69-17,831

SOLOMON, Lura Ann, 1942-

MECHANISMS OF CANINE ENDOTOXIN SHOCK: MODIFICATION OF THE SHOCK STATE BY SALICYLATES.

The University of Oklahoma, Ph.D., 1969
Physiology

University Microfilms, Inc., Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

MECHANISMS OF CANINE ENDOTOXIN SHOCK: MODIFICATION OF THE SHOCK STATE BY SALICYLATES

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

LURA ANN SOLOMON

Oklahoma City, Oklahoma

1969

MECHANISMS OF CANINE ENDOTOXIN SHOCK: MODIFICATION

OF THE SHOCK RESPONSE BY SALICYLATES

APPROVED BY

Lerner B. Hinshaw

Eugene L. Hamilton

Richard A. Bowen

Kurt Weiss

Wesley Kyle

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author wishes to express her sincere appreciation to Dr. Lerner B. Hinshaw for guidance and encouragement throughout the course of this work. Dr. B. J. Gunter is gratefully acknowledged for technical assistance.

Appreciation is due Misses Mary Carol Whittaker and Rosemary Frakes for secretarial assistance.

During this investigation, the author was the recipient of a Predoctoral Fellowship from the National Institutes of Health.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

MECHANISMS OF CANINE ENDOTOXIN SHOCK: MODIFICATION OF THE SHOCK RESPONSE BY SALICYLATES

CHAPTER I

INTRODUCTION

Endotoxin and its toxic manifestations in a wide variety of animals have been the subject of a vast number of scientific publications. The devotion of numerous pages of literature to the subject of endotoxins is well justified in light of the fact that these toxic substances create critical and often lethal clinical situations in human patients (Altemeier, 1967). This dissertation attempts first to review pertinent scientific literature and second to elucidate some of the evasive mechanisms of action of the endotoxins.

All mammalian species studied respond to intravenous endotoxin administration with severe arterial hypotension (Gilbert, 1960). Endotoxin-shocked animals succumb to lethal doses of endotoxin by progressive failure of the circulation, although the precise mechanisms responsible have not been clearly defined (Zweifach, 1965). Endotoxin shock is generally described as a circulatory phenomenon although a variety of nervous, endocrine and metabolic processes have been implicated (Visscher, 1958).

Chemistry of the Endotoxin Complex

Endotoxin is the term commonly employed to designate a lipid-

polysaccharide-protein complex existing in the cell wall of Gram-negative bacteria. From structural analyses and biosynthetic schemes it has been shown that the lipopolysaccharides are long-chain phosphorous-containing heteropolymers constituted of a lipid covalently linked to a core polysaccharide to which are attached serologically different oligosaccharide units (Lüderitz et al., 1958). Because lipopolysaccharides are found in the cell wall of the Gram-negative organisms, they are also referred to as somatic antigens. Endotoxin is resistant to trypsin and high temperatures yet labile to mild acid hydrolysis.

One of the first extractions of the lipopolysaccharide moiety from the bacterial cell wall was that of Boivin and co-workers in 1935. They utilized trichloroacetic acid to extract the toxin from intact cells. Bacteria from 24-hour cultures were washed and suspended in distilled water to a concentration of 200 mg/ml. An equal volume of 0.5N trichloroacetic acid was added and the bacteria were extracted for 3 hours at low temperatures. The bacterial cells, morphologically intact and retaining their staining characteristics, were removed by centrifugation. The toxicity which remained in the supernatant was precipitated by 4 to 5 volumes of alcohol or acetone. The precipitate represented about 5 per cent of the dry weight of the bacteria but the yield could be increased to about 10 per cent if the organisms were digested with trypsin prior to extraction. About 0.1 mg of the precipitate yield resulted in the death of a 20 gm mouse following intraperitoneal injection.

Of the components of the endotoxin substance, the activity of the lipid fraction seems to be the most controversial. Gilbert (1960) and Lüderitz and collaborators (1958) suggest that the lipid is of the utmost

importance in production of toxic manifestations. Lüderitz and co-workers (1968) were able to dissociate the lipopolysaccharide complex into an inactive polysaccharide and an active 'lipid A' which retained many toxic properties when recombined with a natural or artificial protein carrier. Contrary to the previous results, Tal and Olitsky (1948) and Ribi and coworkers (1961) find no evidence that the lipopolysaccharide complex differs quantitatively and qualitatively from one species of Gram-negative bacteria to another. The suggestion was made that these qualitative and quantitative differences in lipid components might influence certain biological activities when bound to the polysaccharide of the lipopolysaccharide unit (Kasai, 1966). The discrepancy between reports which evaluated the toxic properties of the lipid fraction might be attributed to use of different extraction procedures.

Most investigators currently feel that the major endotoxic activity cannot be attributed to the lipid component and is due to the polysaccharide (Raškovà and Vaněček, 1964; Burrows, 1951). The polysaccharide, which may or may not be esterified, functions as a haptene and is responsible for the immunological specificity of the toxic complex (Burrows, 1951; Staub, 1964). Ribi and co-workers have presented evidence that the polysaccharide moiety is largely responsible for both the toxic and antigenic effects of endotoxin from Salmonella enteriditis (Ribi et al., 1960). Still another communication reports that lipopolysaccharide modified by periodate treatment, resulting in oxidation of the polysaccharide fraction, loses its antigenic specificity and many of its toxic properties though its pyrogenicity is retained (Neter et al., 1956). This apparent dissociation of biological effects induced by chemical modification of the

molecule suggests that there may be specific reactive groupings responsible for various toxic properties (Atkins, 1960). Alaupovic et al. (1968) implicate a macromolecular fatty acid-carbohydrate complex of unknown structure and size as the chemical requirement for elicitation of the characteristic hemodynamic response to endotoxin.

Hemodynamic Response to Endotoxin

The appearance of a shock-like state in association with an infection was first described by Laennec in 1831. In the late nineteenth century, Boise (1897) contrasted special features of septic shock to vascular collapse following hemorrhage. Since the middle 1950's, there has been considerable interest in the special type of bacterial shock due to Gram-negative enteric bacteria, first described as a clinical entity by Waisbren (1951) and by Borden and Hall (1951). These historical scientific communications established systemic hypotension as a characteristic feature of the endotoxic condition while also dividing the syndrome into two distinct clinical pictures: one being the toxic state produced by the invading bacteria and the second being the hypotensive state initiated by the effects of the endotoxin. Waisbren (1951) also noted that the clinical syndrome produced by the Gram-negative bacteria was essentially the same regardless of the bacterial species involved. A subsequent review of clinical shock associated with Gram-negative bacteremia advocated the use of norepinephrine to support blood pressure and antibiotics to combat the underlying infection (Wise et al., 1952).

Since the first clinical observations of the Gram-negative septic shock state were made at the University of Minnesota (Waisbren, 1951; Borden and Hall, 1951; Wise et al., 1952), it is not surprising that many

of the early research investigations of shock were also carried out there. It soon became common practice to evaluate septic shock by administering endotoxin, prepared according to the method of Boivin, to the anesthetized laboratory dog. Therefore, the canine hemodynamic reaction following injection of endotoxin has been extensively studied during the past few years. MacLean and Weil (1956) at the University of Minnesota, documented the pattern of shock, elicited by injection of Escherichia coli endotoxin, as characterized by an almost immediate and precipitous decline in systemic arterial blood pressure with a concomitant rise in portal venous pressure. Subsequently, numerous investigations corroborated these early reports (MacLean and Weil, 1956; MacLean et al., 1956; Weil et al., 1956a; Lillehei and MacLean, 1958). These hemodynamic alterations were evident within the first two minutes after the toxin was given. At approximately 15 to 20 minutes postendotoxin, the portal venous pressure declined to near control levels accompanied by a partial recovery of systemic blood pressure. These findings together with pathological changes of congestion and hemorrhage in the mesenteric vascular bed made it appear that shock was at least partially due to trapping of blood in the hepato-splanchnic vascular bed (MacLean and Weil, 1956; Weil et al., 1956a).

The liver soon became suspect as the site of sequestration of blood. The immediate systemic hypotensive response to endotoxin was avoided by excluding the liver from the circulation either by hepatectomy or devascularization (MacLean and Weil, 1956). Subsequent studies of in situ perfused canine livers revealed that following injection of E. coli endotoxin, there was a prompt rise in liver weight. The perfused liver also responded with an increase in vascular resistance (Hinshaw et al., 1966).

The combination of increased weight and vascular resistance results from hepatic venous constriction (Weil et al., 1956a). This is a transient constriction of the hepatic veins and is usually concluded from 5 to 25 minutes after endotoxin administration.

In situ perfusion studies of the canine intestine revealed small early rises in intestinal weight (MacLean et al., 1956). It is probable that the early and simultaneous increases in liver and intestinal weights represent a significant pooling of blood and may well be the major repository of the venous blood in the canine species. Comparison of hepatic and intestinal weight responses to endotoxin reveals that the liver soon returns to steady control levels while the intestine exhibits a continuous yet slow and steady gain in weight. After 60 minutes, this rise in intestinal weight represents a significant amount of sequestration of blood. The increase in intestinal weight is probably due in large part to an increase in interstitial fluid (Gilbert, 1960) as suggested by reports that after endotoxin there is an accumulation of I^{131} tagged albumin in the intestinal wall which corresponds to a 7 to 22 per cent increase in plasma content (Aust et al., 1957). The later phase of weight gain presumably cannot be attributed to increased portal pressure because portal pressure has returned to normal levels prior to this event. Yet livers at postmortem examination are congested even though portal pressures have returned to control levels (Brunson et al., 1966). Therefore, a disparity may exist between portal pressure and liver pooling, particularly following the acute response.

It was found that endotoxin elicited a large fall in cardiac output approximately 2 minutes after injection (Weil et al., 1956a). The

decline in cardiac output was accompanied by a slight rise in total peripheral resistance. Thus it appeared that the decrease in blood pressure was the result of a diminished total blood flow and was not due to peripheral dilation. An experiment using an external reservoir, which received all venous flow and from which blood was pumped at a constant rate into the right atrium, revealed that endotoxin administration produced no immediate fall in canine blood pressure when venous return was held constant (Weil et al., 1956b). Therefore, it was concluded that the early hypotensive condition was due to a lowered cardiac output caused by trapping of blood in the hepatic bed. Were the hypotension the result of dilation of peripheral vessels, the arterial pressure would be expected to decline despite a constant cardiac output. It was also evident that venous return to the heart was decreased because there was a rapid fall of the blood level in the venous reservoir. The inadequate cardiac output was obviously not the result of myocardial failure since the heart was quite capable of pumping the constant amount of blood returned to it (Weil et al., 1956b).

The reaction of the renal vascular bed to the lethal effects of endotoxin has also been evaluated. It was found that the kidney, too, was involved in the early phases of canine endotoxin shock. Renal perfusion studies revealed a fall in kidney weight which commenced 15-35 seconds postendotoxin (Hinshaw and Bradley, 1957). Since this early decline in renal weight occurred prior to systemic arterial hypotension, the weight loss appeared due to arteriolar constriction. When a kidney was perfused at constant arterial pressure, it was found that an initial decrease in renal blood flow was followed by an increase in flow (Hinshaw et al.,

1959). Thus, the renal vascular response to endotoxin is immediate arteriolar constriction which precedes vasodilatation. Altered renal functions postendotoxin appear to be secondary to hemodynamic derangement and not a direct nephrotoxic action (Hinshaw et al., 1961c; Gillenwater et al., 1963; Hinshaw et al., 1959).

Hemodynamic alterations in the lung are also evident when endotoxin is given to an anesthetized dog. An intact dog responds with an increase in pulmonary vascular resistance (Kuida et al., 1961). The pulmonary vascular response is usually evident at 90 seconds postendotoxin (Hinshaw et al., 1957). Results obtained in an isolated lung perfusion system were as follows: increases in pulmonary artery pressure, pulmonary artery wedge pressure and lung weight. When the pre- and post-capillary vascular beds were examined, calculated venous resistances rose more than arterial resistances (Kuida et al., 1958). These changes observed in the vascular system of the lung were rapid in onset and disappeared by approximately 30 minutes after the endotoxin was given. Thus, the lung presents an example of local pooling and venous constriction (Gilbert, 1960) during the early stage of endotoxin shock.

Unfortunately, there seems to be a paucity of information on the cerebral vascular bed during endotoxin shock. A report by Lillehei and MacLean (1958) states that no great increases in resistance were found in the carotid artery postendotoxin. It is possible that blood flow to the brain is diminished due to lowered cardiac output and systemic hypotension. Since the terminal phase of endotoxin shock exhibits respiratory arrest while the heart is still beating, severe medullary depression resulting from cerebral ischemia is suggested (Gilbert, 1960).

The spleen, too, has been shown to participate in the canine vascular response to endotoxin. An early contraction of the splenic red cell reservoir causes an abrupt outpouring of concentrated blood directly into the portal vein (Boruchow and Abel, 1966). It is thought that this contraction response is related to the baroreceptor stimulus to the adrenals following acute arterial hypotension. It is evident that the state of the splenic blood volume at the time endotoxin is administered influences the early changes in hematocrit (Chien et al., 1965a).

Despite the fact that the shock syndrome often terminates with cardiac failure (Crowell and Guyton, 1962), there is evidence that endotoxin exerts no direct effects upon the myocardium. Frohlich and co-workers (1962) reported a decrease in coronary vascular resistance when the whole animal was included in the perfusion circuit while others (Londe et al., 1967) demonstrated that the vasculature of the isolated perfused heart is unaffected by endotoxin. In the intact animal, ventricular function may actually be enhanced during the early hypotensive phase due to increased sympathetic drive. Thus, it is suggested that the irreversibility is primarily determined by peripheral mechanisms (Visscher, 1965), although cumulative myocardial damage or progressive deterioration of sympathetic drive may contribute to the ultimate heart failure observed when shock is prolonged (Goodner, 1967). In contrast to the previous findings, Moses and MacIntyre (1963) feel that the primary vascular episode in endotoxemia is a generalized impairment of cardiac function and that changes in other organs, such as the liver, are secondary to the diminished cardiac output. A similar point of view is expressed by Maxwell and co-workers (1959) who conclude that endotoxemia is associated with marked

impairment of myocardial function.

Endotoxin, often referred to as a "vascular poison," appears to have profound effects at the capillary level of the circulation. The report of an increase in fibrinogen content of the thoracic duct was interpreted to mean that there was a large increase in capillary permeability to high molecular weight proteins (Chien et al., 1965b). Plasma protein concentrations have been shown to decline after endotoxin (Chien et al., 1965b). The report of Lillehei and MacLean (1959) showed the secondary fall in arterial pressure to occur concomitantly with a significant decline in circulating plasma volume. Hematocrit rises after endotoxin administration and is partially ascribed to extravasation of vascular fluid (Chien et al., 1965a). It is concluded that endotoxin poisoning includes, as either a direct or an indirect effect, permeability changes (Visscher, 1965). The indirect action on the capillary bed might be attributed to liberated chemical mediators or to neural mechanisms.

The nervous system has been implicated as a site of increased permeability by Eckman and co-workers (1958). They demonstrated that endotoxin rendered cerebral vessels of rabbits permeable to injected colloidal dyes. A more recent publication (Clawson et al., 1966), utilizing the electron microscope to study the distribution of colloidal iron oxide in cerebral capillaries of rabbits, found that two hours after endotoxin administration there was a significant accumulation of iron oxide particles in the cerebral capillary epithelial cells of endotoxin-shocked animals. The authors concluded that endotoxin caused the cerebral capillary endothelial cells to become more actively phagocytic.

In summary, the early hemodynamic responses of the dog associated with endotoxin administration are as follows: (1) systemic hypotension;

(2) diminished cardiac output due to decreased venous return; (3) vascular pooling, especially in the hepatic bed; (4) arteriolar constriction in the kidney; and (5) pulmonary venous constriction. Thus, the early results of endotoxin appear to be redistribution of blood in the vascular bed. The primary hypotensive state might be described best by saying that a disparity exists between the circulating blood volume and the capacity of the vascular bed. In contrast, the late phase of endotoxin shock is characterized by a gradual decline in systemic arterial pressure, extravasation of fluid into the intestinal area, diminished venous return and evidences of dilatation or circulatory failure. The decrease in venous return can be explained in part by progressive pooling in the gut wall. It is important to note that this cannot be the only site of loss of vascular fluid since the eviscerated dog also becomes hypotensive and dies when endotoxin is administered (MacLean and Weil, 1956). Pooling does occur in the eviscerated dog but its site is unknown (Hinshaw et al., 1958).

Other Canine Reactions to Endotoxin

It should be noted that although many toxic reactions to endotoxin occur in the circulatory system, not all of the lethal effects of endotoxin occur there. The endotoxin-shocked dog exhibits severe bloody diarrhea, retching and vomiting, acidosis and other metabolic disturbances, oliguria, coagulation disturbances, and leucopenia followed by leucocytosis. Copious diarrhea is observed 30 to 60 minutes postendotoxin (MacLean and Weil, 1956). Loss of fluid due to diarrhea tends to promote late increases in hematocrit (Chien et al., 1965a). At postmortem examination, the bowel reveals numerous congested and necrotic areas which suggest that a loss of

fluid into the tissue has occurred (Penner and Bernheim, 1942; Burrows, 1951).

The dog responds to endotoxin with rapid development of a metabolic acidosis characterized by a marked and significant increase in lactic acid and a fall in bicarbonate ion and pH. These changes occur within 14 minutes after endotoxin. The metabolic acidosis appears to be the result of stagnation of the circulation and accumulation of metabolites as a consequence of low blood pressure (Hardaway et al., 1961; Rosenberg and Rush, 1966). Most of the metabolic disturbances seem to be associated with derangements in carbohydrate metabolism which are thought to be the result of two mechanisms: one being a direct action of endotoxin on cellular metabolic processes and the other being the inadequate tissue perfusion (Rosenberg and Rush, 1966). An initial hypoglycemia is followed by hyperglycemia, with depletion of liver glycogen (Berry and Smythe, 1959). The hyperglycemia and glycogen depletion are thought to be the result of increased hepatic phosphorylase activity because endotoxin seems to convert most of the liver phosphorylase to the active form (Hamosii and Shapiro, 1960). High levels of extracellular glucose are maintained until the animals begin a slow recovery or suffer a rapid death (Green and Stoner, 1954).

Urine output is often severely diminished during shock (Vick et al., 1963). This appears to be the result of an embarrassed filtration rate caused by the intense arteriolar constriction secondary to systemic hypotension (Visscher, 1965; Emerson et al., 1966).

Abnormal alterations in the coagulation mechanisms are evident soon after endotoxin is injected (Cavanagh and DeCenzo, 1963; Gans et al.,

1963). There is a rapid and dramatic fall in blood fibrinogen levels within 20 minutes but normal levels are regained within four to six hours. There is also a temporary depletion of platelets and prothrombin time is prolonged (Corrigan et al., 1968). These changes are attributed to depletion of blood coagulation factors in an intravascular clotting episode (Hardaway and Johnson, 1963). Activation of the Hageman factor has been suggested as the initial step in the resultant coagulation process (Nies et al., 1968).

Endotoxin activity elicits several changes in the formed elements of the blood. Endotoxin particles adhere to platelets and ultimately result in platelet destruction (Spielvogel, 1967; Des Prez, 1967). A profound polymorphonuclear leucopenia occurs within one hour after endotoxin and persists for two or three hours (Weil and Spink, 1957). This phenomenon, described by Delaunay in 1943, was attributed to retention of leucocytes in capillaries of the lung, liver, and spleen. Neither leucopenia or thrombocytopenia is a specific manifestation of endotoxin. Both have been observed in anaphylactic shock (Rocha e Silva, 1950).

It has been hypothesized that endotoxin acts upon granulocytes to release intracellular enzymes, collectively known as lysosomes by Nies and co-workers (1968). These authors reported increased plasma levels of free lysosomal enzymes postendotoxin. Lysosomes may play an essential role in pathogenesis of the shock state, producing the necrotic lesions associated with endotoxin administration. The special significance of lysosomal enzymes rests in their role in autophagocytosis and potential initiation of more distant cellular injury (Berman et al., 1969). Since lysosomal activity is enhanced in an acid medium, the acidotic condition

of the shocked animal provides optimum conditions for these functions (Sutherland et al., 1968). There are reports of especially accelerated lysosomal release from splanchnic tissue (Sutherland et al., 1968), notably acidotic in the shocked dog, as evidenced by elevation of acid phosphate levels in thoracic duct lymph (Berman et al., 1969).

Many of the characteristic pathologic consequences of endotoxin are also elicited in anaphylactic or allergic states. It has been reported that the initial phase of canine endotoxin shock involves an immune mechanism of the anaphylactic type in which endotoxin, acting as an antigen, reacts with antibody in the presence of complement resulting in liberation of vasotoxic substances (Spink et al., 1964). It has been shown that fibrin participates in the pathogenesis of the Generalized Shwartzman Reaction (GSR) by Bohle and co-workers (1959). Subsequently, Lee (1964) revealed that a decisive feature of the GSR is the inability of the reticuloendothelial system (RES) to clear the blood of fibrin. Endotoxin attenuates the phagocytic ability of the RES (Arredondo, 1963). It appears that the intravascular fibrin deposition occurring in association with endotoxin injection is very similar to the pathologic condition produced by the classical GSR (Stetson, 1955). Endotoxin apparently causes fibrin to collect in capillary beds, especially in the kidney and lungs (McKay et al., 1966). The subsequent plugging of capillaries could cause the tissues to become hypoxic (Braude, 1964). However, it seems unlikely that vessel plugging plays a critical role in shock, since it has not been noted as a prominent feature during in vivo microscopic studies (Gilbert, 1960). Extensive studies of canine endotoxin shock have fortified the concept that endotoxin shock is an expression of acquired bacterial hypersensitivity (Kim and Watson, 1966). The severity of the shock appears dependent

upon two factors: the dose of the antigen (endotoxin) and the degree of hypersensitivity (Spink, 1967).

