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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

EFFECT OF METHYLPREDNISOLONE AND PROSTAGLANDIN E_1 ON METABOLIC AND CARDIOVASCULAR RESPONSES TO *E. COLI* ENDOTOXIN

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

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BY

ARTHUR V. PRANCAN

Oklahoma City, Oklahoma

1973

EFFECT OF METHYLPREDNISOLONE AND PROSTAGLANDIN E_1

ON METABOLIC AND CARDIOVASCULAR RESPONSES

TO *E. COLI* ENDOTOXIN

APPROVED BY

DISSERTATION COMMITTEE

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TO CATHERINE NASH ELLZEY, MY FAMILY and MY FRIENDS

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADP	Asenosine 5'-diphosphate
AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
β-NAD, NAD^+	β-Nicotinamide adenine dinucleotide
β-NADH, NADH	β-Dihydronicotinamide adenine dinucleotide
c	Curie
cm	Centimeter
CPM	Counts per minute
Cyclic AMP	Cyclic 3',5'-Adenosine monophosphate
°C	Degrees Centigrade
dl	Deciliter
dp/dt	Maximum rate of rise in ventricular pressure
DPM	Degradations per minute
Endotoxin	*E. coli* lipopolysaccharide
FFA	Free fatty acid
g	Gram
Hg	Mercury
^3H-PGE_1	^3H-Prostaglandin E_1
HR	Heart rate
hr	Hour
i.p.	Intraperitonealy
i.v.	Intravenously
kg	Kilogram

L	Liter
LAP	Left atrial pressure
LD_{50}	Dose which is lethal to 50% of the animals in 48 hours
LD_{60}	Dose which is lethal to 60% of the animals in 48 hours
LD_{80}	Dose which is lethal to 80% of the animals in 48 hours
LDH	Lactate dehydrogenase
M	Molar
mc	Millicurie
meq, mEq	Milliequivalent
µc	Microcurie
µg	Microgram
µl	Microliter
µM	Micromolar
µmoles	Micromoles
min	Minute
mg	Milligram
ml	Milliliter
mm	Millimole
mM	Millimolar
mmoles	Millimoles
mm Hg	Millimeters mercury
M-P, MP	Methylprednisolone
NAD^+, β-NAD	β-Nicotinamide adenine dinucleotide

NADH, β-NADH	β-Dihydronicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
n,N	Number of animals per experimental group
N	Normal
ng	Nanogram
nm	Nanometer
O.D.	Optical density
PAP	Pulmonary arterial pressure
PCr	Phosphocreatine
PG	Prostaglandin
PGA_1	Prostaglandin A_1
PGA_2	Prostaglandin A_2
PGDH	Prostaglandin dehydrogenase
PGE_1	Prostaglandin E_1
PGE_2	Prostaglandin E_2
PGE_3	Prostaglandin E_3
$PGF_{1\alpha}$	Prostaglandin $F_{1\alpha}$
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
POPOP	1,4-bis-[2-(5-phenyloxazolyl)]-benzene
PPO	2,5-diphenyloxazole
RAP	Right atrial pressure
RF	Resolution factor
rpm	Revolutions per minute

SAP	Systemic arterial pressure
s.c.	Subcutaneously
std	Standard
Tris	Tris (hydroxymethyl) amino methane HCl
v/v	volume to volume
WBC	White blood cell

EFFECT OF METHYLPREDNISOLONE AND PROSTAGLANDIN E_1 ON METABOLIC AND CARDIOVASCULAR RESPONSES TO *E. COLI* ENDOTOXIN

CHAPTER I

INTRODUCTION

Endotoxin shock has gained much interest during the past two decades because of its consistent lethality in patients and because of its elusive nature. Investigators have been unable to define the precise pathophysiology of shock or to recommend a suitable treatment. Many investigators, however, have been deeply involved in the search for agents which will exert beneficial effects on patients in endotoxic shock. Their efforts have raised a controversy which is primarily focused on the corticosteroids and only recently on the prostaglandins as well. With metabolic, cardiovascular and hematological information during endotoxin shock as a background, the effects of methylprednisolone and prostaglandin E_1 on these parameters were evaluated in laboratory animals. These studies were undertaken in order to better define the effects of these drugs on animals during endotoxin shock.

