J., Bell, B.M.: Comparison between the polymixins and gentamicin in preventing endotoxin-induced intravascular coagulation and leukopenia. Inf. Immun. 4:563-566, 1971.

41. Bergstein, J.M., Michael A.F.: Effect of polymixin B and endotoxin on renal cortical fibrinolysis. Proc. Soc. Exp. Med. 144:369-372, 1973.

42. Craig, W.A., Turner, J.H., Kunin, C.M.: Prevention of the generalized Schwartzman reaction and endotoxin lethality by polymixin B localized in tissues. Inf. Immun. 10:287-292, 1974.

43. Bannatyne, R.M., Harnett, N.M., Cheung, R.: Protective effect of polymixin B sulfate in experimental meningococcal infection in mice. Can. J. Microbiol. 23:1526-1528, 1977.

44. Liehr, H., Grun, M., Brunswig, D., Saulter, T.H.: Endotoxaemia in liver cirrhosis: Treatment with polymixin B. Lancet. I:810-811, 1975.

45. Kwapinski, J.B.: Methods of Serological Research. New York, John Wiley and Sons, Inc., 1965, pp. 59-60.

46. Niemetz, J., Morrison, D.C.: Lipid A as the biologically active moiety in bacterial endotoxin (LPS) - initiated generation of procoagulant activity by peripheral blood leucocytes. Blood. 49:947-956, 1977.

47. Rickles, F.R., Rick. P.D.: Structural features of Salmonella typhinurium lipopolysaccharide required for activation of tissue factor in human mononuclear cells. J. Clin. Invest. 59:1188-1195, 1977.

48. Horowitz, H.J., Des Prez, R.M., Hook, E.W.: Effects of bacterial endotoxin on rabbit platelets. II. Enhancement of factor 3 activity in vivo and in vitro. J. Exp. Med. 116:619-633, 1962.

49. Des Prez, R.M., Horowitz, H.J., Hook, E.W.: Effects of bacterial endotoxin on rabbit platelets. I. Platelet aggregation and release of platelet factors in vitro. J. Exp. Med. 114:857-874, 1961.

50. Des Prez, R.M.: Effects of bacterial endotoxin on rabbit platelets. III. Comparison of platelet injury induced by thrombin and by endotoxin. J. Exp. Med. 120:305-313, 1964.

51. Des Prez, R.M., Bryant, R.E.: Effects of bacterial endotoxin on rabbit platelets. IV. The divalent ion requirements of endotoxin-induced platelet injury. J. Exp. Med. 124:971-982, 1966.

52. Des Prez, R.M.: The effects of bacterial endotoxin on rabbit platelets. V. Heat labile plasma factor requirements of endotoxin-induced platelet injury. J. Immunol. 99:966-973, 1967.

53. Meagher, D.M.: Clinical evaluation and management of shock in the equine patient. Vet. Clinics of N. Am. 6:245-255, 1976.

54. Walker, R.J.: The contribution of intestinal endotoxin to mortality in hosts with compromised resistance: A review. Exp. Hemat. 6:172-184, 1978.

55. Frank, M.M., May, J.E., Kane, M.A.: Contributions of the classical and alternate complement pathways to the biological effects of endotoxin. J. Inf. Dis. 128:176-181, 1973.

56. Cluff, L.E.: Effects of endotoxins on susceptibility to infection. J. Inf. Dis. 122:205-215, 1970.

57. Morrison, D.C., Kline, L.F.: Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS). J. Immunol. 118:362-368, 1977.

58. Brown, D.L., Lachmann, P.J.: The behavior of complement and platelets in lethal endotoxin shock in rabbits. Int. Arch. Allergy Appl. Immunol. 45:193-205, 1973.

59. Wolff, S.M., Rubenstein, M., Mulholland, J.H., Alling, D.W.: Comparison of hematologic and febrile response to endotoxin in man. Blood. 26:190-201, 1965.

60. Spielvogel, A.R.: An ultrastructural study of the mechanism of platelet-endotoxin interaction. J. Exp. Med. 126:235-250, 1967.

61. Burrows, G.E.: Hematologic alterations associated with acute E. coli endotoxemia, in Proceedings. Int. Symp. Equine Hemat. 1:505-515, 1975.

62. Meyers, K.M., Lindner, C., Grant, B.: Characterization of the equine platelet aggregation response. Am. J. Vet. Res. 40:260-264, 1979.

63. Meyers, K.M., Lindner, C., Katz, J., Grant, B.: Phenylbutazone inhibition of equine platelet function. Am. J. Vet. Res. 40:265-270, 1979.