Endotoxins are commonly described as poor antigens and unable to stimulate production of high titers of antibodies. Actually, relatively small doses of purified endotoxin result in high antibody titers. The poor reputation of the endotoxins as antigens rests largely on their inability to stimulate formation of high titer antitoxic sera (Morgan, 1941). Thus, agglutinin and precipitin titers may be high but the antiserum will not neutralize the toxic effects of endotoxin (Burrows, 1951; Thomas, 1954).

Role of the Nervous System

The nervous system is occasionally implicated in the pathogenesis of Gram-negative shock. Reilly et al. (1934) injected small amounts of endotoxin into the perisplanchnic area and observed hemorrhagic necrosis in the bowel wall. They reported injection of endotoxin about the splanchnic nerve to be more effective than its intravenous administration. Fine and Minton (1966) asserted that death from endotoxin is caused by the loss of integrity of the blood vessels in the splanchnic area as a result of excessive and unrelenting release of catecholamines produced by the splanchnic sympathetic nerves. Fine's thesis is that the lethal effect of endotoxin requires an intact sympathetic nerve supply to the splanchnic tissues and that the injury to tissues in the splanchnic region after endotoxin is caused by norepinephrine. He further proposes that endotoxin does not kill the host by direct infliction of injury on blood or tissues. Palmerio et al. (1963) proposed that the refractory state of shock is

the result of a reaction of the nervous system to prolonged hypovolemia or to bacterial toxins. The result of this, the authors proposed, was that the nervous system evoked excessive catecholamine activity and a reduction of flow everywhere in the peripheral circulation. The consequence of this reduction of flow was a generalized functional damage with the most critical disturbance occurring in the organs of the splanchnic area, particularly the intestines and liver. They found that celiac blockade relieved vasoconstriction in the splanchnic area and promoted survival after endotoxin. These results, however, were not confirmed by Emerson et al. (1965) in a series of chronic splanchnic denervated dogs administered endotoxin. Their results showed no difference in the hemodynamic response in splanchnic denervated or non-denervated dogs given endotoxin, and there were no survival benefits in the denervated group. In summary, these authors observed that denervation of the abdominal visceral sympathetic nervous supply gave no apparent protection in endotoxin shock.

It has been suggested that the hypothalamus plays a role in mediating the pyrogenic effects of endotoxin (Bard and Woods, 1959). Bennett and co-workers (1957) observed that intracisternal injection of only one thousandth of the usual intravenous dose produced fever in rabbits. Porter and Kass (1965) concluded that there are graded cerebral sites of sensitivity to endotoxin and that the posterior hypothalamic area is the most sensitive. Their findings indicate that the lethal action of endotoxin is manifested, in part at least, through mediation of the posterior hypothalamus, but they failed to determine whether this was an indirect or direct action of the toxin. Braude (1964) discussed one

possibility of an indirect action of endotoxin on hypothalamic centers following release of a pyrogen from white cells and its subsequent circulation to the brain, and another possibility of a direct action of endotoxin on the hypothalamus after reaching it from the blood stream via the cerebrospinal fluid. Others have postulated an action of endotoxin in the central nervous system leading to altered hemodynamics (Block et al., 1966). Kovats (1967) has proposed that the primary toxic action of endotoxin is in the autonomic nervous system. He has stated that experimental observations show that the autonomic centers of the central nervous system have a particularly marked sensitivity to endotoxin. He has concluded that one of the main targets of endotoxin is the central nervous system, especially the brain, and that the vascular effects of endotoxin may be of a pure adrenergic nerve-dependent character. Although the nervous system may participate in the response to endotoxin, it appears that the initial and direct effects of endotoxin in the dog and cat are not mediated through the central nervous system (Ravdin and Hardy, 1962), since intact nervous pathways from the brain to the periphery are not necessary for the elicitation of endotoxin shock (Weil et al., 1956a). Shock and the classical pathological changes are not limited to an animal with an intact brain.

Role of Vasoactive Agents

In attempting to elucidate the mechanisms by which endotoxin exerts its toxicity, many investigators have incriminated numerous vasoactive agents as the culprits responsible for the pathogenesis of endotoxin shock. In vivo and in vitro studies have reported that endotoxin elicits the release of histamine (Schayer, 1960), serotonin (Rosenberg et al., 1961)

and a vasoactive polypeptide-like substance (Kobold et al., 1964). Fine and Minton (1966) implicated catecholamines released in the splanchnic tissues as the initiators of the lethal effects of endotoxin. The renin-aldosterone system is also activated during the course of shock (White et al., 1967).

Vick (1965), utilizing a bioassay consisting of an isolated vein in a perfusion circuit with endotoxin-shocked dogs, presented evidence that the response to endotoxin can be divided into two phases, each initiated by a separate vasoactive substance. Histamine is said to predominate during the primary phase and catecholamines during the second. It has been repeatedly reported that plasma levels of histamine are elevated post-endotoxin in the dog (Hinshaw et al., 1960; Hinshaw et al., 1961a; Hinshaw et al., 1962a; Kobold et al., 1964; Spink et al., 1964); and in the primate (Hinshaw et al., 1961b). The mechanism responsible for elevated histamine levels is said to be acceleration of the rate of histamine synthesis due to increased activity of histidine decarboxylase (Greisman, 1960; Schayer, 1960). There is a progressive increase of the histamine:histidine ratio which is related to the relative rates of formation, conversion, and destruction of the two components (Hinshaw et al., 1961a). The rise in plasma histamine might be attributed to the marked decrease in numbers of circulating platelets and white blood cells (Graham et al., 1955) found during early endotoxin shock (Weil and Spink, 1957). Comparisons of the actions of histamine and endotoxin have disclosed definite similarities of action in a number of parameters: early increase in portal venous pressure coincident with a decrease in venous return, pooling, and a rapid decrease in systemic arterial pressure;

lowered pH; and elevated hematocrit (Hinshaw et al., 1962a). Post-mortem findings were also similar (Jordan et al., 1965).

Contrary to the reports that histamine levels are pronounced post-endotoxin, Corrado et al. (1964), and Jacobson et al. (1964) found no elevation of plasma histamine levels after endotoxin. Jacobson and co-workers (1964) also reported decreased vascular responsiveness to histamine during endotoxemia and found anti-histamine drugs to be ineffective in modifying the hemodynamic response to endotoxin. These investigators used a less toxic dose of endotoxin and suggested that elevated histamine levels postendotoxin were elicited by anaphylactic reactions to massive endotoxin doses.

Serotonin has been postulated as another vasoactive substance active in promotion of the shock state accompanying injection of endotoxin, (Rosenberg et al., 1959; Rosenberg et al., 1961). A marked decline in serum serotonin occurs concomitantly with platelet clumping and breakdown postendotoxin (Davis et al., 1960). This decreased serotonin level persists throughout the postendotoxin period. It is important to note the type of anticoagulant used in these studies when interpreting data on serotonin levels. Heparin produces spontaneous platelet aggregation; hence, citrate or EDTA is to be preferred (Raškova and Vaněček, 1964). The report of Davis and co-workers (1960) used EDTA as the anticoagulant yet communnications from Jacobson et al. (1964) and Rosenberg et al. (1961) utilized heparin in assays of serotonin levels. Rosenberg and co-workers (1959) failed to report the anticoagulating agent employed in their studies.

The catecholamines have been suspected as being responsible for

the pathogenesis of Gram-negative shock since Penner and Bernheim (1942) pointed out the similarity between the pathologic changes of endotoxin shock and the lesions produced by epinephrine infusion. Frank (1944-45) found that epinephrine augmented the intestinal congestion associated with canine endotoxemia. The work of Zweifach et al. (1956) promoted the concept that an abnormal response to the catecholamines might be an important factor in the lethal effects of endotoxin. They concluded that endotoxin alters the reactivity of the blood vessels to epinephrine and norepinephrine. However, Meyer and Ballin (1959) and Hinshaw et al. (1958b) failed to confirm the previous findings. It has subsequently been revealed that no significant changes in epinephrine levels are found until the pre-terminal stages while norepinephrine levels are elevated throughout the period postendotoxin (Ravdin and Hardy, 1962; Rosenberg et al., 1959; Rosenberg et al., 1961). Kovats, in a review article, postulated that catecholamines initiate the primary toxic events in the endotoxin reaction (Kovats, 1967). It would be difficult, however, to harmonize his view with recent findings in the endotoxin-shocked primate which does not show elevated catecholamine levels until at least 2 hours after endotoxin administration (Hinshaw et al., 1967; Guenter et al., in press). A major unanswered question relates to whether the release of catecholamines is "damaging" or "protective." Studies in dogs showed adrenalectomized dogs exquisitely sensitive to the deleterious effects of endotoxin (Hinshaw et al., 1964), suggesting a possible protective role of catecholamines in the shock reaction.

The polypeptide bradykinin has been suspected of playing a prominent role in endotoxin shock (Beraldo, 1950; Aldrete et al., 1966; Gilbert, 1960). Polypeptides are frequently released by proteolytic

enzymes (Gaddum, 1955), and there is evidence of proteolysis after endotoxin (Lockwood, 1946; Westphal, 1957). Aldrete et al. (1966) discussed the possible roles of vasoactive polypeptides in septic shock, suggesting that local release of polypeptides might produce low vascular resistance at sites of inflammation. In effect, this would function like a large arteriovenous shunt, increasing flow to the inflamed site. Polypeptide release may possibly account for local and generalized vascular changes in endotoxin shock including alterations in flow, vascular resistances, and capillary permeability. The actions of bradykinin resemble those of histamine (Kontos et al., 1964). It stimulates visceral smooth muscle, but relaxes vascular smooth muscle, dilates capillaries and increases capillary permeability (Aldrete et al., 1966; Kontos et al., 1964). These alterations have all been reported to result from injection of endotoxin and may occur after the release of both histamine and bradykinin. Kobold et al. (1964) reported the releases of histamine, serotonin, and a vasoactive polypeptide-like substance after endotoxin in dogs.

The renin-angiotensin system is reportedly activated after endotoxin in dogs (Vaughn et al., 1967). It is considered to be responsible for a large part of the recovery phase of shock by effecting an increase in blood pressure.

Substantial evidence has been presented that administration of bacterial endotoxin results in the release of several vasoactive materials. The most probable explanation of events occurring at the vascular level postendotoxin will be an interaction of endotoxin and the various substances released. It appears that none of the previously mentioned agents

can be indicted as the primary mediator of early endotoxin shock (Jacobson et al., 1964). It should be considered that vascular reactivity may be substantially altered when serotonin levels are significantly lowered in the face of elevated catecholamine and histamine values (Schayer, 1960). The increased catecholamine levels may have a greater effect than their concentrations tend to indicate (Rosenberg et al., 1959).

This review has demonstrated the great complexity of the vascular responses to endotoxin. There are without doubt a multitude of endogenously released vasoactive agents activated in response to injections of endotoxin. These may be released into circulating blood or activated locally at sites of vascular smooth muscle. The kinds and concentrations of vasoactive agents may vary between species of animals, may be modified as a function of the dose of endotoxin (Davis et al., 1961) and probably vary in accordance with time of release during the postendotoxin period. The canine response to endotoxin has been emphasized because of the great bulk of research carried out on this species. Caution should be continually exercised however, in extrapolating data to another species because of marked species differences, exhibited most dramatically in the primates (Kuida et al., 1961).

Current Therapy for Endotoxin Shock

Endotoxin often initiates critical clinical situations (Altemeier, 1967). Therefore, exhaustive searches have been undertaken to find drugs capable of reversing the septic shock state. Numerous investigators have reported survival benefits from corticosteroids (Lillehei and MacLean, 1959; Spink and Vick, 1961; Jaques and Jaquet, 1964; Nagy et al., 1964;

Weil and Whigham, 1965; Thomas and Brockman, 1968). Today, steroids are widely employed in treatment of shock patients. The mechanisms by which steroids reverse the shock state are not well defined. It has been well established that supraphysiological doses of the steroids must be utilized in order to obtain beneficial actions (Weil and Whigham, 1965). These compounds apparently act as drugs rather than replacement hormones. Thus, they play a pharmacological rather than a physiological role in combating shock (Weil and Whigham, 1965). Nagy et al. (1965) suggested that recovery from a hypotensive to a normotensive state might be due to an increase in cardiac output. This augmentation of output is reportedly the result of prevention of increased peripheral resistance rather than an inotropic cardiac action (Nagy et al., 1965). High doses of corticosteroids, vasodilators in both dogs and humans, may oppose the compensatory constriction of the shock state and thus prevent ischemic tissue injury (Sambhi et al., 1965). The vasodilator activity may also be responsible for decreasing the amount of anaerobic metabolites by promoting a better peripheral circulation (Nagy et al., 1965). Vaughn and others (1967) reported that prednisolone specifically produced an improvement in the mesenteric circulation of shocked animals. Recent work by Dietzman et al. (submitted for publication) has shown the corticosteroids to significantly decrease peripheral vascular resistance and increase systemic oxygen consumption of shock patients. Still another action attributed to the steroids is stabilization of lysosomal membranes (Lefer and Martin, 1969). This could prevent the following sequence of events in the shock state: disruption of lysosomes, release of proteases, and cleavage of plasma proteins. Thus it appears that the actions of steroids

in combating the shock syndrome are numerous and diverse.

Recognition of the presence of numerous vasoactive agents during the septic shock state has stimulated interest in the use of drug antagonists to reverse the shock syndrome (Kalas and Jacobson, 1964). Antihistaminic compounds (Tsagaris et al., 1963), anti-adrenergic substances (Gourzis et al., 1961; Lillehei, 1962), anti-serotonin drugs (Tsagaris et al., 1963), reserpine, which depletes catechol stores (Shimamoto et al., 1958), and anti-inflammatory agents (Erdös et al., 1967) have all been suggested for the therapy regimen of shock. Reports from Northover and Subramanian (1962), and Massion and Erdös (1966) revealed that salicylate pre-treatment prevented the shock-like hypotensive state associated with endotoxin administration in dogs while Meli et al. (1964) disclosed that aspirin effectively reversed endotoxin-induced lung inflammation in mice. Thus, it appeared that an anti-inflammatory agent, i.e. salicylate, was capable of obviating some of the symptoms of Gram-negative shock. This dissertation research project was undertaken to evaluate the actions of salicylates in endotoxin shock in hopes of gleaning information into the evasive mechanisms of action of the endotoxins.

The communication from Northover and Subramanian (1962) pointed out that pre-treatment with salicylates protected dogs from the arterial hypotension associated with endotoxin administration. No subsequent reports of the phenomenon were published by these authors. It appeared that the protective effects of salicylates in endotoxin shock were worthy of further and more detailed investigation.

The Working Hypothesis

The basic observation that salicylates obviated the systemic

hypotension associated with administration of endotoxin was the stimulus for the present dissertation. The research centered on the following question: How do salicylates prevent the endotoxin shock state? Endotoxin was known to be acting at various vascular sites to produce a lowered blood pressure and it was hypothesized that a vascular action of the salicylates might be responsible for reversal of the shock symptoms. Previous research, documented earlier in this paper, has shown that endotoxin acts at the pre-capillary, capillary, and post-capillary level of the vascular system. The results of these actions are ultimately intravascular and extravascular sequestration of blood. Loss of circulating blood volume is said to be responsible for the lowered blood pressure. Therefore, it was hypothesized that the salicylates were preventing the early toxic vascular reactions of endotoxin in the liver and the late deleterious effects which result in extravasation of fluid. A recent symposium on salicylates (Smith, 1966a) revealed anti-inflammatory activity of the salicylates to be due to antagonism of action of bradykinin, histamine and slow reacting substance. An investigation of the capillary bed and post-capillary area following salicylate and endotoxin administration was deemed necessary in order to elucidate some of the actions of salicylates in endotoxin shock.

This work was undertaken to accomplish the following objectives: (1) document the ability of salicylates to protect dogs from endotoxin shock; (2) assertain the role of salicylates in promoting survival after endotoxin; (3) investigate and evaluate the hemodynamic responses following salicylate pre-treatment; and (4) attempt to explain the mechanisms by which salicylates protect the animal from a lethal dose of endotoxin.

It was hypothesized that the salicylates were acting in some way to reverse the effects of endotoxin, and this investigation was designed to elucidate some of the sites of action. Although many researchers have looked in vain for substances possessing the ability to treat patients suffering the effects of endotoxemia, this dissertation was not undertaken to promote the use of salicylates for treatment. The salicylates were merely employed as a tool by which it was possible to examine the actions of endotoxin.

The initial studies undertaken were pilot experiments which consisted of administration of the salicylate (Alka-seltzer was used exclusively in the first studies) prior to injection of an LD_{80} dose of endotoxin. An LD_{80} dose was chosen for several reasons. It simulated the clinical conditions where approximately 80 per cent of patients suffering from Gram-negative bacteremia succumb. This dose also assured lethal effects but was not so potent as to prevent observation of protective effects of the salicylates. Although the LD_{80} dose of endotoxin was routinely employed, the salicylate dose was varied. It soon became evident that the protective effects of the salicylate were directly related to the dose, but as the dose was increased to over 100 mg/kg the dogs began to show evidence of salicylate poisoning: increased body temperature and hyperventilation often followed by respiratory arrest and death. Optimum protective benefits were observed at the highest doses which did not produce toxic manifestations (100 mg/kg).

After the salicylate dose was determined, the actual dissertation protocol was established and begun. A series of survival experiments was carried out which consisted of administration of salicylate

prior to the toxin. The animals were observed for 4 days. Those surviving at 96 hours postendotoxin were called permanent survivors. This time period was chosen on the basis of previous work with endotoxin-shocked dogs. The majority of dogs which succumb to the lethal effects of endotoxin will perish within the first 24 to 48 hours. Any animal living at 96 hours after endotoxin is usually a permanent survivor.

The next phase of this investigation was aimed at the hemodynamic responses. Systemic arterial and portal venous pressures were monitored in intact animals while cardiac output was measured in open-chest animals.

Since the liver was known to be a major site of pathological changes in endotoxin shock, a series of liver perfusion studies was designed to evaluate the liver response to salicylate pre-treatment. Hepatic arterial and portal venous pressures were monitored.

The final portion of the study was focused on the capillary bed. A technique was employed which allowed the measurement of pressure in the capillary bed. Assessment of the microcirculatory bed was included to evaluate mechanisms of fluid loss during endotoxin shock.

CHAPTER II

MATERIALS AND METHODS

The subjects of this investigation were adult mongrel dogs, all of which were anesthetized with intravenous injections of sodium pentobarbital, 30 mg/kg.

Determination of the Canine Hemodynamic Response to Endotoxin and Acetylsalicylic Acid

One hundred and fifty-six animals were utilized in the initial phase of this investigation. Three major types of studies were employed: (1) survival studies; (2) hemodynamic evaluations on intact animals; and (3) venous return preparations. The distribution of animals was as follows: 45 animals for establishment of the LD_{80} dose of endotoxin, 51 for studying effects of acetylsalicylic acid (ASA) on survival after endotoxin, 44 for determination of the hemodynamic response, and 16 for measurement of venous return. In each case a sufficient number of animals was employed to permit statistical evaluation of data.

An approximate LD_{80} dose of endotoxin was established in a group of 45 dogs. Animals living 4 days after intravenous Escherichia coli endotoxin (Difco Laboratories, Detroit, Mich.) administration were considered permanent survivors. The endotoxin doses utilized varied between 0.4 and 1.0 mg/kg.

The effects of pre- and post-treatment with acetylsalicylic acid

(ASA) (aspirin, U.S.P., Merck and Company, Inc., Rahway, N.Y.) or buffered acetylsalicylic acid (Alka-Seltzer, Miles Laboratories, Inc., Elkhardt, Ind.) on survival after endotoxin were studied in a group of 51 dogs.

Again, 4 days was used to designate permanent survival. Buffered ASA was prepared by dissolving six Alka-Seltzer tablets in distilled water and adjusting to a final volume of 60 ml. The resulting concentration was 32 mg of ASA per ml. The 60 ml solution also contained the following constituents: buffer (citrate), 6.64 g; bicarbonate, 11.52 g; and monocalcium phosphate, 1.23 g. The pH of the solution was 7.07. A dose of 100 mg/kg, calculated on the basis of an ASA concentration of 32 mg/ml was infused intravenously over a 30 minute period by means of an infusion pump (Harvard Infusion Apparatus). The infusion rate varied between 1-2 ml/min. Infusion was carried out slowly because deleterious effects were encountered at higher rates of infusion. The predominant harmful effect observed by rapid infusion was marked elevation of body temperature. In animals receiving endotoxin alone, saline was infused in a volume equal to that given animals receiving buffered ASA. In pre-treatment studies, endotoxin was injected after total administration of the ASA. In post-treatment experiments, infusion of buffered ASA was commenced 15 minutes after endotoxin injection, when dogs were usually in the most marked hypotensive condition; the infusion continued for 40 minutes. In additional experiments, ASA (aspirin) was infused during the pre-endotoxin period. Aspirin powder was dissolved in 90% ethyl alcohol to achieve a final concentration of 150 mg/ml. The pH was 7.03.

Approximately 45 mg/kg was infused intravenously. This dose was used because the aspirin powder was not buffered. The alcohol and ASA

solution was infused with a saline flush (50-80 ml) at a constant rate during a 30 minute period. The volume of alcohol infused varied between three and four and one-half ml.

Hemodynamic Measurements in Intact Animals

Forty-four animals were used in this phase of the study. A saline-filled cannula, attached to a Statham pressure transducer, was introduced into the femoral artery. A similar cannula was inserted into a splenic vein of 10 animals, exposed after a midline laparotomy, and advanced to the portal vein. These procedures permitted the measurement of femoral artery and portal vein pressures, respectively, which were recorded on a Sanborn direct writing recorder. Hematocrit and pH values were determined at various intervals. The 44 animals were administered endotoxin alone, buffered ASA alone or in combination with endotoxin or ASA alone or in association with the Gram-negative toxin. The endotoxin dose was 0.4 mg/kg (LD_{82}, as determined in the previously mentioned survival study). Experiments were carried out for 120 minutes postendotoxin administration.