Cardiovascular, Cellular and Metabolic Character of Endotoxin Shock

The hemodynamic response to *E. coli* endotoxin injection is biphasic, with both changes resulting in hypotension. The first phase is the initial reaction to endotoxin seen during the reversible stage of shock and the second phase is the prolonged hypotension of irreversible shock. Studying the hemodynamic alterations seen in the early phase of shock, Hinshaw and his co-workers (1958) found that systemic arterial pressure falls within 30 min after endotoxin in eviscerated dogs in which the cardiac inflow was kept constant. They concluded that a decrease in vascular tone occurs after endotoxin and that it probably has a significant effect on later phases of endotoxin shock. In the intact dog, the initial response to endotoxin was a sudden, precipitous drop in systemic arterial pressure and a simultaneous elevation of portal venous pressure (MacLean and Weil, 1956; Weil *et al.*, 1956 b). With the subsequent decline of portal venous pressure, the systemic arterial pressure, partially recovered, and gradually entered a slow decline that lasted throughout the duration of the shock state. MacLean and co-workers (1956) associated a large increase in liver and intestine weight, due to pooling of blood 1-2 min after endotoxin, with the pronounced fall in systemic arterial pressure and the rise in portal venous pressure. Hepatectomy or circulatory liver by-pass prevented hypotension (MacLean and Weil, 1956). Weil and co-workers (1956) also found a decrease in venous return, and they concluded that the sharp hypotension following endotoxin was caused by venospasm in the liver and elsewhere, which caused pooling of large amounts of blood, resulting in decreased venous return and thereby

decreasing cardiac output. Reversible endotoxin shock was later described as an ischemic state characterized by a high peripheral resistance due to relex release of catecholamines in response to low circulating blood volume, and a diminished venous return and low cardiac output, resulting in decreased organ perfusion (Lillehei et al., 1964). The ischemic phase can progress to irreversible stagnant shock which leads to death.

Irreversibility in endotoxin shock is considered to occur sometime after 1 hour following endotoxin injection (Hinshaw et al., 1962; Fine, 1966). An early factor in the progress of shock which no doubt influences the later developments, is the sympathetic overactivity which causes vascular injury severe enough to produce necrosis (Thomas, 1956; Horowitz, 1972). Lillehei and MacLean (1958) described an action of endotoxin on the bowel in dogs which precipitated characteristic features of irreversible shock. In their animals, hemorrhagic necrosis of the bowel resulted in an increase in hematocrit and plasma hemoglobin, mirrored by a loss of plasma. Later studies described the endotoxin injury as an intense vasospasm with pooling of blood in the viscera and the skin which leads to stagnant anoxia and profound metabolic disturbances (Lillehei et al., 1964; Lillehei et al., 1967; Roberts and Laros, 1971). The hemodynamic alterations during late endotoxin shock in rabbits have been reported as a progressive decrease in systemic arterial pressure, decrease in central venous pressure, decrease in venous return, decrease in cardiac output, increase in circulation time, decrease in mesenteric blood flow and a decrease in plasma volume (Ashford, et al., 1966). Spink (1962) described this situation as peripheral vascular collapse. Hinshaw and co-workers (1966) concluded that one of the

primary causes of hypotension after endotoxin in subhuman primates was a continuous decline in venous return, as shown earlier in dogs. More recently (Hinshaw et al., 1970) the reduction of venous return in endotoxemic rhesus monkeys was associated with decreased systemic venous resistance in promoting the hypotensive response. In addition to systemic venous pooling, pulmonary pooling of blood was also found to be a significant factor in decreased venous return. The pulmonary obstruction was produced by the sequestration of polymorphonuclear leukocytes in pulmonary capillaries and by a humoral venous constriction.

These peripheral vascular changes assume central importance as shock progresses. Prolonged increase in capillary hydrostatic pressure, decrease in colloid osmotic pressure and changes in capillary permeability may result in extravascular fluid accumulation (Hinshaw, 1969). Isogravimetric capillary pressure in the isolated canine forelimb decreased markedly 4 hours after endotoxin, indicating an increase in capillary permeability (Solomon and Hinshaw, 1968). Other investigators found similar results in isolated forelimb and intestine studies (Motsay et al., 1970, 1971).