64. Burrows, G.E.: Equine Escherichia coli endotoxemia: Comparison of intravenous and intraperitoneal endotoxin administration. Am. J. Vet. Res. 40:991-998, 1979.

65. Davis, R.B., Meeker, W.R., Baily, W.L.: Serotonin release by bacterial endotoxin. Proc. Exp. Biol. Med. 108:774-776, 1961.

66. Davis, R.B., Meeker, W.R., McQuarrie, D.G.: Early changes in intravascular pressure, platelets, and serotonin after E. coli endotoxin administration. Surg. Forum. 10:401-405, 1959.

67. Morrison, D.C., Ulevitch, R.J.: A review: The effects of bacterial endotoxins in host mediation systems. Am. J. Path. 93:527-617, 1978.

68. Goodman, M.L., Way, B.A., Irwin, J.W.: The inflammatory response to endotoxin. J. Path. 128:7-14, 1979.

69. Stein, M., Thomas, D.P.: Role of platelets in the acute pulmonary response to endotoxin. J. Appl. Physiol. 23:47-52, 1967.

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

71. Hinshaw, L.B., Kuida, H., Gilbert, R.P., Visscher, M.B.: Influence of perfusate characteristics on pulmonary vascular response to endotoxin. Am. J. Physiol. 191:293-295, 1957.

72. Shwartzman, G.: Studies on Bacillus typhosus toxic substances. I. Phenomenon of local skin reactivity to B. typhosus culture filtrate. J. Exp. Med. 48:247-268, 1928.

73. Shwartzman, G.: A new phenomenon of local skin reactivity to B. typhosa culture filtrate. Proc. Soc. Exp. Biol. Med. 25:560-561, 1928.

74. Cochrane, C.G.: 20. The Hageman factor pathways of kinin formation, clotting, and fibrinolysis, in Beers, R.F., Basset, E. (eds): The Role of Immunological Factors in Infectious, Allergic, and Autoimmune Processes. New York, Raven Press, 1976, pp. 237-246.

75. Revak, S.D., Cochrane, C.G., Griffin, J.H.: Initiation of coagulation by contact activation of Hageman factor, in McIntosh, R.M., Guggenheim, S.J., Schrier, R.W. (eds): Kidney Disease: Hematologic and Vascular Problems. New York, John Wiley and Sons, Inc., 1977, pp. 29-40.

76. Kisiel, W., Fujikawa, K., Davie, E.W.: Activation of bovine factor VII (pro-convertin) by factor XIIa (activated Hageman factor). Biochemistry. 16:4189-4194, 1977.

77. Radcliffe, R., Bagdasarian, A., Colman, R., Nemerson, Y.: Activation of bovine factor VII by Hageman factor fragments. Blood. 50:611-617, 1977.

78. Alexander, J.W.: Function of leucocytosis in defense mechanisms. See ref. 4. pp. 190-197.

79. MacGregor, R.R.: Granulocyte adherence changes induced by hemodialysis, endotoxin, epinephrine, and glucocorticoids. Ann. Int. Med. 86:35-39, 1977.

80. Fehr, J., Jacob, H.S.: In vitro granulocyte adherence and in vivo margination. Two associated complement-dependent functions. J. Exp. Med. 146:641-652, 1977.

81. Mechanic, R.C., Frei, E., Landy, M., Smith, W.W.: Quantitative studies of human leucocytic and febrile response to single and repeated doses of purified bacterial endotoxin. J. Clin. Invest. 41:162-172, 1962.

82. Athens, J.W., Haab, O.P., Raab, S.O., Mauer, A.M., Ashenbrucker, H., Cartwright, G.E., Wintrobe, M.M.: Leukokinetic studies. IV. The total blood circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. J. Clin. Invest. 40:989-995, 1961.

83. Barth, R., Schauer, A., Hubner, G., Burkhardt, R.: Changes of the bone marrow in endotoxin shock. See ref. 4. pp. 206-209.

84. Kirkpatrick, C.H., Rosenthal, A.S.: Glucocorticoid and allergic reactions. See ref. 26. pp. 245-246.

85. Mergenhagen, S.E., Rosenstreich, D.L., Wilton, M., Wahl, S.M., Wahl, L.M.: 3. Interaction of endotoxin with lymphocytes and macrophages. See ref. 74. pp. 17-27.

86. Chien, S., Dellenbach, R.J., Usami, S.: Hematologic changes in endotoxin shock. Proc. Soc. Exp. Bio. Med. 118:1182-1185, 1965.

87. Chien, S., Dellenbach, R.J., Usami, S., Tritell, K., Chang, C., Gregerson, M.J.: Blood and its distribution in endotoxin shock. Am. J. Physiol. 210:1411-1418, 1966.