Measurement of Venous Return

A group of 16 dogs were utilized for this study. Each animal was placed in a supine position on a canine surgery table. Pressure measurements were made in the femoral artery as previously described. Following insertion of an endotracheal tube, the animal was respired on room air by means of a Starling constant volume respirator adjusted to 14 to 16 strokes/min and 300 to 400 ml/stroke. The chest was then opened with a mid-sternal incision and retractors were employed to hold the chest in

an open position.

The extracorporeal blood circulation was established according to the procedure described by Weil and co-workers (1956a) and later modified by Hinshaw and co-workers (1961a). The azygous vein was ligated so that all venous blood returned to the heart via the superior and inferior vena cavae. A slit was made in the pericardium to expose the heart and the phrenic nerves were ligated and severed. Large polyethylene cannulae were inserted upstream into the venae cavae, thus collecting all venous return and depositing it in a reservoir maintained at 40°C by means of a constant temperature water bath. A Sigmamotor pump was placed on the outflow side of the reservoir which pumped the blood through another cannula to the right atrium of the dog. The atrial cannula was introduced and securely tied into the auricle of the right atrium. Therefore, all venous return was collected in a reservoir and returned, by means of a pump, to the right atrium. Speed of the atrial inflow pump was repeatedly adjusted during each experiment so that venous return and cardiac inflow were maintained equal at all times. Central venous pressure was maintained at atmospheric pressure (0 mm Hg) by adjusting heights of vena caval drainage cannulae so that a repetitive flutter of the orifices of the central veins continually occurred throughout the experiments.

Following termination of surgical and technical procedures, 10 to 15 minutes stabilization periods were allotted. Cardiac output (venous return) was periodically determined through the study by use of a graduated plastic cylinder and stop watch, measuring blood draining from the inferior and superior and venae cavae. These experiments were carried out for only one hour owing to deterioration of the preparation beyond this

period.

These 16 animals were divided into three groups. Five dogs were administered only endotoxin, 0.4 mg/kg. Six animals were given buffered ASA, 100 mg/kg, prior to endotoxin. The remaining 5 dogs received buffered ASA and no endotoxin.

Total peripheral resistance was calculated by dividing mean systemic arterial pressure by cardiac output (venous pressure = zero mm Hg). Standard deviations (S.D.) were derived utilizing the following equation.

$$S.D. = \sqrt{\frac{\Sigma(x^2)}{(N-1)}}$$

In this equation x equals the deviations from the arithmetic mean and N is equal to the total number of experimental subjects. The standard deviation is a special form of average deviation from the mean. Standard errors (S.E.) were computed by dividing the S.D. by the square root of N. Significance levels were evaluated with the modified t-test (Arkin and Colton, 1958). This was a test for comparing means of unpaired observations and unequal variances, based on the assumption that the two samples from which the means were taken were uncorrelated.

The following formula was employed to calculate the p values:

$$p = \sqrt{\frac{(S.D._1)^2}{N_1} + \frac{(S.D._2)^2}{N_2}}$$

where $S.D._1$ = standard deviation of the first sample, $S.D._2$ = standard deviation of the second sample, N_1 = number of items in the first sample, and N_2 = number of item in the second sample.

Both S.D. and S.E. were computed because the S.D. was utilized in significance calculations while S.E. was employed in graphic illustrations of data.

Perfusion of the Liver

A group of 19 dogs, weighing between 9 and 14 kg, comprised the subject population of the second phase of the present study. Each animal was placed in a supine position on a canine surgery table. Following a mid-line laparotomy incision, a splenctomy and gastrectomy were performed. The hepatic artery was isolated and carefully denervated surgically. The hepatic artery was cannulated and perfused with blood obtained from the femoral artery while the portal vein was cannulated with a perfusion system from the inferior vena cava. The portal venous inflow catheter tip was placed just downstream from exiting veins. Sigmamotor pumps were interposed in each perfusion set-up. A constant blood flow to the hepatic artery was delivered at physiological pressures (100-140 mm Hg), while constant flow to the portal circuit was delivered at pressures between 6 and 15 mm Hg. The celiac, superior mesenteric, and inferior mesenteric arteries were separately ligated following removal of the spleen and stomach and cannulation of the hepatic artery. Care was taken to insure that blood flow to the hepatic artery and portal vein was only separately interrupted (less than 60 sec.) during the cannulation procedures. A continuous flow to the liver through at least one inflow vessel was maintained at all times during insertion of the cannulae.

Pressures were monitored via Statham pressure transducers on a Sanborn direct-writing recorder. Systemic arterial pressure was obtained by advancing a polyethylene catheter from the femoral artery into the aorta. Similar catheters were placed in the tips of the hepatic artery and portal vein inflow tubing to record pressures at those sites.

A period of 20 minutes was allowed to permit stabilization of all

parameters after completion of surgical procedures. Fifty mg/kg of ASA dissolved in 90% ethyl alcohol (150 mg/ml) was administered intravenously to 12 animals. The ASA and alcohol (3-4.5 ml volume) infusion, administered via a Harvard infusion apparatus into the jugular vein, was simultaneously accompanied by a 30 to 40 ml saline flush. ASA injection was accomplished in 15 minutes. Seven of these animals then received an LD_{82} dose of endotoxin (0.4 mg/kg) while the remaining 5 animals received no endotoxin and served as ASA controls. Seven other animals were administered alcohol and saline (no ASA) followed by endotoxin. All preparations were observed for 30 minutes after endotoxin was injected. The results obtained were statistically evaluated with an analysis of variance calculation (Steel and Torrie, 1960). This calculation gave identical results to those obtained with the modified student t-test (Arkin and Colton, 1958).

Determination of Isogravimetric Capillary Pressure

Thirty-seven anesthetized canine subjects were employed in the final episode of the investigation. The study was comprised of 10 endotoxin-shocked preparations and 7 sodium salicylate-treated (sodium salicylate, USP, obtained from Mallinckrodt Chemical Works, St. Louis, Missouri) and endotoxin-injected dogs, all of which were observed at 4 hours following endotoxin administration plus 15 control (no endotoxin) dogs. Another group of 5 animals was studied at 20 hours postendotoxin. At 4 hours postendotoxin the animal is usually in a deep state of shock. The 20 hours observation group consisted of animals which might have been survivors of the toxin injection. The sodium salicylate, 80 mg/kg, was administered intravenously in 10 ml of saline 3 minutes prior to endotoxin

injection. A single shot injection was employed. The pH of the sodium salicylate solution was 7.71.

In each animal, one forelimb was surgically removed above the elbow. The area was shaved and all hair removed prior to surgery. A circular incision was made through the skin approximately 1 inch above the elbow. Surgical removal was carried out by sectioning muscle tissue between double ligatures. Care was taken to avoid ligation of lymphatics. All nerves were tied and severed while the brachial artery, brachial vein and cephalic vein were carefully dissected free for several centimeters. The bone was severed with an autopsy saw and the opening of the bone was immediately sealed with bone wax.

Following intravenous heparization, 5 mg/kg, the brachial artery of the leg was cannulated with plastic tubing extending through a Sigmamotor pump, connected at its proximal end to the femoral artery of the donor dog. The leg veins were separately cannulated with plastic tubing leading to a reservoir placed in a constant temperature water both maintained at 40°C. Blood was returned to the femoral vein of the donor dog by means of another Sigmamotor pump. At the time of cannulation of the leg vessels, the dog side of each vessel was securely ligated to avoid bleeding.

The leg was then placed in a metal tray, suspended from one arm of a strain gauge weighing device and positioned above the venous drainage reservoir. The weighing apparatus consisted of a Wheatstone bridge attached to a strain gauge recording device which permitted continuous monitoring of changes in limb weight (Stish et al., 1956).

Brachial artery pressure, cephalic vein and brachial vein

pressures were monitored at the orifices of the vessels by means of needle-tipped catheters attached to Statham pressure transducers. Systemic arterial pressure was obtained via another cannula introduced into the femoral artery and attached to another transducer. All pressures and changes in limb weight were registered on a Sanborn 6-channel recorder. Foreleg blood flows were measured with a graduated cylinder and stop watch. Initial flow rates to the limb were adjusted to the highest values at which the leg remained in an isogravimetric state. Screw clamps were placed on the venous outflow catheters so that adjustment of these clamps permitted alterations in venous pressures. An equilibration period of approximately 20 minutes was permitted prior to commencement of the experimental procedures.

Hematocrit and pH values were determined periodically.

The limb was then perfused isogravimetrically at various rates of arterial inflow, according to a technique described by Pappenheimer and Soto-Rivera (1948). The isogravimetric condition was established by simultaneous adjustment of inflow rate and venous outflow pressures until the limb neither gained nor lost weight. Each isogravimetric state was maintained for a period of 5 minutes, and 5 to 10 minutes were allowed between each readjustment of flow and pressures. When a condition of isogravimetricity was attained, the foreleg blood flow and associated venous outflow pressures were recorded. Flows were randomly varied between 0 and the highest possible flow attainable in the absence of weight gain. Venous pressures were separately adjusted to be equal in value. The 0 flow technique was previously described by Johnson et al. (1966).

The isogravimetric capillary pressure (Pc_i) is a value which is

determined at a condition of momentary zero flow to the perfused leg. When no blood is flowing to the limb and the limb is neither gaining nor losing weight, the arterial and venous pressures are equal. It is assumed that all pressures in the limb are equal at this time. Thus the arterial or venous pressure measured at an isogravimetric and zero flow state should represent the pressure in a capillary.

Statistics were performed by completely random design analysis of variance and Duncan's multiple-range test for difference between means (Steel and Torrie, 1960).

CHAPTER III

EXPERIMENTAL RESULTS

Canine Hemodynamic Response to Endotoxin

Survival Studies

The results of the survival studies are depicted in Table 1. All tables and several figures presented in this dissertation were previously published in the following journals: J. Pharm. Exp. Ther. 157: 665, 1967; Proc. Soc. Exp. Biol. and Med. 127: 332, 1968; and Am. J. Physiol. 214: 443, 1968. Permission was obtained from each journal to reproduce these tables and figures.

Dogs living 4 days after administration of endotoxin were considered permanent survivors. Fifty-one animals were utilized for this portion of the study. Of the 18 animals receiving only endotoxin (0.4 mg/kg, an LD_{82} dose as previously established in a series of 45 animals), 22 per cent were permanent survivors. When 13 dogs were pre-treated with buffered ASA prior to endotoxin injection, the survival rate was 62 per cent. Fifty-eight per cent of another group of 12 dogs receiving aspirin (ASA, 45 mg/kg) survived the subsequent insult of endotoxin. Statistical analysis, comparing each pre-treated group to the endotoxin shocked group, revealed the p value to be less than 0.05. Another series of 8 dogs was administered endotoxin and then post-treated with buffered ASA. The

TABLE 1

EFFECTS OF ASA ON SURVIVAL IN DOGS ADMINISTERED ENDOTOXIN (0.4 mg/kg)

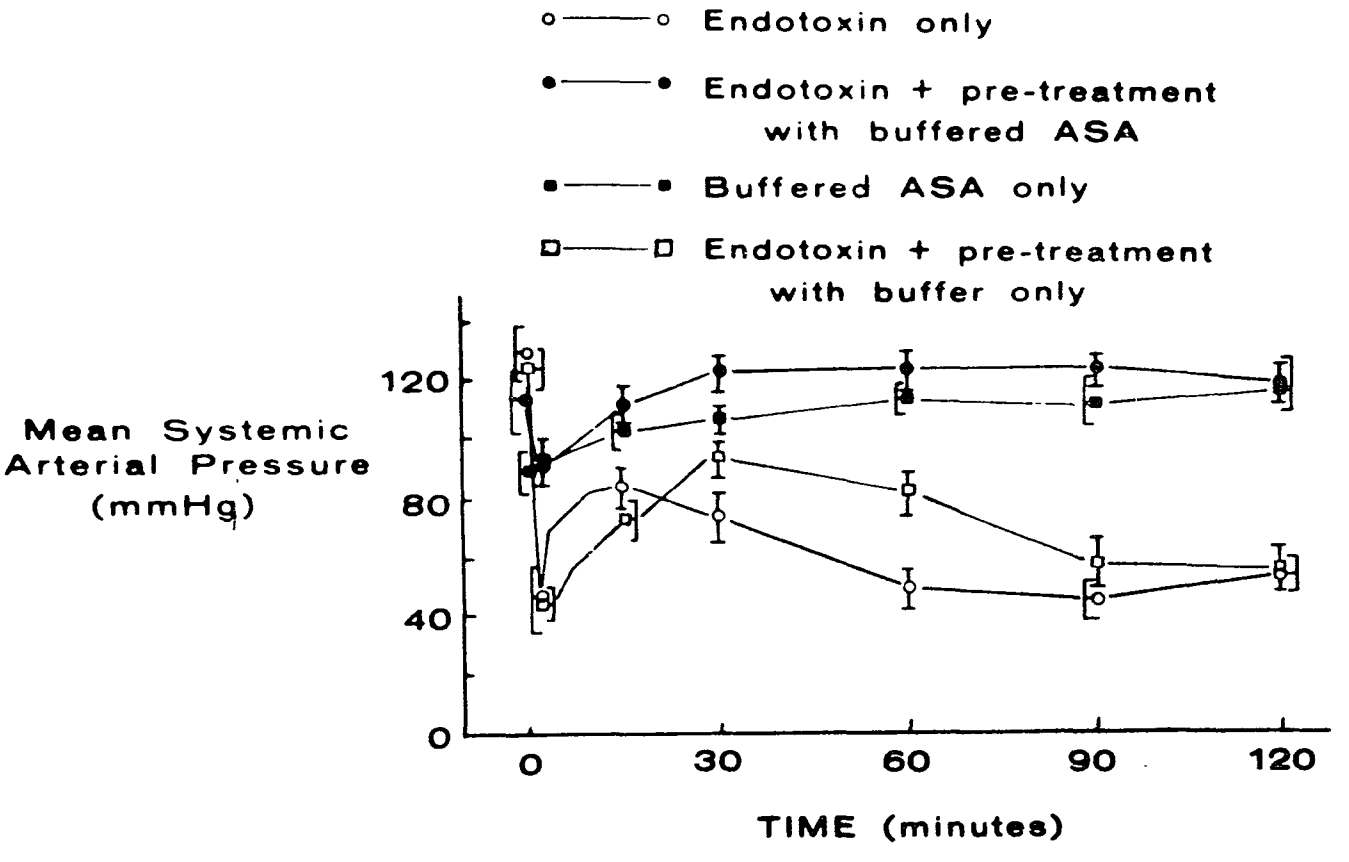
Groups	Number of Animals	Survival Rate
Endotoxin only	18	22%
Treated Animals:		
(1) Pre-treated with buffered ASA (100 mg/kg)	13	62% ($p<.05$)
(2) Pre-treated with ASA (45 mg/kg)	12	58% ($p<.05$)
(3) Post-treated with buffered ASA (100 mg/kg)	8	37% (not significant)

survival rate in this group was 37 per cent, not statistically significant from the endotoxin group. Therefore, pre-treatment with buffered ASA or ASA had a significant effect on increasing the survival rate after endotoxin.

Hemodynamic Response of the Intact Animal

Thirty-seven animals, divided into 4 groups, comprised this phase of the study. The results are shown in Figures 1, 2 and 3. Endotoxin (0.4 mg/kg) was administered to each of the animals in 3 of the 4 groups at zero time. Mean values plus or minus standard errors are represented for each of the 4 groups. In some instances, standard errors are not shown because they are smaller than the symbols. The systemic arterial responses are seen in Figure 1. Ten animals, given only endotoxin and represented by the open circles, responded with an initial precipitous decline in arterial pressure within 2 minutes. Pressures had recovered to near normal levels by 30 minutes but tended to decrease from 30 to 120 minutes. A second group, consisting of 12 dogs, was pre-treated with buffered ASA (100 mg/kg) prior to endotoxin administration. This group, depicted by the closed circles, revealed a slight drop in pressure at 2 minutes but control levels were regained by 15 minutes and maintained throughout the experimental period. Statistical analysis of these first two groups revealed significant differences ($p<.05$) in pressure at all points past zero time. Solid squares represent the mean pressure values of 10 animals receiving only the buffered ASA (no endotoxin). Initial pressures were lower in this group than the other 3 groups and showed a slight tendency to increase throughout the 120 minutes. The fourth group (5 animals) was included to study the effects of the buffer components of

Figure 1. Effects of Endotoxin and Buffered ASA Pre-Treatment on Systemic Arterial Pressure. Mean systemic arterial pressure values, plus or minus standard errors, are presented for four groups of animals (total number of animals = 37). Ten dogs were administered only endotoxin (0.4 mg/kg) at zero time. Twelve dogs received buffered ASA (100 mg/kg) prior to endotoxin injection. Buffered ASA alone was given to another series of ten animals. The fourth group consisted of five dogs which were given buffer prior to endotoxin administration.


Endotoxin only
Endotoxin + pre-treatment with buffered ASA
Buffered ASA only
Endotoxin + pre-treatment with buffer only
Mean Systemic Arterial Pressure (mmHg)
120
80
40
0
0
30
60
90
120
TIME (minutes)

the ASA mixture. The buffer solution was infused in an amount equal to that used in the previous pre-treatment studies but without ASA. Open squares show the response to endotoxin with buffer pre-treatment. The pressure response of this group was very similar to that of the endotoxin-shocked group.

Figure 2 shows the hematocrit changes associated with endotoxin and ASA administration. The animals which received only endotoxin (open circles) exhibited an 18% rise in hematocrit while those dogs pre-treated with buffered ASA prior to endotoxin (closed circles) showed only a 9% increase. These 2 groups were significantly different at 60, 90, and 120 minutes postendotoxin. The dogs getting only buffered ASA (solid squares) revealed a very slight rise in hematocrit which had returned to control levels by the end of the experimental period. Elevations in hematocrit readings were also seen in the endotoxin plus buffer group (open squares).

Periodic pH values are revealed in Figure 3. Acidosis was evident by 30 minutes and marked by 60 minutes in the endotoxin-shocked group of dogs (open circles). In contrast, dogs given buffered ASA before endotoxin showed a slight elevation in pH values. Again, these 2 groups were significantly different ($p<.05$) at all points beyond zero time. Buffered ASA caused a rise in pH in animals not subjected to endotoxin (solid squares). Dogs which were given buffer before endotoxin (open squares) responded with a decline in pH, somewhat similar to that seen in the endotoxin-shocked group.

Figure 4 presents portal venous pressure results from 10 intact animals in which the effects of unbuffered ASA (aspirin) were evaluated.

Figure 2. Effects of Endotoxin and Buffered ASA Pre-treatment on Hematocrit. Mean hematocrit values, plus or minus standard errors, are presented for four groups of animals (total number of animals = 37). Ten dogs were administered only endotoxin (0.4 mg/kg) at zero time. Twelve dogs received buffered ASA (100 mg/kg) prior to endotoxin injection. Buffered ASA alone was given to another series of ten animals. The fourth group consisted of five dogs which were given buffer prior to endotoxin administration.

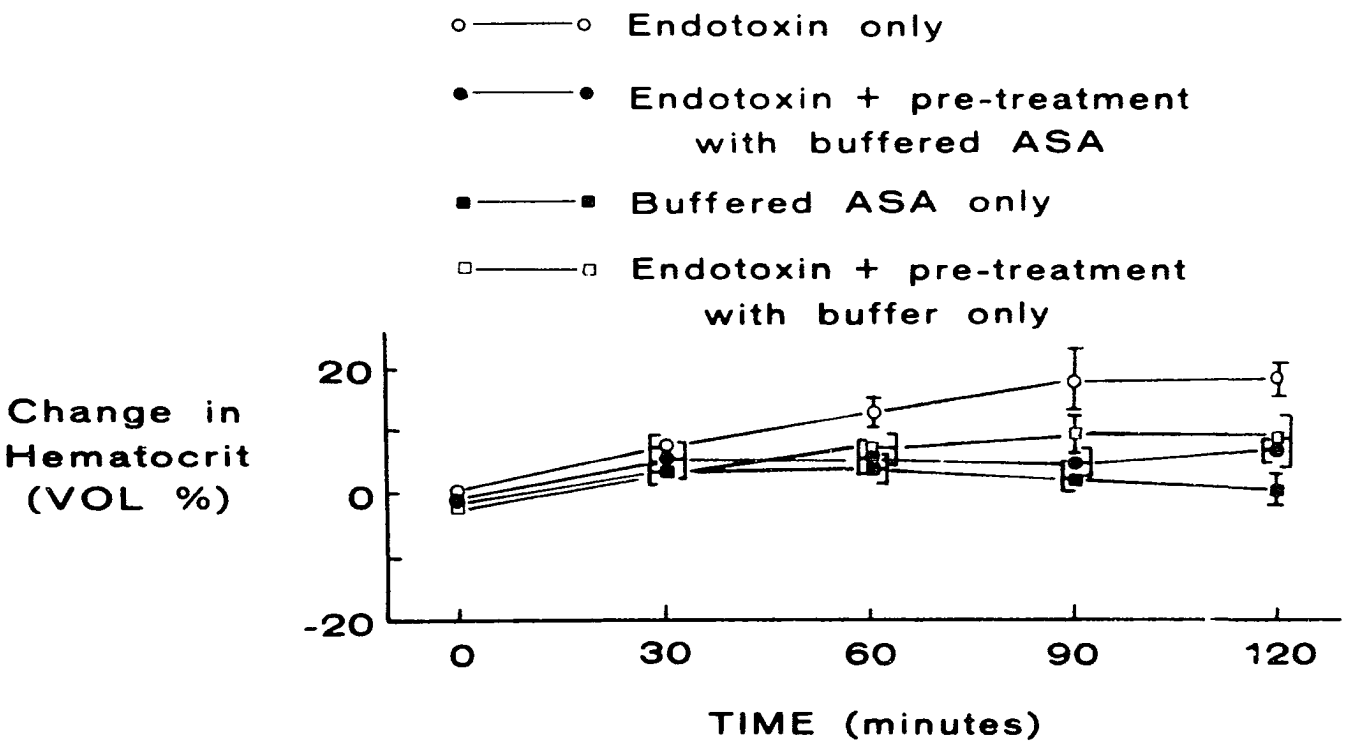

Endotoxin only
Endotoxin + pre-treatment with buffered ASA
Buffered ASA only
Endotoxin + pre-treatment with buffer only
Change in Hematocrit (VOL %)
20
0
-20
0
30
60
90
120
TIME (minutes)

Figure 3. Effects of Endotoxin and Buffered ASA Pre-treatment on pH. Mean pH values, plus or minus standard errors, are presented for four groups of animals (total number of animals = 37). Ten dogs were administered only endotoxin (0.4 mg/kg) at zero time. Twelve dogs received buffered ASA (100 mg/kg) prior to endotoxin injection. Buffered ASA alone was given to another series of ten animals. The fourth group consisted of five dogs which were given buffer prior to endotoxin administration.