The role of the heart in endotoxin shock remains controversial. Myocardial failure in septic shock patients was reported by Siegel and co-workers (1967), and depressed myocardial function followed by heart failure was described by Bell and Thal (1970). Solis and Downing (1966) studied the function of the left ventricle in cats and they concluded that reduced left ventricular contractility and perhaps depressed myocardial function are directly due to endotoxin. Londe and his associates (1967) found no effect of endotoxin on heart rate, heart rhythm, coronary

perfusion pressure or coronary blood flow in the isolated canine heart. Priano and co-workers (1970) also concluded that endotoxin has no detrimental effect on the isolated dog heart. Hinshaw and co-workers (1971) studied the isolated dog heart perfused by a donor dog, measuring left ventricular end diastolic pressure, myocardial contractile force, cardiac power (work/sec), left ventricular dp/dt, O_2 uptake and CO_2 production for 3 hours following endotoxin. These investigators concluded that endotoxin had no effect on the isolated dog hearts. In a recent study during a later stage of shock, however, Hinshaw and co-workers (1972), using an isolated dog heart preparation, observed heart failure 6-9 hours after endotoxin. The results in this study included elevated left ventricular end diastolic pressure and decreased maximum change in left ventricular pressure (dp/dt).

Pulmonary cellular damage during shock has recently been described by several investigators. In 1971 Ratliff and co-workers found a sharp decrease in circulating white blood cells, especially neutrophils, during hemorrhagic shock. Meanwhile, the neutrophils appeared elevated in the lungs. These cells also exhibited altered structure, loss of density in the cell sap, increased density in the granules and unusual apposition between the plasma membrane of the neutrophil and the adjacent endothelium of lung vessels. Ratliff and his associates concluded that the neutrophils become sticky in some way during shock and they collect in the lung, thereby accounting for some abnormalities in pulmonary circulation. Other investigators described similar changes during endotoxin shock (Coalson et al., 1970; Massion et al., 1972). Endotoxin caused a

decrease in circulating polymorphonuclear leukocytes and platelets in dog lung. These cells probably adhered to the pulmonary vasculature, causing an increase in pulmonary vascular resistance following endotoxin. Massion and his co-workers also found an increase in β-Glucuronidase activity in the lung during shock due to lysosomal disruption. Wilson and co-workers (1972 a, b) found that in addition to disrupting platelets and polymorphonuclear leukocytes, endotoxin disturbed the phagocytic ability of the polymorphonuclear leukocytes. Pronounced endothelial and epithelial swelling was also observed in the lung, as well as the destruction of mitochondria in alveolar cells.

The liver has also been described as a focus of endotoxin-related damage. In 1962, Weissman and Thomas observed a pronounced elevation in β-glucuronidase and cathepsin activity in the supernatant of the large granular fraction of rabbit liver shortly after administration of endotoxin. Janoff and co-workers (1962) also localized a hepatic origin for plasma lysosomal enzymes. More recently, Rangel and his associates (1970 a) concluded that the massive release of hepatic lysosomal enzymes into the general circulation may account for the development of irreversibility in endotoxin shock. The mechanisms involved may be the release of kinins by the enzymes, causing hypotension and capillary leakage, and enzyme-induced hypercoagulability of the blood, followed by disseminated intravascular clotting (Massion and Blümel, 1971).

Structural changes have also been found in the liver following endotoxin, and they may be related to the functional alterations reported by other investigators. A consistent observation has been swollen or distorted mitochondria. Boler and Bibighaus (1967) saw swollen mito-

chondria 15 min after endotoxin in the dog liver, spherical mitochondria at 75 min and severely swollen mitochondria with a loss of interior structure at 135 min. Other workers also observed swollen mitochondria in rat liver following endotoxin (DePalma et al., 1970; Mela et al., 1971). In addition, DePalma and co-workers noticed engulfed mitochondria within autophagic vacuoles. Stewart (1970) observed further effects of endotoxin on the ultrastructure of the liver in hamsters 24 hours after endotoxin. He reported hepatic cellular changes such as gaps between cells, discontinuous basement membranes, swelling of platelets and red blood cell aggregation in the capillaries.