88. Giertz, H.: Pharmacological aspects of endotoxin shock. See ref. 4. pp. 311-314.

89. Schauer, A.: Initial liberation of biogenic amines and effect of further mediators following application of endotoxins. See ref. 4. pp. 315-322.

90. Trank, J.W., Visscher, M.B.: Carotid sinus baroreceptor modifications associated with endotoxin shock. Am. J. Physiol. 202:971-972, 1962.

91. Hopkins, R.W., Damewood, C.A.: Septic shock: Hemodynamics of endotoxin and inflammation. Am. J. Surg. 127:476-483, 1974.

92. Kuida, H., Hinshaw, L.B., Gilbert, R.P., Visscher, M.B.: Effect of gram-negative endotoxin on pulmonary circulation. Am. J. Physiol. 192:335-344, 1958.

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

94. Weil, M.H., MacLean, M.B., Visscher, M.B., Spink, W.W.: Studies on the circulatory changes in the dog produced by endotoxin from gram-negative microorganisms. J. Clin. Invest. 35:1191-1198, 1956.

95. Brobmann, G.F.: Mesenteric hemodynamics in endotoxin shock. See ref. 4. pp. 265-270.

96. Hawiger, J., Hawiger, A., Steckley, S., Timmons, S., Cheng, C.: Membrane changes in human platelets induced by lipopolysaccharide endotoxin. Br. J. Haematol. 35:285-299, 1977.

97. Neuhof, H.: Changes in hemodynamics and gas metabolism after endotoxin injection. See. ref. 4. pp. 256-264.

98. Urbaschek, B., Urbaschek, R.: Some aspects of the effects of endotoxin, endotoxoid, and biogenic amines. See ref. 4. pp. 323-328.

99. Eyre, P., Lewis, A.J.: Acute systemic anaphylaxis in the horse. Br. J. Pharmac. 48:426-437, 1973.

100. Burka, J.F., Deline, T.R., Holroyde, M.C., Eyre, P.: Chemical mediators of anaphylaxis (histamine, 5-HT, and SRS-A) released from horse lung and leukocytes in vitro. Chem. Path. Pharm. 13:379-388, 1976.

101. Chand, N., Eyre, P.: Classification and biological distribution of histamine receptor sub-types. Agents and Actions. 5:277-295, 1975.

102. Al-Khayyet, A.A., Aronson, A.L.: Pharmacologic and toxicologic studies with the polymixins. Chemotherapy. 19:98-108, 1973.

103. Kunin, C.M., Bugg, A.: Binding of polymixin antibiotics to tissues: The major determinant of distribution and persistance in the body. J. Inf. Dis. 124:394-400, 1971.

104. Tizard, J.R., Holmes, W.L.: Degranulation of sensitized rat peritoneal mast cells in response to antigen, compound 48/80 and polymixin B. Int. Arch. Allergy. 46:867-879, 1974.

105. Cooperstock, M., Baublis, J.V.: Deactivation of endotoxin by serum and polymixin B. J. Pediatrics, Obs. 83:155-156, 1973.

106. Cooperstock, M.S.: Inactivation of endotoxin by polymixin B. Antimicrob. Agents and Chemotherapy. 6:422-425, 1974.

107. Weisel, R.D., Berger, R.L., Hecktman, H.B.: Measurement of cardiac output by thermodilution. N. Eng. J. Med. 292:682-684, 1975.

108. Weisel, R.D., Vito, L., Dennis, R.C., Berger, R.L., Hecktman, H.B.: Clinical applications of thermodilution cardiac output determinations. Am. J. Surg. 129:449-452, 1975.

109. Muir, W.W., Sharda, R.T., Milne, D.W.: Estimation of cardiac output in the horse by thermodilution techniques. Am. J. Vet. Res. 37:697-700, 1976.

110. Goldstein, A.: Biostatistics: An Introductory Text. New York, Macmillan Publishing Co., Inc., 1964.

111. Steel, G.D., Torrie, J.H.: Principles and Procedures of Statistics. New York, McGraw-Hill Book Company, 1960.

112. White, G.L., Archer, L.T., Beller, B.K., Holmes, D.D., Hinshaw, L.B.: Leukocyte response and hypoglycemia in superlethal endotoxin shock. Circ. Shock. 4:231-239, 1977.

113. Barrier, M.J., Ellison, N.: Platelet function. Anesthesiology. 46:202-211, 1977.

114. Burrows, G.E.: The effects of repeated administration of Escherichia coli lipopolysaccharides to ponies. Can. J. Com. Med. 43:321-327, 1979.