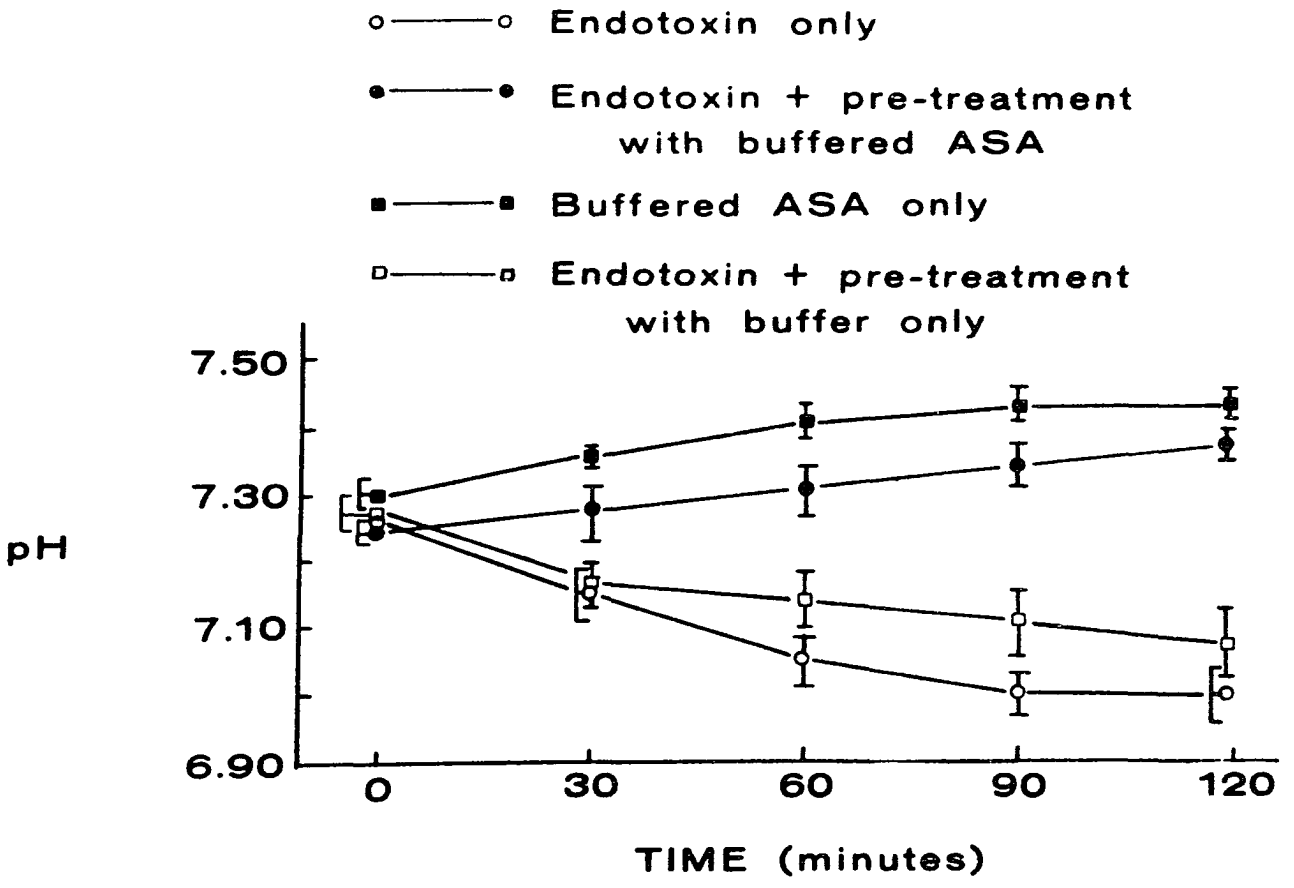
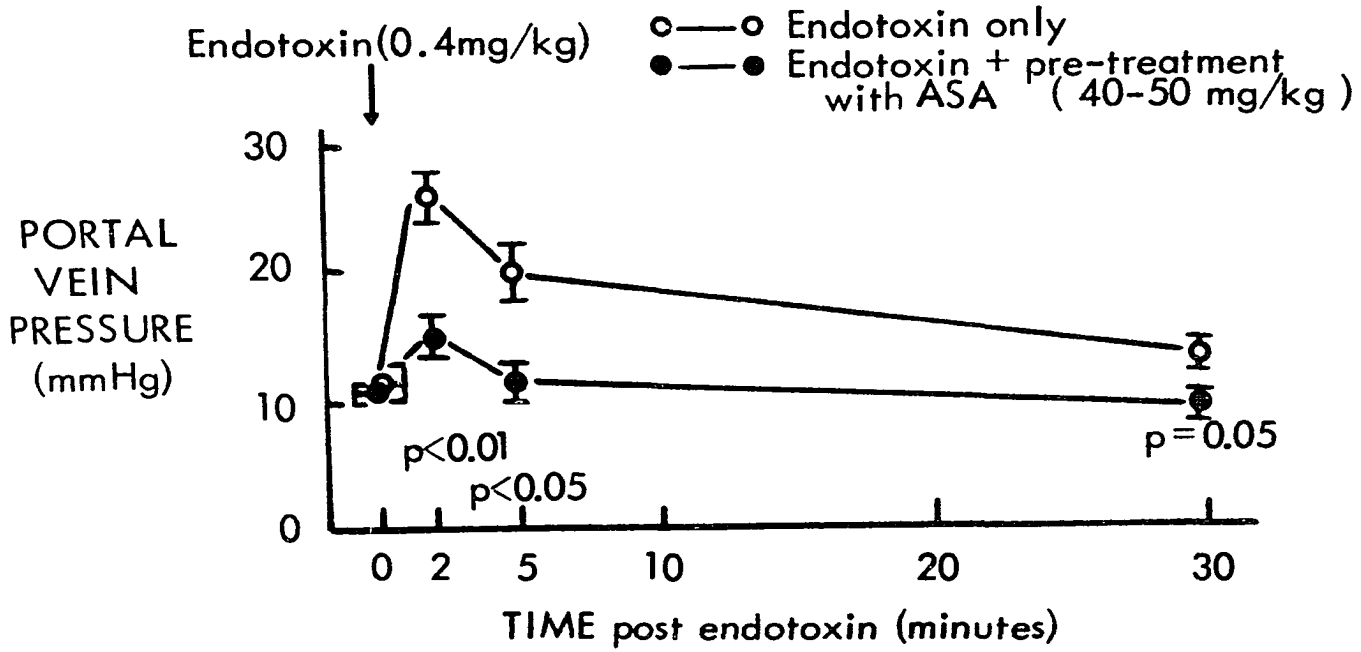

Endotoxin only
Endotoxin + pre-treatment with buffered ASA
Buffered ASA only
Endotoxin + pre-treatment with buffer only
pH
7.50
7.30
7.10
6.90
0
30
60
90
120
TIME (minutes)

Figure 4. Effects of Endotoxin and ASA Pre-treatment on Portal Vein Pressure. Mean portal vein pressure values, plus or minus standard errors, are shown for fifteen animals. Five dogs were given only endotoxin (0.4 mg/kg) at zero time. The remaining ten dogs received ASA (40-50 mg/kg) prior to endotoxin injection.


Endotoxin(0.4mg/kg)
o—o Endotoxin only
●—● Endotoxin + pre-treatment with ASA (40-50 mg/kg)
PORTAL VEIN PRESSURE (mmHg)
30
20
10
0
p<0.01
p<0.05
p=0.05
0 2 5 10 20 30
TIME post endotoxin (minutes)

Five animals were administered intravenous infusions of ASA during the 30 minute period prior to endotoxin while the remaining 5 animals received only endotoxin (no ASA pre-treatment). Portal vein pressures were noticeably elevated in the animals receiving only endotoxin (open circles) while the ASA pre-treated dogs (closed circles) revealed only slight increases. The portal venous pressure responses of the 2 groups were significantly different at each point beyond endotoxin administration.

Thus, at the conclusion of this phase of the study, the classical canine hemodynamic response to endotoxin had been reproduced and it was observed that buffered ASA pre-treatment obliterated systemic hypotension, portal venous hypertension, and acidosis while diminishing hemoconcentration usually associated with the Gram-negative toxin.

The effects of post-treatment with buffered ASA were evaluated. Buffered ASA infusion was begun at 15 minutes after endotoxin administration. Figures 5, 6 and 7 present the effects of post-treatment on systemic arterial pressures, pH, and hematocrit, respectively. Mean values, plus or minus standard errors, are shown. In some instances, the standard errors were smaller than the symbols and are not shown. Ten dogs received only endotoxin and their responses are shown by the open circles (these same 10 dogs are the endotoxin-shocked animals shown in Figures 1, 2 and 3). A group of 7 animals receiving the buffered ASA postendotoxin is depicted by the solid squares. The characteristic abrupt fall in systemic arterial pressure, at 2 minutes after endotoxin, was followed by recovery to control levels by 60 minutes (Figure 5). The pH levels were essentially constant throughout the 120 minutes (Figure 6). Buffered ASA post-treatment also depressed the rise in hematocrit (Figure 7).

Figure 5. Effects of Endotoxin and Buffered ASA Post-treatment on Systemic Arterial Pressure. Mean systemic arterial pressure values, plus or minus standard errors, are shown for seventeen animals. Ten animals received only endotoxin (0.4 mg/kg) while the other seven dogs were given buffered ASA (100 mg/kg) at 15 minutes after endotoxin.

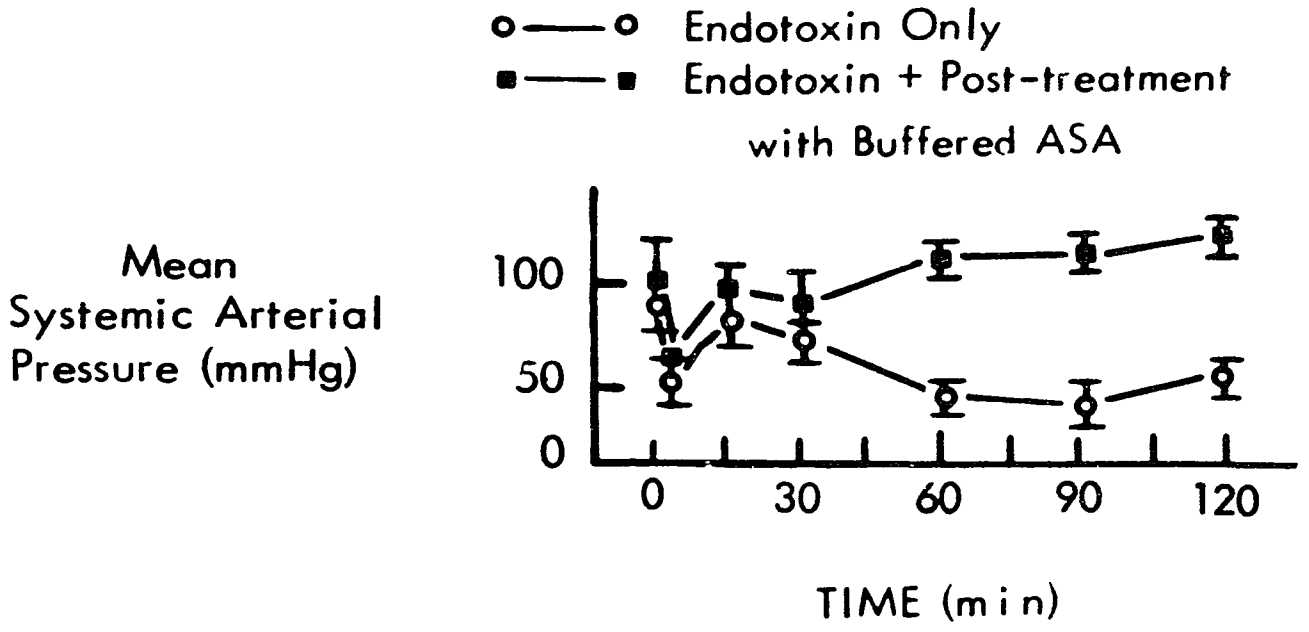

o—o Endotoxin Only
■—■ Endotoxin + Post-treatment with Buffered ASA
Mean Systemic Arterial Pressure (mmHg)
100
50
0
0
30
60
90
120
TIME (min)

Figure 6. Effects of Endotoxin and Buffered ASA Post-treatment on pH. Mean pH values, plus or minus standard errors, are presented for seventeen animals. Ten dogs received only endotoxin (0.4 mg/kg). Seven animals were given buffered ASA (100 mg/kg) at 15 minutes postendotoxin.

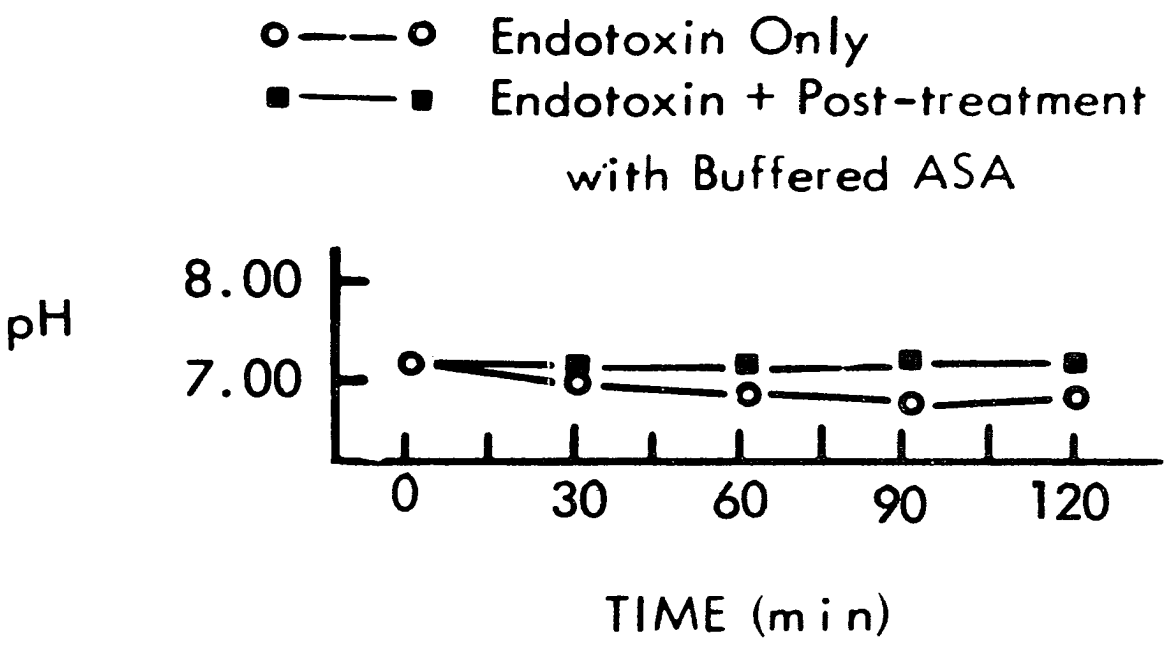
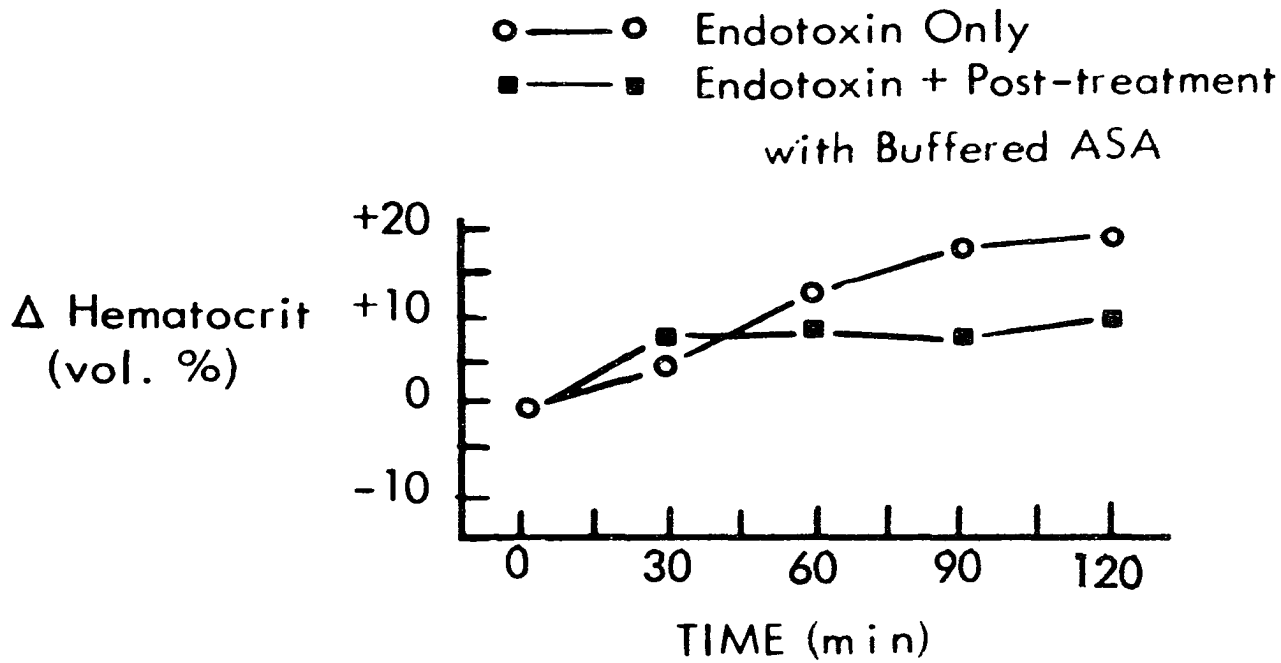

Endotoxin Only
Endotoxin + Post-treatment with Buffered ASA
pH
8.00
7.00
0
30
60
90
120
TIME (min)

Figure 7. Effects of Endotoxin and Buffered ASA Post-treatment on Hematocrit. Mean hematocrit values, plus or minus standard errors, are presented for seventeen animals. Ten dogs received only endotoxin (0.4 mg/kg). Seven animals were given buffered ASA (100 mg/kg) at 15 minutes postendotoxin.


Endotoxin Only
Endotoxin + Post-treatment with Buffered ASA
Δ Hematocrit (vol. %)
+20
+10
0
-10
0
30
60
90
120
TIME (min)

Venous Return Preparations

The results of 16 venous return experiments are shown in Figure 8. Infusions of ASA were carried out in two of the three groups between 0 and 30 minutes. Endotoxin was administered intravenously in two of the three groups at 30 minutes. Mean values are shown for all three groups and standard errors are indicated where they are large enough to be shown. Open circles indicate the 5 animals which received only endotoxin (administered at the 30 minute time interval). These animals revealed the typical early response to endotoxin: (1) systemic arterial hypotension, (2) diminished cardiac output, and (3) increased calculated total peripheral resistance. The 6 dogs which were infused with buffered ASA before endotoxin was administered at 30 minutes are shown by the closed circles. During the pre-treatment period, these animals exhibited essentially constant pressures, but a rise was noted in cardiac output. Thus, total peripheral resistance levels declined. Following endotoxin injection, systemic arterial pressure, cardiac output and resistance values revealed little or no change. The third group, consisting of 5 dogs, received only the buffer solution (no endotoxin, no ASA). These animals, represented by solid squares, exhibited responses which were very similar to those seen in the ASA plus endotoxin group.

Effect of ASA on the Liver Response to Endotoxin

The livers were perfused at constant rates of inflow in each study. Table 2 presents the mean values, plus or minus standard errors, of the flows in each of the three groups of experiments. Very similar flows were established in all 3 groups.

Figure 8. Effects of Endotoxin and Buffered ASA Pre-treatment on Venous Return Preparations. Means values, plus or minus standard errors, for systemic arterial pressure, cardiac output and total peripheral resistance are presented. Five dogs received only endotoxin at zero time. Six animals were given buffered ASA (100 mg/kg) prior to endotoxin. The other five dogs received buffered ASA and no endotoxin.