The liver probably makes a protective response to endotoxin early in shock as evidenced by an increase in smooth endoplasmic reticulum (Boler and Bibighaus, 1967; Rangel et al., 1970). Rangel and co-workers found a more severe hypotension when endotoxin was injected in the systemic circulation as compared to the portal vein. They concluded that the liver may metabolize or remove the endotoxin during the initial phase of shock, and that this ability probably deteriorates as shock progresses, eventually leading to hepatic injury.

Changes in carbohydrate metabolism have also been associated with endotoxin administration in animals and with septic shock in patients. Early studies with gram-negative bacteria in rabbits revealed a hyperglycemia in the initial response to infection (Menten and Manning, 1924; Zeckwer and Goodell, 1925). Delafield (1932), however, observed a prompt fall of blood glucose to hypoglycemic levels in rabbits injected with gram-negative bacteria. Subsequent work has also shown a hypoglycemic response to endotoxin. Berry and co-workers (1959) found a decrease in

blood glucose in mice following endotoxin injection. Other investigators found a hypoglycemic activity of endotoxin in rats (LaNoue et al., 1968 a, b; Shands et al., 1969). They attributed the low blood glucose before death in these rats to an alteration in hepatic gluconeogenesis.

Later work, however, revealed a biphasic change in blood glucose concentration. Berk and his associates (1970) studied the hypoglycemia of shock in 129 dogs and reported a significant decrease in blood glucose immediately after endotoxin in 42% of their animals. The remaining 58% of the group exhibited an initial increase in blood glucose to 143% of the control level and a subsequent decline, 1-2 hours after endotoxin which also resulted in a significant hypoglycemia. Williamson and co-workers (1970) found similar results in endotoxemic rats. One-half of their animals showed an initial hyperglycemia which culminated in a progressive hypoglycemia after 1 hour, leading to death 5-6 hours after endotoxin. Furthermore, these investigators studied specific metabolic changes in the liver which might account for such a profound alteration in glucose metabolism. They observed an initial increase in gluconeogenesis following endotoxin, which progressively dropped to sub-normal levels within 1 hour. The decrease in gluconeogenesis was associated with an accumulation of fructose-1.6-diphosphate and with a corresponding depletion of fructose-6-phosphate and glucose-6-phosphate, suggesting either an increase in phosphofructokinase activity or a decrease in fructose diphosphatase activity. Williamson and his associates concluded that some derangement during endotoxin shock, perhaps a decrease in adenosine 5'-triphosphate, which regulates phosphofructokinase, results in a block of gluconeogenesis and consequently stimulates glycolysis.

Hyperglycemia following endotoxin has been reported in recent work, but it appears to be related to the early or mild hyperglycemia shown in some of the animals studied by the Berk and Williamson groups (Rangel *et al.*, 1970; Cryer *et al.*, 1971). The characteristic response of blood glucose concentration to endotoxin appears to be a mild early increase followed by a profound decrease throughout the duration of the shock state. The hypoglycemia is probably due to an inability of the liver to conduct adequate gluconeogenesis in the face of both depletion of glycogen stores and increased glucose needs of ischemic tissues (Filkins, 1973).

Glycogen depletion in the liver is another defect of carbohydrate metabolism which has been consistently associated with endotoxin shock. Delafield (1932) reported a decrease in liver glycogen in rabbits which had been injected with a suspension of gram-negative bacteria. Berry and co-workers (1959) published similar results following studies on different strains of mice given endotoxin. Later work confirmed these findings in a variety of animals. Boler and Bibighaus (1967) observed ultrastructurally a loss of glycogen in dog liver within 15 min after endotoxin. In other ultrastructure studies, glycogen was depleted in hamster liver 24 hours after endotoxin (Stewart, 1970). Glycogen loss was also measured in rabbit liver 8 hours after endotoxin (Lundsgaard-Hansen *et al.*, 1972). Most recently, Filkins (1973) showed a sharp decrease in hepatic glycogen content in rats following endotoxin.