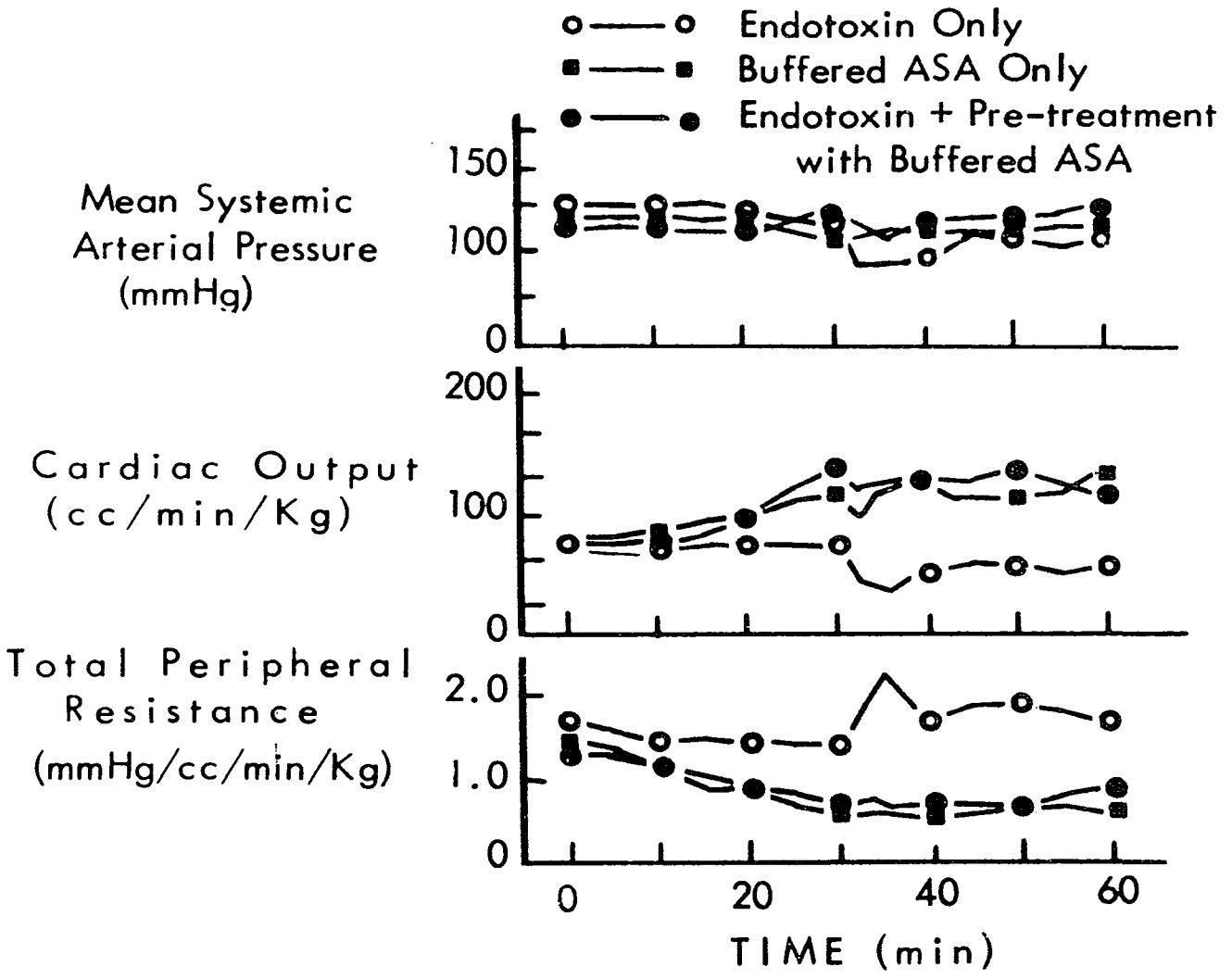

Endotoxin Only
Buffered ASA Only
Endotoxin + Pre-treatment with Buffered ASA
Mean Systemic Arterial Pressure (mmHg)
150
100
0
Cardiac Output (cc/min/Kg)
200
100
0
Total Peripheral Resistance (mmHg/cc/min/Kg)
2.0
1.0
0
0
20
40
60
TIME (min)

TABLE 2

LIVER BLOOD FLOW (MEANS ± SE)

Group (number of animals)	Blood Flow (ml/kg body wt.)
ASA Pre-endotoxin (7)	17.9 ± 1.4
Alcohol blank Pre-endotoxin (7)	20.8 ± 1.5
ASA (5)	19.4 ± 3.3

Mean values and standard errors of systemic arterial and portal venous pressures are seen in Figure 9. The classical response to endotoxin was observed in the 7 animals receiving only endotoxin; i.e., systemic arterial hypotension was associated with portal venous hypertension.

In contrast, these responses were obliterated in the 7 animals which received ASA prior to endotoxin administration. Comparison of these two groups with statistical analysis showed significant differences in arterial pressures at 3, 15 and 30 minutes postendotoxin. Portal venous pressures were found to be significantly different at 3 and 15 minutes after the toxin. Constant arterial and portal venous pressures were seen in the 5 dogs receiving only ASA (no endotoxin).

Hepatic artery pressure responses are found in Figure 10. The results are presented as changes elicited by endotoxin. Pressure was significantly elevated at 3 minutes after endotoxin in the 7 animals not pre-treated with ASA. Yet those 7 dogs which received ASA pre-treatment failed to show this increase in pressure. The five control studies (ASA, no endotoxin) also exhibited constant hepatic artery pressures.

Absolute hepatic artery pressures (means and standard errors) of the 3 groups are displayed in Table 3.

Effect of Endotoxin and Sodium Salicylate on the Isogravimetric Capillary Pressure in the Forelimb

Figure 11 shows the pressure-flow relationship obtained from a typical control (no endotoxin) experiment. Isogravimetric venous pressures (Pv_i) are plotted versus blood flows. Numbers indicate the sequence in which the points were obtained. The isogravimetric capillary pressure

Figure 9. Effects of Endotoxin and ASA Pre-treatment on Systemic Arterial and Portal Venous Pressure. Mean values, plus or minus standard errors, are shown for nineteen animals. Seven dogs received only endotoxin (0.4 mg/kg) at zero time. Another seven animals were pre-treated with ASA (40-50 mg/kg) prior to endotoxin injection. Five dogs were given only ASA.

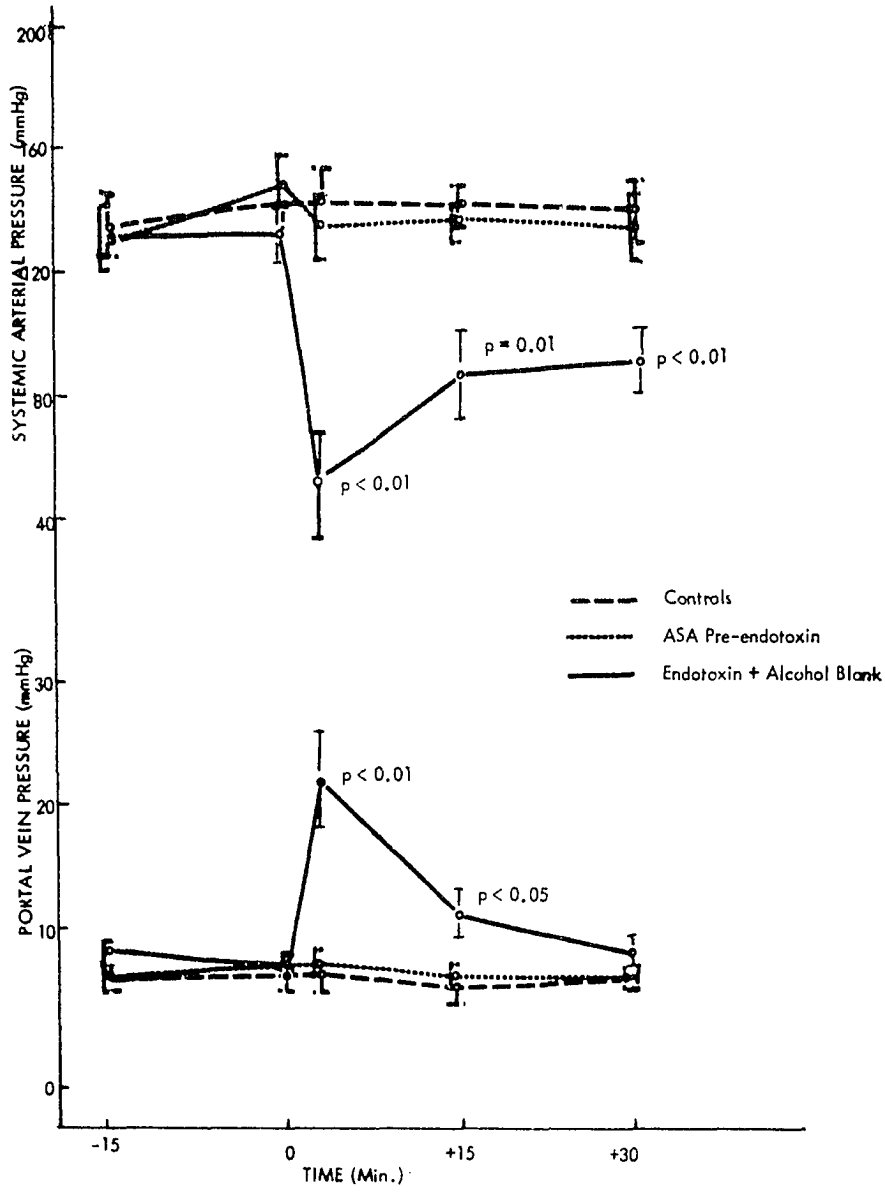

SYSTEMIC ARTERIAL PRESSURE (mmHg)
200
160
120
80
40
p = 0.01
p < 0.01
p < 0.01
Controls
ASA Pre-endotoxin
Endotoxin + Alcohol Blank
PORTAL VEIN PRESSURE (mmHg)
30
20
10
0
p < 0.01
p < 0.05
-15
0
+15
+30
TIME (Min.)

Figure 10. Effects of Endotoxin and ASA Pre-treatment on Hepatic Artery Pressure. Mean values, plus or minus standard errors, are shown for nineteen animals. Seven dogs received only endotoxin (0.4 mg/kg) at zero time. Another seven animals were pre-treated with ASA (40-50 mg/kg) prior to endotoxin injection. Five dogs were given only ASA.

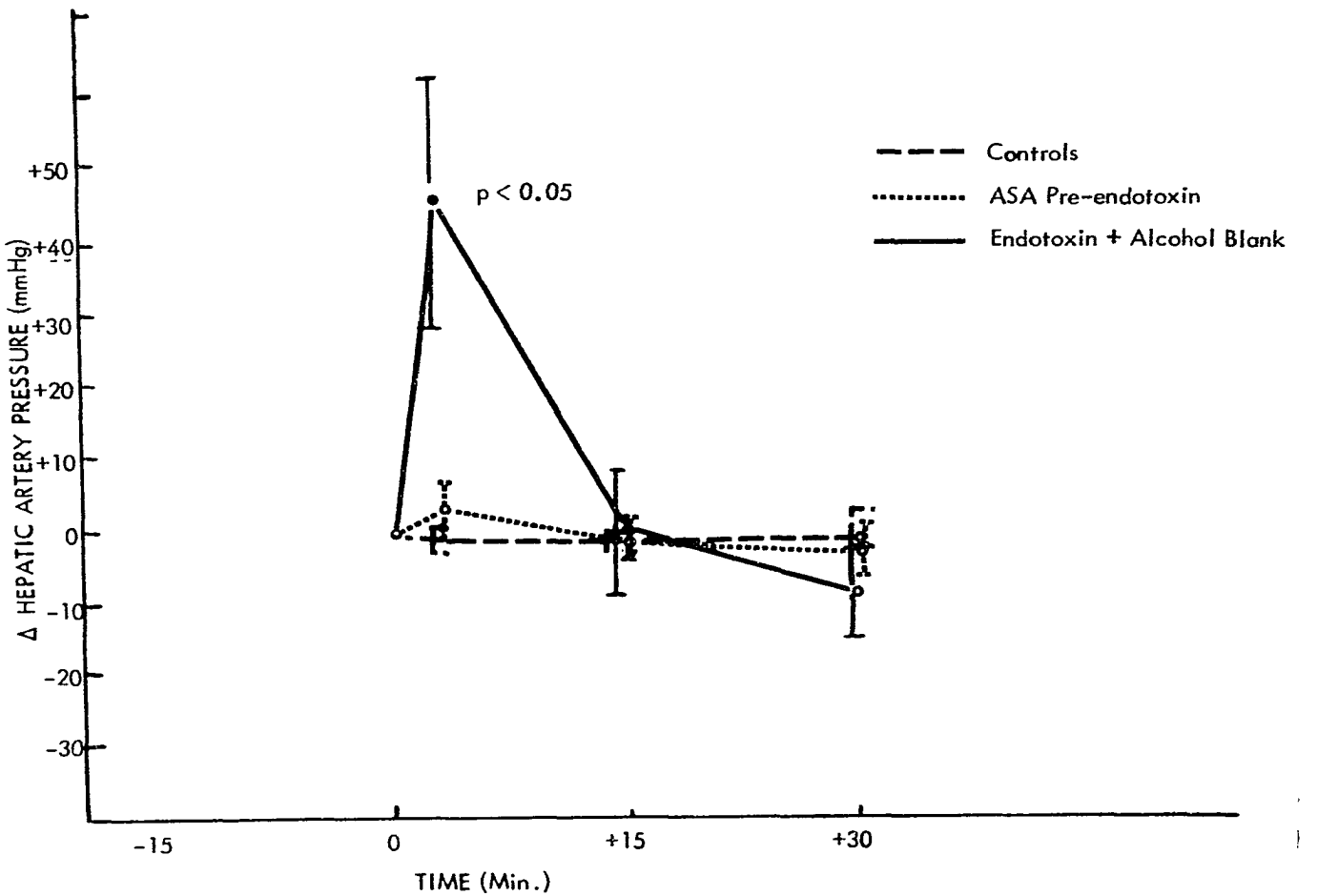
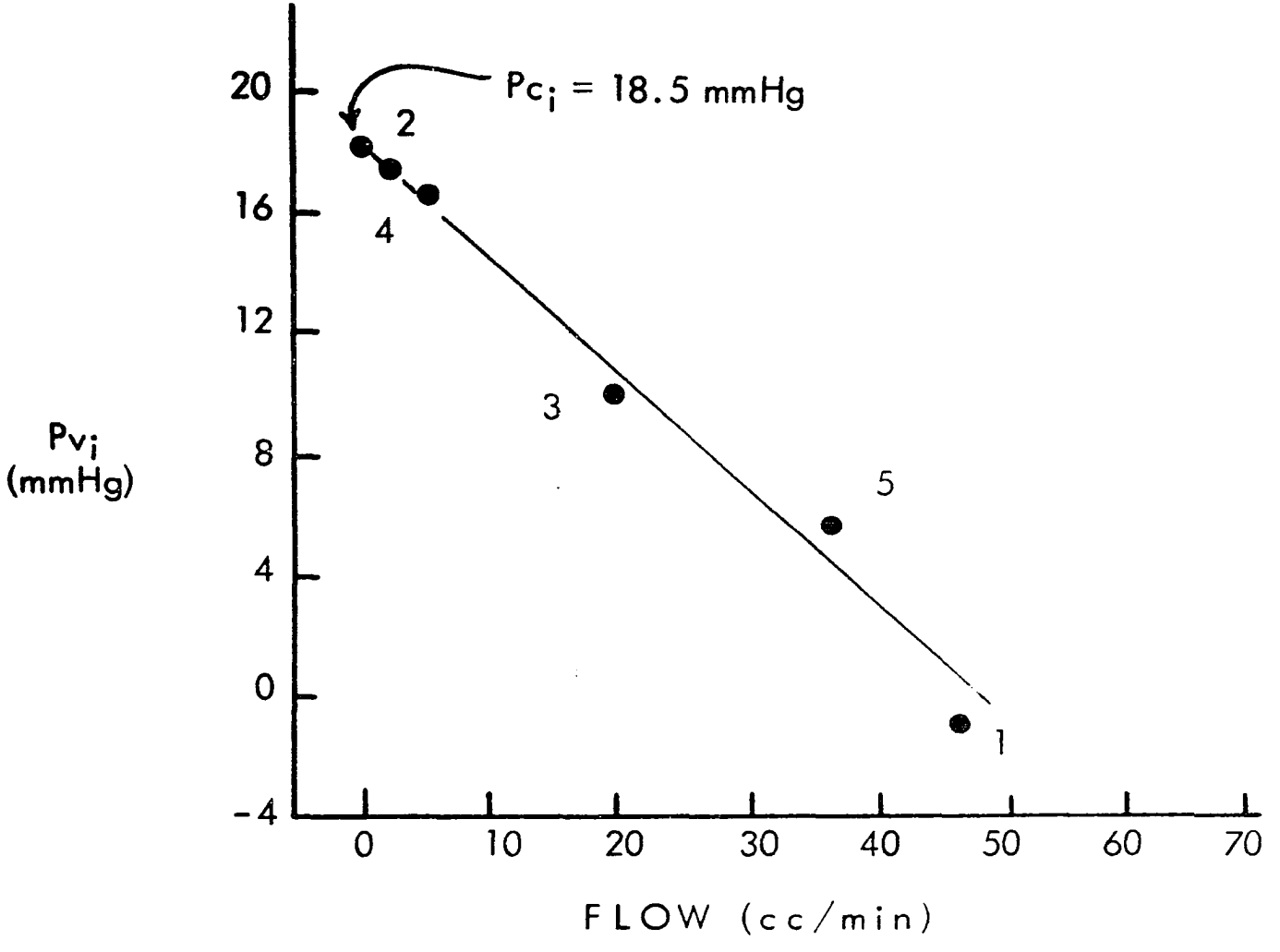

Controls
ASA Pre-endotoxin
Endotoxin + Alcohol Blank
p < 0.05
Δ HEPATIC ARTERY PRESSURE (mmHg)
+50
+40
+30
+20
+10
0
-10
-20
-30
-15
0
+15
+30
TIME (Min.)

TABLE 3

HEPATIC ARTERY PRESSURES (mm Hg; means ± SE)

Group (Number of animals)	Time (min)				
	Pre-Infusion	0	+3	+15	+30
Controls (5)	65 ± 6	64 ± 7	63 ± 6	61 ± 7	62 ± 6
ETOH + endotoxin (7)	80 ± 6	74 ± 8	119 ± 22	82 ± 17	66 ± 7
ETOH + endotoxin + ASA (7)	82 ± 13	79 ± 13	81 ± 15	77 ± 12	76 ± 11

Figure 11. Determination of Isogravimetric Capillary Pressure in a Control Study. The pressure-flow relationship obtained in a control (no endotoxin) animal is shown.


Pc_i = 18.5 mmHg
2
4
3
5
1
20
16
12
8
4
0
-4
0
10
20
30
40
50
60
70
Pv_i (mmHg)
FLOW (cc/min)

(Pc_i), the venous pressure recorded at a condition of zero flow, was 18.5 mm Hg in this experiment. A linear relationship is noted between the Pc_i and other points. This straight line relationship was previously reported by Johnson et al. (1966).

Figure 12 contrasts the pressure-flow relationships obtained in a control versus an endotoxin-shocked animal (4 hours after endotoxin). Best-fit linear relationships are shown. The control study shows higher pressures and flows than the shocked preparation. A Pc_i value of 17 mm Hg was exhibited by the control leg while the endotoxin injected limb had a Pc_i of only 4 mm Hg. The flat type of curve shown by this shocked preparation was characteristic of all 10 dogs receiving only endotoxin. If venous pressures were elevated above the low values shown, steady gains in limb weight ensued.

The typical pressure-flow relationship represented in Figure 13 was obtained in one of the 7 animals given sodium salicylate prior to endotoxin. The Pc_i value was 16 mm Hg. The results obtained are very similar to those seen in control (no endotoxin) animals. Similar responses were observed in the other 6 animals of this treated group.

Figure 14 shows the Pc_i values obtained in all three groups of animals. The mean Pc_i value found in control (no endotoxin) dogs was 16 mm Hg while the mean Pc_i 4 hours after endotoxin was 7 mm Hg. Animals receiving ASA pre-treatment failed to demonstrate the lowered Pc_i values associated with endotoxin. The mean Pc_i level in preparations given both ASA and endotoxin was 16 mm Hg, the same value found in control (no endotoxin) studies.

The results found when the pressure-flow relationship was determined in one of the five dogs at 20 hours postendotoxin are seen in

Figure 12. Determination of Isogravimetric Capillary Pressure in a Control Versus an Endotoxin-Shocked Animal (4 hours postendotoxin). The pressure-flow relationships obtained in a control (no endotoxin) and in an endotoxin (0.4 mg/kg) injected dog are shown.

DETERMINATION OF ISOGRAVIMETRIC CAPILLARY PRESSURE

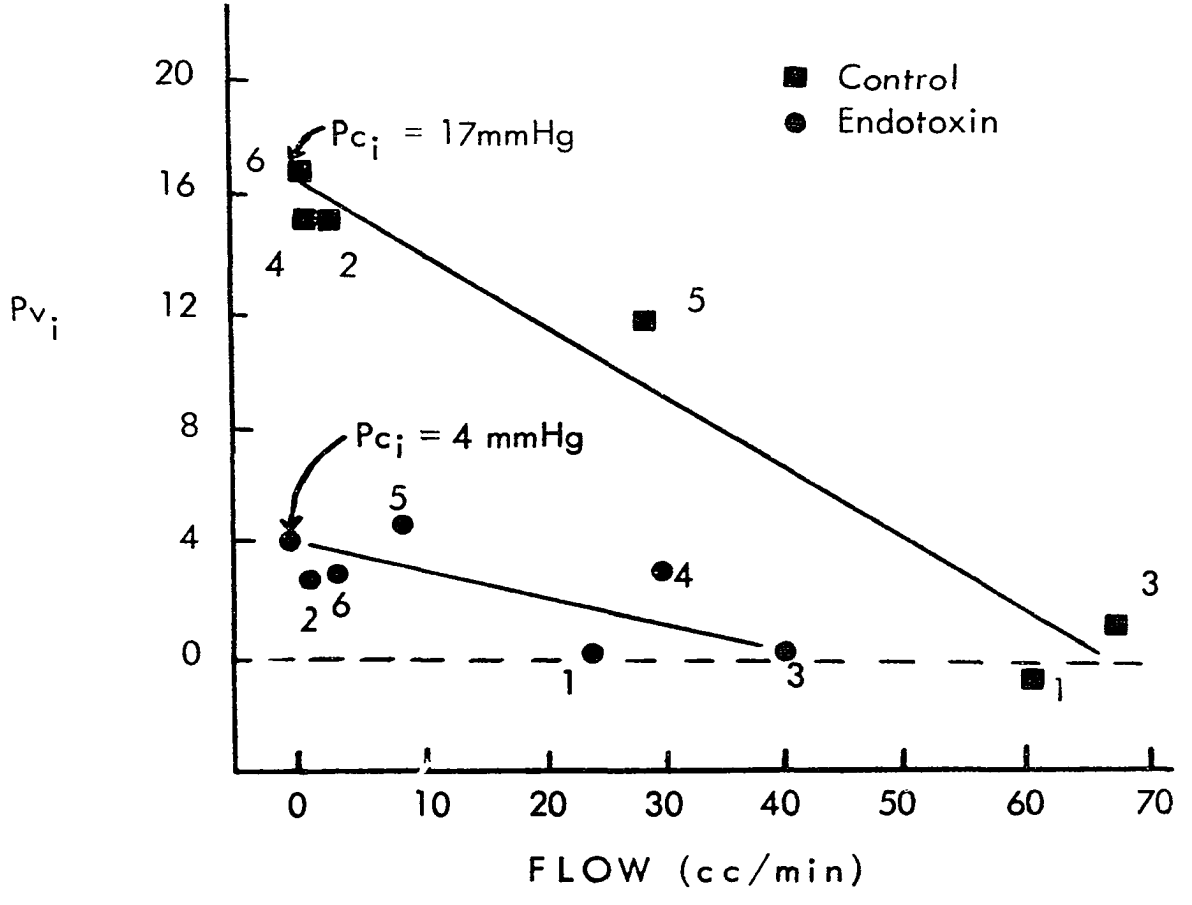


Figure 13. Determination of Isogravimetric Capillary Pressure in a Sodium Salicylate and Endotoxin Injection Animal. The pressure-flow relationship is presented for an animal given sodium salicylate (80 mg/kg) prior to endotoxin (0.4 mg/kg) administration.

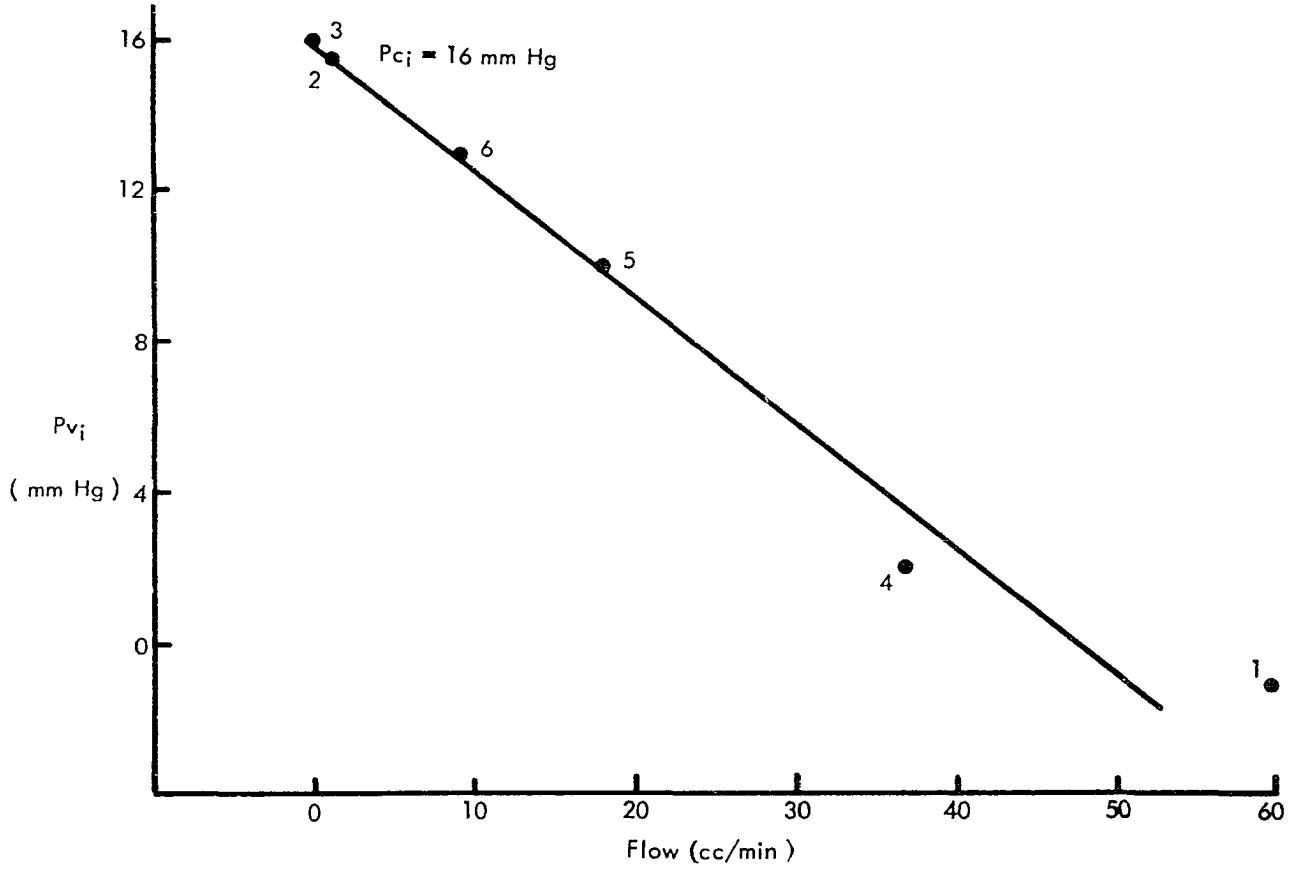

16
12
8
4
0
P_{v_i}
(mm Hg)
P_{c_i} = 16 mm Hg
1
2
3
4
5
6
0
10
20
30
40
50
60
Flow (cc/min)

Figure 14. Summary of Isogravimetric Capillary Pressures Obtained in Control, Shocked, and Sodium Salicylate Pre-treated Animals. The isogravimetric capillary pressure values are shown for fourteen control (no endotoxin), ten endotoxin-shocked (0.4 mg/kg), and seven sodium salicylate (80 mg/kg) pre-treated dogs. Each line represents the isogravimetric capillary pressure value obtained in one experiment.

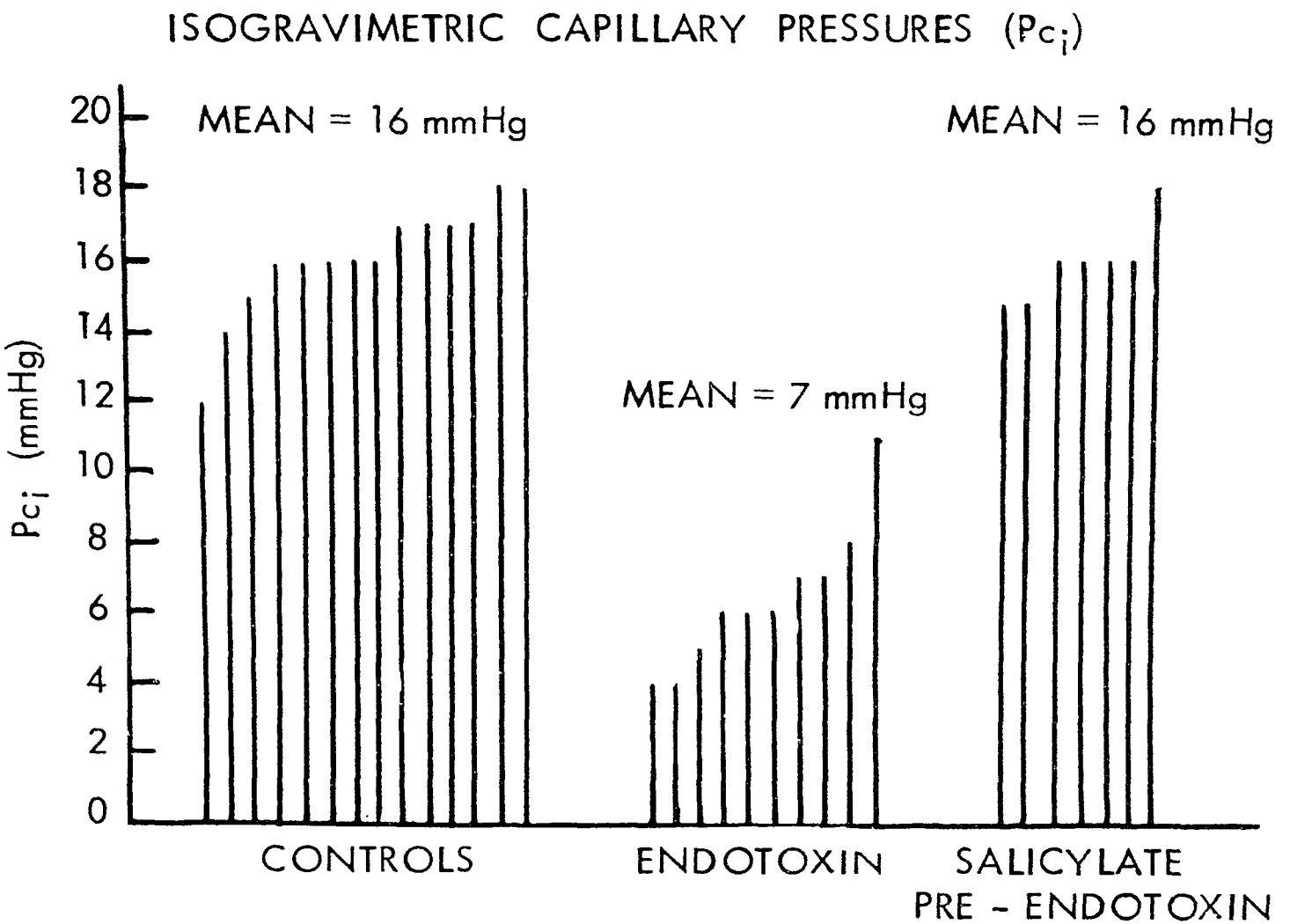

ISOGRAVIMETRIC CAPILLARY PRESSURES (Pc_i)
MEAN = 16 mmHg
MEAN = 16 mmHg
MEAN = 7 mmHg
Pc_i (mmHg)
20
18
16
14
12
10
8
6
4
2
0
CONTROLS
ENDOTOXIN
SALICYLATE
PRE - ENDOTOXIN

Figure 15. Similar results were obtained from the other 4 dogs. The Pc_i was lowered as previously noted at 4 hours after endotoxin. It was impossible to establish high rates of blood flow in this preparation without inducing a gain in limb weight.

Table 4 summarizes a number of parameters in 15 control experiments and in 17 studies in which endotoxin had been administered 4 hours previously. The endotoxin group is divided into 2 series: (1) those receiving only endotoxin; and (2) those which were pre-treated with sodium salicylate followed by endotoxin. The results show that endotoxin significantly lowered Pc_i, mean systemic arterial pressure (MSAP), pH, and forelimb blood flows while significantly elevating hematocrit values. Sodium salicylate pre-treatment prevented all of these changes except hemoconcentration. It should also be noted that diarrhea was routinely observed in endotoxin shocked dogs yet sodium salicylate pre-treatment obliterated the diarrhea.

The results obtained 20 hours post-endotoxin are summarized in Table 5. Again, there were significant decreases in Pc_i and mean systemic arterial pressure. Hematocrit values were increased significantly. Forelimb blood flows and pH readings were not altered significantly at 20 hours after endotoxin. No pre-treated animals were included in the long term shock studies.

Figure 15. Determination of Isogravimetric Capillary Pressure in an Endotoxin-Shocked Animal (20 hours postendotoxin). The pressure-flow relationship is presented for an animal studied at 20 hours after endotoxin injection.

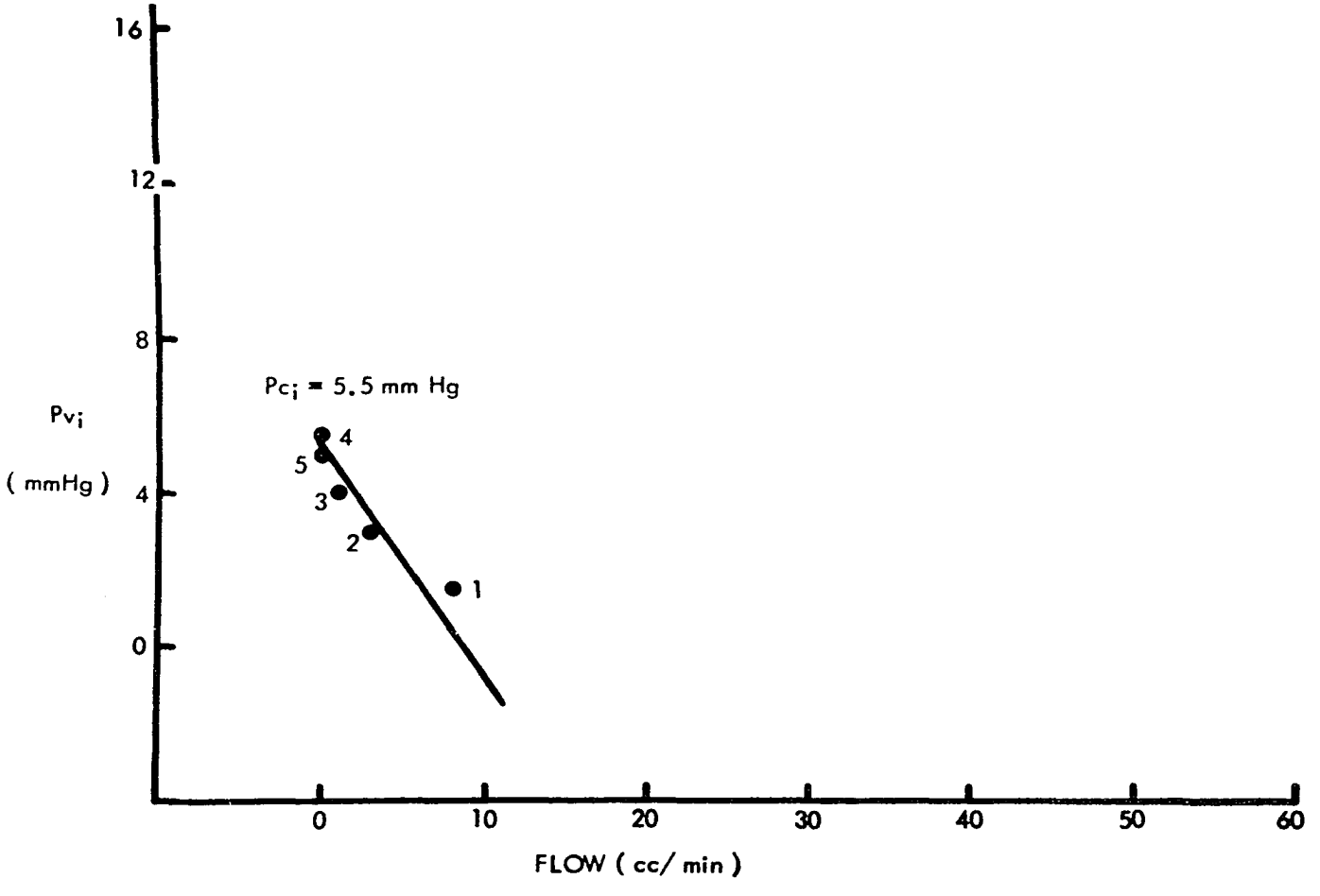

16
12
8
4
0
P_{v_i}
(mmHg)
P_{c_i} = 5.5 mm Hg
1
2
3
4
5
0
10
20
30
40
50
60
FLOW (cc/ min)

TABLE 4

EFFECT OF ENDOTOXIN ON DOG PERFUSED FORELIMB WITH SPECIAL REFERENCE TO SALICYLATE PRETREATMENT

Experiment	Pc_i (mm Hg)	MSAP (mm Hg)	pH	Hct (Vol.%)	Forelimb Blood Flow (ml/min per 100 g)
Control, no endotoxin, N = 15	16±0.6	131±5.2	7.28±0.01	38±1.3	16±1.1
Endotoxin, LD_{82} 4 Hr. Postendotoxin, 0.4 mg/kg, N = 10	6±0.7 $p<0.01$	92±10.0 $p<0.01$	7.13±0.05 $p<0.05$	52±1.8 $p<0.01$	7±1.1 $p<0.01$
4 Hr. Postendotoxin, 0.4 mg/kg, pre-treatment with sodium salicylate--80 mg/kg N = 7	16±0.5 NS	132±6.4 NS	7.25±0.05 NS	51±1.2 $p<0.01$	13±1.8 NS

TABLE 5

EFFECT OF ENDOTOXIN ON DOG PERFUSED FORELIMB PREPARATION

Experiment	Pc_i (mm Hg)	MSAP (mm Hg)	pH	Hct (Vol.%)	Forelimb Blood Flow (ml/min per 100 g)
Control, no endotoxin, N = 15	16±0.6	131±5.2	7.28±0.01	38±1.13	16±1.1
20 Hr. postendotoxin, N = 5	9±2.2 $p<0.01$	98±9.3 $p<0.01$	7.23±0.02 NS	43±2.3 $p = 0.05$	12±4.6 NS

CHAPTER IV

DISCUSSION

The results of the present investigations reveal that pre-treatment with salicylate obviates many of the pathological responses to endotoxin. Acetylsalicylic acid (ASA) can prevent early hemodynamic reactions elicited by endotoxin and increase the survival rate. Although the precise mechanisms whereby salicylates interrupt the toxic processes are not clearly evident, several possible explanations are available. Systemic arterial hypotension, characteristically identified with the endotoxin shock state, was averted because venous return did not decline. Prevention of the fall in venous return was attributed to preclusion of pooling of blood in the liver. ASA blocked portal venous and hepatic arterial hypertension. It is important to note that the agent responsible for prevention of the vascular effects of endotoxin shock was ASA and not the buffer solution. Infusion of the buffer alone failed to modify the response to endotoxin.

Constriction of the hepatic artery postendotoxin is reportedly due to a myogenic response triggered by hepatic venous constriction. Portal vein pressure then rises as a passive response to hepatic vein constriction (Hinshaw et al., 1965). Avoidance of portal venous hypertension indicates that ASA acted at the site of the hepatic veins to prevent constriction. It can be speculated that ASA might act in one of the following ways: (1) directly block the action of endotoxin at the hepatic veins;

(2) interfere with the action of a vasoactive agent released by the endotoxin; or (3) prevent the release of certain humoral agents. Liberation of histamine is reportedly accelerated by endotoxin administration (Hinshaw et al., 1961a) and histamine elicits portal venous hypertension Hinshaw et al., 1962a; Jordan et al., 1965). The catecholamines have also been implicated in the elevation of portal vein pressure (Weil and Spink, 1957). High levels of plasma serotonin reported in the portal vein postendotoxin suggested that serotonin was being released from bowel deposits (Davis et al., 1960).

Swyer (1948) was able to show that administration of 100 mg/kg body weight of sodium salicylate significantly reduced the effect of histamine in rabbits. It has also been reported that salicylates inhibit antigen-induced histamine release from guinea pig lung (Mongar and Schild, 1957). Spector and Willoughby (1963) produced evidence that large systemic doses of salicylate suppressed the rat's response to histamine. Rocha e Silva (1963) showed that salicylate interfered with histamine release from the isolated rat diaphragm while a communication from Schayer (1964) suggested inhibition of the histidine-decarboxylase system. Strom and Coffman (1963) found that aspirin attenuated the peripheral circulatory response to epinephrine and norepinephrine in human subjects. Vasoconstriction in the human forearm produced by intra-arterial serotonin infusion was shown to be inhibited by therapeutic levels of intravenous sodium salicylate by Glover and co-workers (1957). The authors considered this a specific inhibition of serotonin because they observed no significant effect on vasoconstriction produced by intra-arterial norepinephrine or histamine. Thus, the reports of Strom and Coffman (1963) and Glover et al.

(1957) seem to be at variance with one another, but the report from Glover and co-workers employed intravenous infusions of salicylate while Strom and Coffman administered the salicylate orally. A subsequent communication has divulged that aspirin antagonized the vasoconstrictor effect of histamine but had no effect upon responses to serotonin in the isolated perfused guinea pig lung (Greeff and Moog, 1964). Therefore, it is apparent that species differences and route of administration must be considered in an evaluation of salicylate action.

During the thirty-minute infusion of ASA to venous return preparations, an increase in the volume of blood returning to the heart was noted. There appear to be several explanations for the increase in venous return. Large doses of sodium salicylate reportedly elicit a prompt increase in contractile force of the heart (Walton and Darby, 1959). The increase in force is short-lived, declining to control levels within 20 minutes after completion of salicylate administration. Another reason for an augmented cardiac output may be an indirect result of profound metabolic stimulation. The increase in metabolic rate is effected by uncoupling of oxidative phosphorylation (Smith, 1966b). A two-fold increase in oxygen consumption was found when large doses of sodium salicylate were administered to anesthetized dogs (Tenney and Miller, 1955). The source of the previously mentioned increase in metabolism was found to be skeletal muscle. The authors concluded that the circulatory system met the increased level of oxygen consumption by widening the auriculoventricular oxygen difference and increasing cardiac output (Tenney and Miller, 1955). A third reason for a rise in the venous return is thought to be the stimulation of the pituitary-adrenal system (Done et al., 1958). This could

cause a contraction of the spleen and emptying of the hepatic bed, effecting an increase in blood returning to the heart.

Salicylate pre-treatment obliterated the acidosis frequently found in endotoxin-shocked dogs. Hyperventilation, promoted directly by stimulation of the respiratory center and indirectly by the increased metabolic production of carbon dioxide (Tenny and Miller, 1955) may account for prevention of a drop in pH levels. Large doses of salicylates thus produce a primary hyperventilation with lowering of the carbon dioxide tension in the blood leading to an alkalotic tendency. Respiratory stimulation has been found with massive salicylate administration in dogs (Boyle et al., 1947; Tenney and Miller, 1955) and in humans (Ghose, 1967).

The final phase of this research endeavor was aimed at investigation of the mechanism of fluid loss in endotoxin shock. The capillary bed of an isolated perfused forelimb of the dog was chosen as the research model. The results obtained suggest that endotoxin administration modified certain properties of the capillary membrane. The isogravimetric capillary pressure, defined as the highest capillary hydrostatic pressure at which edema formation (gain in leg weight) does not occur, was significantly lowered by 4 hours after endotoxin injection.

The technique utilized to obtain isogravimetric capillary pressures (Pc_i) was originally described by Pappenheimer and Soto-Rivera (1948) and later modified by Johnson and co-workers (1966). The Pc_i values obtained in control (no endotoxin) animals in this study (16 mm Hg) are in close agreement with those values reported by Pappenheimer and Soto-Rivera (1948) utilizing an extrapolation to zero flow to get Pc_i levels (17 mm Hg). Johnson et al. (1966) extended the technique to include direct measurement

of Pc_i at conditions of zero flow. The present study employed the zero flow method and obtained Pc_i results (16 mm Hg) very similar to those reported by Johnson and co-workers in 1966 (15 mm Hg). In contrast to the mean value of 16 mm Hg seen in control (no endotoxin) animals, a mean Pc_i value of 7 mm Hg was observed in endotoxin-shocked dogs. Since the capillary bed of the forelimb was unable to support the higher levels of pressure seen in control dogs, it appeared that capillary permeability had been increased. Any attempt to raise the pressure in shocked preparations elicited an extravasation of fluid, as evidenced by a gain in weight.

Some of the changes seen in the forelimb postendotoxin might be attributed to the 4 hour period of ischemia. The limb was autoperfused by the endotoxin-shocked dog for 4 hours prior to perfusion with the pump. Since cardiac output and systemic arterial pressure fell to shock levels, it is reasonable to assume that blood flow to the leg was diminished. This condition of low flow rate could have permitted accumulation of metabolites which may have effected changes in the limb capillary bed.

An evaluation of the method employed to ascertain extravasation and absorption of fluid is pertinent to this discussion. The criterion for extravasation of fluid is an increase in limb weight. Conversely, a decline in limb weight is said to signify absorption of fluid from tissue spaces into the capillary vessels. The previous papers describing this experimental technique (Pappenheimer and Soto-Rivera, 1948; Johnson et al., 1966) and the present investigation provide no direct evidence that a weight gain actually represents loss of vascular fluid to tissue spaces and not the filling of empty intravascular capillary spaces. The validity of the results obtained using this investigative technique depend

on the assumption that an increase in weight of the perfused limb is equated with extravasation of vascular fluid. The best evidence supporting this assumption comes from work by Johnson and Hanson (1962) in which protein concentrations were measured simultaneously on the arterial and venous sides of the capillary bed being examined. The authors reported increases in venous protein concentrations which were correlated with increases in weight. The elevation in venous protein levels was ascribed to a loss of plasma water to extravascular spaces.

The Starling hypothesis (Starling, 1896) describing transcapillary fluid exchange, is helpful in evaluating the results obtained in the limb perfusion study. The Starling hypothesis delineates three factors involved in transcapillary fluid exchange: (1) the differences in hydrostatic pressure across the capillary membrane; (2) protein osmotic pressures acting within the vascular bed and in the extravascular compartment; and (3) the properties of the capillary membrane which influence fluid transfer from intravascular to extravascular regions. The hydrostatic pressure across the membrane was zero when Pc_i values were determined in this study. A report that plasma protein concentrations were at normal levels 4 hours postendotoxin (Chien et al., 1966) suggests that the second factor of the Starling hypothesis is not involved in the alteration of the capillary bed. Thus it appears that endotoxin administration modifies certain physical properties of the capillary membrane. It can only be speculated as to whether this is the result of a direct action of the endotoxin on the capillary membrane or due to action of vasoactive agents released by endotoxin administration. The failure of isolated organs perfused by a pump-lung apparatus (Hinshaw et al., 1962b) to respond to

endotoxin tends to implicate released neurohumoral agents.

Venous constriction, previously reported in the forelimb (Hinshaw et al., 1962b), would tend to elevate capillary pressures, thus promoting extravasation of plasma fluid. Small vein constriction postendotoxin did not appear to interfere with estimation of Pc_i, since at zero flow, vascular constriction would have no influence on the value of isogravimetric capillary pressure. Conversely, venous dilatation would not interfere with this technique as suggested in a report that sympatholytic agents did not appreciably alter Pc_i levels, although postcapillary resistance declined (Hanson and Johnson, 1962).

Administration of sodium salicylate prevented the decrease in Pc_i levels. Pre-treated dogs revealed a mean Pc_i value of 16 mm Hg at 4 hours after endotoxin, the same mean value obtained in control studies. The salicylates may act by directly stabilizing the walls of the microcirculation, or may interfere with the release or antagonize the action of vasoactive substances released by endotoxin. An interference with the release of histamine from mast cells (Rocha e Silva, 1963) or action of histamine (Spector and Willoughby, 1963) is possible. There are reports that toxic vascular changes are partly due to destruction of vasoconstrictor amines which would otherwise constrict and reduce permeability, hence opposing the action of histamine and kinins (Cameron and Spector, 1961). It has been suggested that salicylates interfere with sympathomimetic amine destruction (Smith, 1966a). Endotoxin may also cause the release of kinins (Nies et al., 1968). It appears that histamine is only active in the early response to toxin and that other factors, such as kinins, are responsible for the acute inflammatory response (Smith,

1966a). Work by Spector and Willoughby (1963) and Northover and Subramanian (1961) suggests that salicylates inhibit the activity of the plasma kinin-forming enzymes, thus preventing the release of kinins. Bhoola and collaborators (1962) reported that aspirin antagonized injected bradykinin. Slow reacting substance-A (SRS-A), a substance often released in anaphylactic reactions and associated with edema formation is also reportedly antagonized by salicylates (Collier, 1962). The most definite statement that can be made about the action of salicylates is that they are able to stabilize capillary permeability in the face of endotoxin administration.

In conclusion, several critical evaluations of the data presented here are appropriate. This research has centered only on the response of the dog to endotoxin. It has been clearly established that many differences occur in the species response to endotoxin (Kuida, 1961; Guenter et al., 1969). Therefore, application of the canine response to other species, especially the primates, should be carefully examined. The majority of the experimental techniques employed here were very traumatic to the animal. Thus, experiments were carried out on anesthetized abnormal animals. Swan and Jacobson (1967) have shown that the hemodynamic response of the conscious animal varies from that of the anesthetized dog. This does not necessarily invalidate the results because all experiments were controlled and all results were compared only to the control (no endotoxin) studies. Recent reports have suggested that the response to endotoxin injection may vary from the response to a Gram-negative septicemia (Waisbren, 1964; Hinshaw et al., 1968). This would indicate that the experimental administration of purified endotoxin to an anesthetized

dog might be at great variance with the response seen in a patient with a Gram-negative infection. More studies are required in which the primate is the shock model and live organisms are used in place of endotoxin.

CHAPTER V

SUMMARY

This dissertation has presented an evaluation of the hemodynamic responses to endotoxin. The laboratory shock model employed was the anesthetized mongrel dog. Salicylates were utilized to modify the response to Gram-negative toxin in an attempt to elucidate some of the basic mechanisms operating during the shock state. The basic goal was determination of how salicylates act to prevent pathogenesis of the shock state. Beneficial actions were found in the capillary and post-capillary vascular areas.

The acute response of the anesthetized dog to a lethal dose of endotoxin is characterized by systemic arterial hypotension, sequestration of blood in the liver and diminished venous return accompanied by acidosis and hemoconcentration. Infusion of large doses of buffered ASA, prior to administration of the endotoxin, obliterated the usual shock-like state: (1) systemic arterial pressure remained significantly elevated; (2) portal hypertension did not occur; (3) venous return was maintained at normal levels; and (4) acidosis was prevented. Ablation of the decrease in cardiac output and systemic arterial pressure was attributed primarily to prevention of hepatic pooling. Stimulation of the myocardium, resulting in a short-term enhancement of contractile force, may also have aided in maintaining a normal state in the circulatory system. Respiratory

stimulation, attributed to the salicylates, probably reversed the acidotic tendency. Administration of buffered ASA 15 minutes after injection of the toxin resulted in restoration of systemic arterial pressure and pH values. Pre-treatment with buffered and unbuffered ASA prior to endotoxin administration significantly increased the survival rate over the untreated endotoxin-shocked group of animals. The results obtained suggested that the lethal effects of endotoxin are related to its hemodynamic alterations.

Experiments were performed on in situ liver perfusions with controlled hepatic arterial and portal venous flows. Intravenous injections of ASA prior to administration of endotoxin prevented the constriction observed in both the hepatic artery and hepatic vein of animals receiving only endotoxin. It was suggested that ASA was acting in one of the following ways: (1) at the site of the hepatic veins to reverse the constrictor effects of endotoxin or vasoactive agents, such as histamine, released by endotoxin or (2) preventing the release of humoral agents capable of eliciting venous constriction.

An evaluation of the canine forelimb capillary bed indicated that endotoxin promotes an increase in capillary permeability. Isogravimetric capillary pressures obtained in endotoxin-shocked preparations were significantly lower than those found in control (no endotoxin) studies. That is, the capillary bed of shocked animals was unable to support the higher pressures seen in control dogs. It has been suggested that fluid loss due to increased permeability of the capillary system might be at least partially responsible for the late phase of decreased venous return seen during endotoxin shock. The apparent increase in permeability is thought to be due to a direct action of endotoxin or released vasoactive agents

on the capillary membrane. Sodium salicylate pre-treatment averted the permeability changes associated with endotoxin administration. The salicylates are said to stabilize the capillary membrane.

BIBLIOGRAPHY

Alaupovic, P., Solomon, L. A., Olson, A. C., Jordan, M. M. and Hinshaw, L. B. Relationship of Chemical Structure to Hemodynamic Properties of Endotoxins. Proc. Soc. Exp. Biol. and Med. 127: 253, 1968.

Aldrete, J. S., Sheps, S. G., Bernatz, P. E. and Didier, E. P. Vasoactive Polypeptides of Surgical Significance. Mayo Clin. Proc. 41: 399, 1966.

Altemeier, W. A., Todd, J. C. and Inge, W. W. Gram-Negative Septicemia; A Growing Threat. Ann. Surg. 166: 530, 1967.

Arkin, H. and Colton, R. R. Statistical Methods. p. 121. Barnes and Noble, Inc., New York, 1958.

Atkins, E. Pathogenesis of Fever. Physiol. Rev. 40: 580, 1960.

Arrendondo, M. I. and Kampschmidt, R. F. Effect of Endotoxins on Phagocytic Activity of the Reticuloendothelial System of the Rat. Proc. Soc. Exp. Biol. and Med. 112: 78, 1963.

Aust, J. B., Johnson, J. A. and Visscher, M. B. Plasma Sequestration in Endotoxin Shock. Surg. Forum. 8: 8, 1957.

Bard, P. and Woods, J. W. Central Site of Pyrogenic Action of Bacterial Endotoxin. Physiologist. 3: 5, 1959.

Bennett, I. L., Jr., Petersdorf, R. G. and Keene, W. R. Pathogenesis of Fever: Evidence for Direct Cerebral Action of Endotoxins. Jr. A. Am. Physicians. 70: 64, 1957.

Beraldo, W. T. Formation of Bradykinin in Anaphylactic and Peptone Shock. Am. J. Physiol. 163: 283, 1950.

Berman, I. R., Moseley, R. V., Lamborn, P. B. and Sleeman, H. K. Thoracic Duct Lymph in Shock. Ann. Surg. 169: 202, 1969.

Berry, L. J. and Smythe, D. S. Effects of Bacterial Endotoxin on Metabolism. II. Protein-Carbohydrate Balance Following Cortisone. Inhibition of Intestinal Absorption and Adrenal Response to ACTH. J. Exp. Med. 110: 407, 1959.

Bloch, J. H., Pierce, C. H. and Lillehei, R. C. Adrenergic Blocking Agents in the Treatment of Shock. Ann. Rev. Med. 17: 483, 1966.

Bohle, A., Krecke, H. J., Miller, J. and Sitte, H. In Intern. Symp. Immunopathology. 1st edited by Grabar, P., Miescher, P. B. and Schwabe, B., 1959.

Boise, E. The Differential Diagnosis of Shock, Hemorrhage, and Sepsis. Tr. Am. A. Obst. 9: 433, 1897. (Reviewed by Shubin, H., Weil, M. H. and Udhoji, V. N. Septic Shock. Shock and Hypotension. 463-469, Grune and Stratton, New York, 1965.)

Boivin, A., Mesrobeanu, L. and Juster, P. Sur l'existence dans le Bacillus tumefaciens, d'une endotoxine capable de provoquer la formation de tumeurs chez les végétaux. Compt. rend.acad. sc. Paris. 201: 984, 1935.

Borden, C. W. and Hall, W. H. Fatal Transfusion Reactions from Massive Bacterial Contamination of Blood. New Eng. J. Med. 245: 760, 1951.

Boruchow, I. B. and Abel, M. Role of the Spleen and Portal Hypertension in Canine Endotoxin Shock. Surg. 60: 1195, 1966.

Boyle, M. N., Smull, K. and Wegria, R. The Effect of Sodium Salicylate on the Acid-Base Balance of the Blood. Am. J. Med. 3: 31, 1947.

Braude, A. I. Bacterial Endotoxins. Scientif. American. 210: 36, 1964.

Brunson, J. G., Schulz, D. M., Angevine, D. M., Imrie, S. T. and Douglas, B. H. Evaluation of Therapeutic Agents. J. Okla. State Medical Association. 59: 479, 1966.

Bhoola, K. D., Collier, H. O. J., Schacter, M. and Shorley, P. G. Actions of Some Peptides in Bronchial Muscle. Brit. J. Pharmacol. 19: 190, 1962.

Burrows, W. Endotoxins. Ann. Rev. Micro. 5: 181, 1951.

Cameron, G. R. and Spector, W. G. The Chemistry of the Injured Cell, pp. 109-130. C. C. Thomas, Springfield, 1961.

Cavanagh, D. and DeCenzo, J. Endotoxin Shock. Am. J. Obst. Gynec. 85: 892, 1963.

Chien, S., Dellenback, R. J., Usami, S. and Gregersen, M. I. Hematocrit Changes in Endotoxin Shock. Proc. Soc. Exp. Biol. and Med. 118: 1182, 1965a.

Chien, S., Dellenback, R. J. and Usami, S. Effect of Endotoxin on the Transfer of Fibrinogen from Plasma to Lymph. Proc. Soc. Exp. Biol. and Med. 118: 1187, 1965b.

Chien, S., Dellenback, R. J., Usami, S., Treitel, K., Chang, C. and Gregersen, M. I. Blood Volume and Its Distribution in Endotoxin Shock. Am. J. Physiol. 210: 1411, 1966.

Clawson, C. C., Hartmann, J. E. and Vernier, R. L. Electron Microscopy of the Effect of Gram-Negative Endotoxin on the Blood-Brain Barrier. J. Comp. Neur. 127: 183, 1966.

Collier, H. O. J. Effects of Salicylates on Inflammation. Salicylates, pp. 120-126. Edited by Dixon, A. St. J., Smith, M. J. H., Martin, B. K. and Wood, P. H. N., Little, Brown and Co., Boston, 1962.

Corrado, A. P., Lima, E. G. and Rothschild, A. M. Study of the Mechanism of the Cardiovascular Shock Produced by Endotoxin on Gram-Negative Bacteria: E. coli and S. typhi. Arch. Int. Pharmacodyn. 150: 462, 1964.

Corrigan, J. J., Walker, W. L. and May, N. Changes in the Blood Coagulation System Associated with Septicemia. New Eng. J. Med. 279: 851, 1968.

Crowell, J. W. and Guyton, A. C. Further Evidence Favoring a Cardiac Mechanism in Irreversible Hemorrhagic Shock. Am. J. Physiol. 203: 248, 1962.

Davis, R. B., Meeker, W. R. and Bailey, W. L. Serotonin Release by Bacterial Endotoxin. Proc. Soc. Exp. Biol. and Med. 108: 774, 1961.

Davis, R. B., Meeker, W. R., Jr. and McOuarrie, D. G. Early Changes in Intravascular Pressures, Platelets, and Serotonin and E. coli Endotoxin Administration. Surg. Forum. 10: 401, 1960.

Delaunay, A. Le Mode d'Action des Endotoxines Bacteriennes. Compt. Rend. Soc. Biol. 147: 589, 1943. (Reviewed by Thomas, L. Physiological Disturbances Produced by Endotoxins. Ann. Rev. Physiol. 16: 467, 1954.)

Des Prez, R. M. The Effects of Bacterial Endotoxin on Rabbit Platelets. J. Immunology. 99: 966, 1967.

Dietzman, R. H., Motsay, G. and Lillehei, R. C. High Output-Low Resistance Gram-Negative Septic Shock in Man. Treatment with Massive Doses of Corticosteroids. (Submitted for Publication)

Done, A. K., Ely, R. S. and Kelly, V. C. Salicylates and the Pituitary-Adrenal System. Metabolism. 7: 52, 1958.

Eckman, P. L., King, W. M., and Brunson, J. G. Studies on the Blood Brain Barrier. I. Effects Produced by a Single Injection of Gram-Negative Endotoxin on the Permeability of the Cerebral Vessels. Am. J. Path. 34: 631, 1958.

Emerson, T. E., Jr., Brake, C. M. and Hinshaw, L. B. Endotoxin Shock in Chronic Splanchnic Denervated Dogs. J. Trauma. 5: 737, 1965.

Erdös, E. G., Hinshaw, L. B. and Gill, C. C. Effect of Indomethacin in Endotoxin Shock in the Dog. Proc. Soc. Exp. Biol. and Med. 125: 916, 1967.

Fine, J. and Minton, R. Mechanism of Action of Bacterial Endotoxin. Nature. 210: 97, 1966.

Franke, F. E. Action of Toxic Doses of the Polysaccharide from Serratia marcescens in the Dog and Guinea Pig. J. Nat. Cancer Inst. 5: 183, 1944-45.

Frohlich, E. D., Scott, J. B. and Dooley, E. S. Hemodynamic Alterations Due to Salmonella typhosa Endotoxin with Special Reference to the Coronary Vascular Bed. J. Clin. Invest. 41: 147, 1962.

Gaddum, J. H. Polypeptides Which Stimulate Pain in Muscle. Livingstone, London, 1955.

Gans, H., Hanson, M. and Krivit, W. Effect of Endotoxin on Clotting Mechanism. III. On the Relationship between Hypercoagulability, Plasminogen Activator Activity, and Antithrombin Activity. Surgery. 53: 793, 1963.

Ghose, R. R. The Significance of Acid-Base Measurements in the Management of Salicylate Intoxication. Postgrad. Med. J. 43: 454, 1967.

Gilbert, R. P. Mechanisms of the Hemodynamic Effects of Endotoxin. Physiol. Rev. 40: 245, 1960.

Gillenwater, J. Y., Dooley, E. S. and Frohlich, E. D. Effects of Endotoxin on Renal Function and Hemodynamics. Am. J. Physiol. 204: 293, 1963.

Glover, W. E., Marshall, R. J. and Whelan, R. F. The Antagonism of the Vascular Effects of 5-HT by BOL 148 and Sodium Salicylate in the Human Subject. Br. J. Pharm. Chem. 12: 498, 1957.

Goodyer, A. V. N. Left Ventricular Function and Tissue Hypoxia in Irreversible Hemorrhagic and Endotoxin Shock. Am. J. Physiol. 212: 444, 1967.

Gourzis, J. T., Hollenberg, M. W. and Nickerson, M. Involvement of Adrenergic Factors in the Effect of Endotoxin. J. Exp. Med. 114: 593, 1961.

Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A. and Parish, H. H., Jr. Distribution of Histamine Among Leucocytes and Platelets. Blood. 10: 467, 1955.

Greeff, K. and Moog, E. Vergleichende Untersuchungen über die bronchoconstrictorische und gefäbconstrictorische Wirkung des Bradykinins, Histamins und Serotonins an isolierten Lungenpräparaten. Naunyn Schmiedeberg Arch. Exp. Path. 248: 204, 1964.

Green, H. N. and Stoner, H. B. Effects of Injury on Carbohydrate Metabolism and Energy Transformation. Brit. Med. Bull. 10: 38, 1954.

Greisman, S. E. Activation of Histamine-Releasing Factor in Normal Rat Plasma by E. coli Endotoxin. Proc. Soc. Exp. Biol. (N.Y.) 103: 628, 1960.

Guenter, C. A., Fiorica, V. and Hinshaw, L. B. Cardiorespiratory and Metabolic Responses to Live E. coli and Endotoxin in the Monkey. J. Appl. Physiol. (In press)

Hamosii, M. and Shapiro, B. The Mechanism of Glycogenolytic Action of Endotoxin. Brit. J. Exp. Path. 41: 372, 1960.

Hanson, K. M. and Johnson, P. C. Evidence for Local Arterio-Venous Reflex in the Intestine. J. Appl. Physiol. 17: 509, 1962.

Hardaway, R. M. and Johnson, D. Clotting Mechanism in Endotoxin Shock. A.M.A. Arch. Intern. Med. 112: 775, 1963.

Hinshaw, L. B. and Bradley, G. M. Alterations in Kidney Weight Produced by Escherichia coli Endotoxin. Am. J. Physiol. 189: 329, 1957.

Hinshaw, L. B., Bradley, G. M. and Carlson, C. H. Effect of Endotoxin on Renal Function in the Dog. Am. J. Physiol. 196: 1127, 1959.

Hinshaw, L. B., Brake, C. M., Emerson, T. E., Holmes, D. D., Jordan, M. M. and Masucci, F. D. Role of the Sympatho-Adrenal System in Endotoxin Shock. Fed. Proc. 23: 416, 1964.

Hinshaw, L. B., Gilbert, R. P., Kuida, H. and Visscher, M. B. Peripheral Resistance Changes and Blood Pooling after Endotoxin in Eviscerated Dogs. Am. J. Physiol. 195: 631, 1958a.

Hinshaw, L. B., Gilbert, R. P., Kuida, H. and Visscher, M. B. Effect of Endotoxin on Vascular Reactivity to Epinephrine in the Perfused Dog Forelimb and Lung. Proc. Soc. Exp. Biol. and Med. 99: 684, 1958b.

Hinshaw, L. B., Jordan, M. M. and Vick, J. A. Mechanism of Histamine Release in Endotoxin Shock. Am. J. Physiol. 200: 987, 1961a.

Hinshaw, L. B., Jordan, M. M. and Vick, J. A. Histamine Release and Endotoxin Shock in the Primate. J. Clin. Invest. 40: 1931, 1961b.

Hinshaw, L. B., Kuida, H., Gilbert, R. P. and Visscher, M. B. Influence of Perfusate Characteristics on Pulmonary Vascular Response to Endotoxin. Am. J. Physiol. 191: 293, 1957.

Hinshaw, L. B., Reins, D. A. and Hill, R. J. Response of the Isolated Liver to Endotoxin. Canad. J. Physiol. Pharm. 44: 529, 1966.

Hinshaw, L. B., Reins, D. A. and Wittmers, L. Venous-Arteriolar Response in the Canine Liver. Proc. Soc. Exp. Biol. and Med. 118: 979, 1965.

Hinshaw, L. B., Solomon, L. A., Reins, D. A. and Fiorica, V. Sympathoadrenal System and Renal Response to Endotoxin in the Primate. Nephron. 4: 394, 1967.

Hinshaw, L. B., Spink, W. W., Vick, J. A., Mallet, E. and Finstad, J. Effect of Endotoxin on Kidney Function and Renal Hemodynamics in the Dog. Am. J. Physiol. 201: 144, 1961c.

Hinshaw, L. B., Vick, J. A., Jordan, M. M. and Wittmers, L. E. Vascular Changes Associated with the Development of Irreversible Endotoxin Shock. Am. J. Physiol. 202: 103, 1962b.

Hinshaw, L. B., Vick, J. A., Carlson, C. H. and Fan, Y. L. Role of Histamine in Endotoxin Shock. Proc. Soc. Exp. Biol. and Med. 104: 379, 1960.

Hinshaw, L. B., Vick, J. A., Wittmers, L. E., Worthen, D. M., Nelson, D. L. and Swenson, O. P. Changes in Total Peripheral Resistance in Endotoxin Shock. Proc. Soc. Exp. Biol. and Med. 108: 24, 1961d.

Jacobson, E. D., Mehlman, B. and Kalas, J. P. Vasoactive Mediators as the "Trigger Mechanism" of Endotoxin Shock. J. Clin. Invest. 43: 1000, 1964.

Jaques, R. and Jaquet, H. The Duration of Action of Certain Corticosteroids in the Acutely Adrenalectomized Rat as Estimated by Reference to their Protective Effect in Endotoxin Shock. Experimentia. 20: 230, 1964.

Johnson, P. C. and Hanson, K. M. Effect of Arterial Pressure on Arterial and Venous Resistance of Intestine. J. Appl. Physiol. 17: 503, 1962.

Johnson, P. C., Hanson, K. M. and Thulesius, O. Pre- and Post-Capillary Resistance in the Dog Forelimb. Am. J. Physiol. 210: 873, 1966.

Jordan, M. M., Holmes, D. D. and Hinshaw, L. B. Pathophysiological Comparisons of Histamine and Endotoxin Shock. J. Trauma. 5: 726, 1965.

Kalas, J. P. and Jacobson, E. D. The Effect of Vasoactive Antogonists in Endotoxin Shock. Am. Heart J. 67: 764, 1964.

Kim, Y. B. and Watson, D. W. Role of Antibodies in Reactions to Gram-Negative Bacterial Endotoxins. Ann. N. Y. Acad. Sci. 133: 727, 1966.

Kobold, E. E., Lovell, R., Katz, W. and Thal, A. P. Chemical Mediators Released by Endotoxin. Surg. Gynec. Obstet. 118: 807, 1964.

Kontos, H. A., Magee, J. H., Shapiro, W. and Patterson, J. L., Jr. General and Regional Circulatory Effects of Synthetic Bradykinin in Man. Circ. Res. 14: 351, 1964.

Kovats, T. G. Endotoxin Susceptibility and Endotoxin Hypersensitivity. pp. 13-28, Studie Medica Szegedinensia, Szeged, Hungary, 1967.

Kuida, H., Gilbert, R. P., Hinshaw, L. B., Brunson, J. C. and Visscher, M. B. Species Differences in Effect of Gram-Negative Endotoxin on Circulation. Am. J. Physiol. 200: 1197, 1961.

Kuida, H., Hinshaw, L. B., Gilbert, R. P. and Visscher, M. B. Effect of Gram-Negative Endotoxin on Pulmonary Circulation. Am. J. Physiol. 192: 335, 1958.

Kasai, N. Chemical Studies on the Lipid Component of Endotoxin with Special Emphasis on its Relation to Biological Activities. Ann. N. Y. Acad. Sci. 133: 486, 1966.

Laennec, R. T. H. Traite de l'Auscultation Mediate et des Maladies des Poumons et due Coeur. p. 138. J. S. Chaude, Paris, 1831. (Reviewed by Shubin, H., Weil, M. H. and Udhoji, V. N. Septic Shock. Shock and Hypotension, pp. 463-469. Grune and Stratton, New York, 1965.)

Lee, L. In Bacterial Endotoxins, p. 648, edited by Braun, W. and Landy, M. Rutgers, Univ. Press, New Brunswick, N. J., 1964.

Lillehei, R. C., Longerbeam, J. K. and Rosenberg, J. C. The Nature of Irreversible Shock: Its Relationship to Intestinal Changes. Shock: Pathogenesis and Therapy (Ciba Sumposium). Edited by Bock, K. D. Academic Press, Inc., New York, 1962, pp. 106-129.

Lillehei, R. C. and MacLean, L. D. The Intestinal Factor in Irreversible Endotoxin Shock. Ann. Surg. 148: 513, 1958.

Lillehei, R. C. and MacLean, L. D. Physiological Approach to Successful Treatment of Endotoxin Shock in the Experimental Animal. Arch. Surg. 78: 464, 1959.

Lockwood, J. S. The Pathogenic Physiology of Infection. Ped. Clin. North Am. 16: 1416, 1946.

Londe, S. P., Massie, H., Monaro, W. W. and Bernard, H. R. Resistance of the Isolated Canine Heart to Endotoxin Shock. Surg. 61: 466, 1967.

Lüderitz, O., Jann, K. and Wheat, R. Somatic and Capsular Antigens of Gram-Negative Bacteria. Comprehensive Biochemistry. 26A: 105-227. Edited by Florkin, M. and Stotz, E. H. Elsevier Publishing Company, Amsterdam, 1968.

Lüderitz, O., Westphal, O., Echenberger, E. and Neter, E. Über die Komplexbildung von bakteriellen Lipopolysacchariden mit Proteinen und Lipoiden. Biochem. Z. 330: 21, 1958. (Reviewed by Rašková, H. and Vaněček, J., in Pharmacology of Bacterial Toxins. Pharm. Rev. 16: 1, 1964.)

MacLean, L. D., Weil, M. H., Spink, W. W. and Visscher, M. B. Canine Intestinal and Liver Weight Changes Induced by E. coli Endotoxin. Proc. Soc. Exp. Biol. and Med. 92: 602, 1956.

Massion, W. H. and Erdös, E. G. The Effects of ATP and a Proteolytic Enzyme Inhibitor in Irreversible Shock. Okla. St. Med. J. 59: 467, 1966.

Maxwell, F. M., Rowe, G. G., Castillo, C. A., Crumpton, C. W., Clifford, J. E. and Afonso, S. Effect of Endotoxin upon the Systemic and Coronary Hemodynamics and Metabolism of the Intact Dog. Physiologist. 2: 81, 1959.

McKay, D. G., Margaretten, W. and Csavossy, I. An Electron Microscope Study of the Effects of Bacterial Endotoxin on the Blood Vascular System. Lab. Invest. 15: 1816, 1966.

Meli, A., Smith, C. R. and Wolff, A. Anti-Inflammatory Assay. An Improved Method Based on the Reversal of Endotoxin-Induced Lung Inflammation in Mice. Proc. Soc. Exp. Biol. and Med. 117: 34, 1964.

Meyer, M. W. and Ballin, H. M. Failure to Observe Alteration of Epinephrine Activity after Endotoxin. Proc. Soc. Exp. Biol. and Med. 100: 288, 1959.

Mongar, J. L. and Schild, H. O. Inhibition of the Anaphylactic Reaction. J. Physiol. 135: 301, 1957.

Morgan, H. R. Immunologic Properties of an Antigenic Material Isolated from Eberthella Typhosa. J. Immunol. 41: 161, 1941.

Moses, J. M. and MacIntyre, W. J. Effect of Endotoxin on Simultaneously Determined Cardiac Output and Hepatic Blood Flow in Rabbits. J. Lab. Clin. Med. 61: 483, 1962.

Nagy, S., Tárnoky, K. and Petri, G. Effect of a Water-Soluble Corticosteroid Analogue in Experimental Hemorrhagic Shock. J. Surg. Res. 4: 62, 1964.

Nagy, S., Tárnoky, K. and Petri, G. Cardiac Output of Bled Dogs after Intravenous Administration of Corticosteroids. Acta Physiologica. 27: 257, 1965.

Neter, E., Westphas, O., Lüderitz, E., Gorzynski, A. and Eichenberger, E. Studies on Enterobacterial Lipopolysaccharides. Effects of Heat and Chemicals on Erythrocyte-Modifying, Antigenic, Toxic, and Pyrogenic Properties. J. Immunol. 76: 377, 1956.

Nies, A. S., Forsyth, R. P., Williams, H. E. and Memmon, K. L. Contribution of Kinins to Endotoxin Shock in Unanesthetized Rhesus Monkeys. Circ. Res. 22: 155, 1968.

Northover, B. J. and Subramanian, G. Analgesic-Antipyretic Drugs as Inhibitors of Kallikrein. Brit. J. Pharmacol. 17: 107, 1961.

Northover, B. J. and Subramanian, G. Analgesic-Antipyretic Drugs as Antagonists of Endotoxin Shock in Dogs. J. Path. Bact. 83: 463, 1962.

Palmerio, C., Zetterstrom, B., Shammash, J., Euchbaum, E., Frank, E. and Fine, J. Denervation of the Abdominal Viscera for the Treatment of Traumatic Shock. New Eng. J. Med. 269: 709, 1963.

Pappenheimer, J. R. and Soto-Rivera, A. Effective Osmotic Pressure of the Plasma Proteins and Other Quantities Associated with the Capillary Circulation in the Hindlimbs of Cats and Dogs. Am. J. Physiol. 152: 471, 1948.

Penner, A. and Bernheim, A. I. Studies in the Pathogenesis of Experimental Dysentery Intoxication. J. Exptl. Med. 76: 271, 1942.

Porter, P. J. and Kass, E. H. Role of the Posterior Hypothalamus in Mediating the Lethal Action of Bacterial Endotoxin in the Rat. J. Immun. 94: 641, 1965.

Raškova, H. and Vaněcek, J. Pharmacology of Bacterial Toxins. Pharm. Rev. 16: 1, 1964.

Ravdin, I. S. and Hardy, J. D. Pathophysiology of Endotoxin Shock. Am. J. Med. Sci. 244: 237, 1962.

Reilly, J., Rivalier, E., Compagnon, A. and Laplane, R. Hémorrhagies, Lésions Vasculaires et Lymphatiques du Tube Digestif Déterminées par l'Injection Péri Splanchnique de Substances Toxiques Diverses. Comp. Rend. Soc. de Biol. 116: 24, 1934. (Reviewed by Gilbert, R. P. Mechanisms of Hemodynamics Effects of Endotoxin. Physiol. Rev. 40: 245, 1960.)

Ribi, E., Haskins, W. T., Landy, M. and Milner, K. C. Symposium on Bacterial Endotoxins. I. Relationship of Chemical Composition to Biological Activity. Bact. Rev. 25: 427, 1961.

Ribi, E., Hoyer, B. H., Milner, K. C., Perrine, T. D., Larson, C. L. and Goode, G. Physical and Chemical Analysis of Endotoxin from Salmonella enteritidis. J. Immunol. 84: 32, 1960.

Rocha e Silva, M. The Role Played by Leucocytes and Platelets in Anaphylactic and Peptone Shock. Ann. N. Y. Acad. Sci. 50: 1045, 1950.

Rocha e Silva, M. The Participation of Substances of Low Molecular Weight in Inflammation with Special Reference to Histamine and Bradykinin. In Injury, Inflammation and Immunity. pp. 220-241. Williams and Wilkins, Baltimore, 1963.

Rosenberg, J. C., Lillehei, R. C., Longerbeam, J. and Zimmermann, B. Studies on Hemorrhagic and Endotoxin Shock in Relation to Vasomotor Changes and Endogenous Circulating Epinephrine, Norepinephrine and Serotonin. Ann. Surg. 154: 611, 1961.

Rosenberg, J. C., Lillehei, R. C., Moran, W. H. and Zimmermann, B. Effect of Endotoxin on Plasma Catecholamines and Serum Serotonin. Proc. Soc. Exp. Biol. and Med. 102: 335, 1959.

Rosenberg, J. C. and Rush, B. F. Lethal Endotoxin Shock. Oxygen Deficit, Lactic Acid Levels and Other Metabolic Changes. J.A.M.A. 196: 767, 1966.

Schayer, R. W. Histamine and Autonomous Responses of the Microcirculation; Relationship to Glucocorticoid Action. Ann. N. Y. Acad. Sci. 116: 891, 1964.

Schayer, R. W. Relationship of Induced Histidine Decarboxylase Activity and Histamine Synthesis to Shock from Stress and from Endotoxin. Am. J. Physiol. 198: 1187, 1960.

Sambhi, M. P., Weil, M. H. and Udhoji, V. N. Acute Pharmacodynamic Effects of Glucocorticoids. Circ. 31: 523, 1965.

Shimamoto, T., Ingue, M., Konishi, T. and Iwahara, S. Preventive Effect of Reserpine Against the Lethality of High Molecular Weight Substances Capable of Eliciting Host Response. I. Prevention of Death due to Shigella Endotoxin by Reserpine Pre-treatment. Arch. Int. Pharmacodyn. 121: 342, 1959.

Smith, M. J. H. Anti-Inflammatory Activity of Salicylates. In The Salicylates, pp. 203-232, edited by Smith, M. J. H. and Smith, P. K. Interscience Publishers, New York, 1966a.

Smith, M. J. H. Metabolic Effects of Salicylates. The Salicylates, pp. 49-105, edited by Smith, M. J. H. and Smith, P. K. Interscience Publishers, New York, 1966b.

Spector, W. G. and Willoughby, D. A. Anti-Inflammatory Effects of Salicylate in the Rat. In Salicylates, pp. 141-147, edited by Dixon, A. St. J., Martin, B. K., Smith, M. J. H. and Wood, P. H. N. Little, Brown and Co., Boston, 1963.

Spielvogel, A. R. An Ultrastructural Study of the Mechanisms of Platelet-Endotoxin Interaction. J. Exp. Med. 126: 235, 1967.

Spink, W. W. Endotoxin Shock. Anaphylaxis and Endotoxin. Calif. Med. 106: 2, 1967.

Spink, W. W., Davis, R. B., Potter, R. and Chartrand, S. The Initial Stage of Canine Endotoxin Shock as an Expression of Anaphylactic Shock: Studies on Complement Titers and Plasma Histamine Concentrations. J. Clin. Invest. 43: 696, 1964.

Spink, W. W. and Vick, J. Evaluation of Plasma, Metaraminol, and Hydrocortisone in Experimental Endotoxin Shock. Circ. Res. 9: 184, 1961.

Starling, E. H. On the Absorption of Fluids from Connective Tissue Spaces. J. Physiol., London. 19: 312, 1896.

Staub, A. The Role of the Polysaccharide Moiety in Determining the Specificity and Immunological Activity of the O-Antigen Complex of Salmonellae. Bacterial Endotoxins, edited by Landy, M. and Braun, W. pp. 38-48. Quinn and Boden Company, Rahway, N.J., 1964.

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

Stetson, C. A., Jr. Studies on the Mechanism of the Shwartzman Phenomenon. Similarities Between Reactions to Endotoxins and Certain Reactions of Bacterial Allergy. J. Exp. Med. 101: 421, 1955.

Stish, R. J., MacLean, L. D. and Visscher, M. B. A Device for Continuous Recording of Weight Changes Suitable for Physiological Measurements. J. Appl. Physiol. 9: 297, 1956.

Strom, E. and Coffman, J. D. Effect of Aspirin on Circulatory Responses to Catecholamines. Arthritis and Rheumatism. 6: 689, 1963.

Sutherland, N. G., Bounous, G. and Gurd, F. N. Role of Intestinal Mucosal Lysosomal Enzymes in the Pathogenesis of Shock. J. Trauma. 8: 350, 1968.

Swan, K. G. and Jacobson, E. D. Hemodynamics of Endotoxin Shock in the Conscious Animal. Surg. Gynec. Obstet. 125: 1041, 1967.

Swyer, G. I. M. Anti-histamine Effect of Sodium Salicylate and Its Bearing Upon the Skin-Diffusing Activity of Hyaluronidase. Biochem. J. 42: 28, 1948.

Tal, C. and Olitski, L. J. The Toxic and Antigenic Properties of Fractions Prepared from the Complete Antigen of Shigella dysenteriae. J. Immunol. 58: 337, 1948.

Tenney, S. M. and Miller, R. M. The Respiratory and Circulatory Actions of Salicylate. Am. J. Med. 19: 498, 1955.

Thomas, L. The Physiological Disturbances Produced by Endotoxins. Ann. Rev. Physiol. 16: 467, 1954.

Thomas, C. S. and Brockman, S. K. The Role of Adrenal Corticosteroid Therapy in Escherichia coli Endotoxin Shock. Surg. Gynec. Obstet. 126: 61, 1968.

Tsagaris, T. J., Koehler, J. A., Kuida, H. and Hecht, H. H. Drug Inhibition of Circulatory Response to Endotoxin in Dogs. Am. J. Physiol. 204: 991, 1963.

Vaughn, D., Kirschbaum, T. H., Bersentes, T. and Assali, N. S. Effects of Corticosteroid Hormones on Regional Circulation in Endotoxin Shock. Proc. Soc. Exp. Biol. and Med. 124: 760, 1967.

Vick, J. A. Bioassay of the Prominent Humoral Agents Involved in Endotoxin Shock. Am. J. Physiol. 209: 75, 1965.

Vick, J. A., Lafave, J. and MacLean, L. D. Renal Failure Associated with Endotoxin Shock. Minn. Med. 46: 855, 1963.

Visscher, M. B. Physiological Factors in Bacterial Endotoxin Shock. Physiol. Phys. 3: 1, 1965.

Visscher, M. B. Shock--With Particular Reference to Endotoxin Shock. Postgrad. Med. 23: 545, 1958.

Waisbren, B. A. Editorial. Gram-Negative Shock and Endotoxin Shock. Am. J. Med. 36: 819, 1964.

Waisbren, B. A. Bacteremia due to Gram-Negative Bacilli Other Than the Salmonella. Arch. Int. Med. 88: 467, 1951.

Walton, R. P. and Darby, T. D. Circulatory Effects of Salicylates. Circ. Res. 6: 155, 1958.

Weil, M. H., MacLean, L. D., Spink, W. W. and Visscher, M. B. Investigations on the Role of the Central Nervous System in Shock Produced by Endotoxin from Gram-Negative Organisms. J. Lab. Clin. Med. 48: 661, 1956a.

Weil, M. H., MacLean, L. D., Visscher, M. B. and Spink, W. W. Studies on the Circulatory Changes in the Dog Produced by Endotoxin from Gram-Negative Microorganisms. J. Clin. Invest. 35: 1191, 1956b.

Weil, M. H. and Whigham, H. Corticosteroids for Reversal of Hemorrhagic Shock in Rats. Am. J. Physiol. 209: 815, 1965.

Westphal, O. Polysaccharides in Biology. p. 208, edited by G. F. Springer, Macy, New York, 1957.

White, F. N., Gold, E. M. and Vaughn, D. L. Renin-Aldosterone System in Endotoxin Shock in the Dog. Am. J. Physiol. 212: 1195, 1967.

Wise, R., Shaffer, J. and Spink, W. The Syndrome of Vascular Collapse due to Gram-Negative Bacteria: Its Management with L-Norepinephrine and Antibiotics. J. Lab. Clin. Med. 40: 961, 1952.

Zweifach, B. W. Relation to the Reticuloendothelial System to Natural and Acquired Resistance in Shock. Int. Anesth. Clin. 2: 271, 1964.

Zweifach, B. W. and Janoff, A. Bacterial Endotoxemia. Ann. Rev. Med. 16: 201, 1965.

Zweifach, B. W., Nagler, A. L. and Thomas, L. The Role of Epinephrine in the Reactions Produced by the Endotoxins of Gram-Negative Bacteria. II. The Changes Produced by Endotoxin in the Vascular Reactivity to Epinephrine in the Rat Mesoappendix and the Isolated, Perfused Rabbit Ear. J. Exp. Med. 104: 881, 1956.