Hamosh and Shapiro (1960) were able to speculate on the mechanism of this loss of glycogen following their studies on glycogenolysis in endotoxin-treated rats. They found almost complete conversion of

phosphorylase to the active form following endotoxin. An increase in phosphorylase-kinase activity precipitated this conversion, which accounted for the accelerated glycogenolysis and the subsequent glycogen depletion. In support of this hypothesis, Gimpel and co-workers (1971) reported direct stimulation of liver adenyl cyclase *in vitro* from guinea pigs by endotoxin. Such an event could rapidly elevate cyclic 3',5'-adenosine monophosphate levels, converting phosphorylase kinase to its active form (Bowness, 1966; Krebs *et al.*, 1966). An additional mechanism which may be instrumental in glycogen depletion was reported by Berry and co-workers (1968), who described an apparent inhibitory effect on hepatic glycogen synthesis by endotoxin in mice.

Further related metabolic disorders have also been described as characteristics of endotoxin shock. Adenosine 5'-triphosphate (ATP) content was shown to be depressed in brain, liver and kidney of endotoxemic rats (Staples *et al.*, 1969). Similarly, heart and liver decrease of ATP associated with a rise in adenosine diphosphate (ADP) and adenosine monophosphate (AMP) was observed in rabbits 4-8 hours after endotoxin (Pappova *et al.*, 1971; Lundsgaard-Hansen *et al.*, 1972).

Blood lactate elevation, which indicates impairment of energy-producing processes, has been shown to occur in septic shock patients as well as in endotoxemic laboratory animals. A marked rise in blood lactate was noted in endotoxic patients who ultimately died during shock (Blair *et al.*, 1969). Viter and Cowley (1971) devised a prognostic method using blood lactate concentration as an indicator of mortality in shock. They ascribed 20% mortality to lactate levels in the range of 1.5-4.5 meq lactate/L blood, 70% to 4.5-9.0, 85% to 9.0-13.5 and

100% mortality to 13.5 meq lactate/L blood or over. Sub-human primates have also been reported to display elevated blood lactate during endotoxin shock (Cavanagh, 1969). High tissue lactate/pyruvate ratios were found in heart and liver of rabbits after endotoxin (Pappova et al., 1971: Lundsgaard-Hansen et al., 1972).

Effect of Corticosteroids on Endotoxin Shock

Corticosteroids have long been used in the treatment of patients with a variety of septic diseases including those caused by gram-negative bacilli (Spink, 1957). Spink suggested that short-term use of adrenocorticotrophic hormone (ACTH) or adrenocorticosteroids in carefully selected patients with severe infections will frequently contribute to the recovery of patients and may prevent permanent disability often associated with these diseases. A controversy, which still exists, was evident at that time, as pointed out by an editorial published in the New England Journal of Medicine in March of 1958. The writer of the editorial stressed that Spink and most of the authors he cited were impressed by the favorable appearance of patients after the corticosteroids were administered, a non-specific, pharmacological effect of the drugs, which was seen as often in those patients which did not survive or had serious complications, as in those which recovered from their infections. The editorial continued by saying that many clinical investigators have adopted a conservative attitude toward the use of corticosteroids because of unfavorable results reported in the literature, the scarcity of supporting clinical evidence from controlled studies, the fact that physicians tend to record what "impresses" them favorably and seldom report failures in similar circumstances, reports of untoward effects of hormone

therapy, and the failure of adrenocortical hormones to yield favorable results in some situations in which they are considered to be clearly indicated by some workers. The conclusion placed the burden of proof that adrenocortical hormones may be beneficial in certain types of infections with the investigators who make such claims.

In the succeeding years many clinical observations and experimental studies were published, yet the nature of the claimed beneficial effect of corticosteroids on gram-negative bacterial sepsis is still unknown. Altemeier (1958) suggested a plan of treatment for septic shock which included the use of cortisol only if blood pressure could not be maintained by other means. He did, however, observe an apparent life-saving effect of cortisol on 3 patients. Subsequent clinical reports and laboratory investigations have also described an increase in patient and laboratory animal survival when corticosteroids were used. Lillehei and MacLean (1959) reported that dogs pretreated with cortisol tolerated shock due to endotoxin much better than did untreated controls. The survival of endotoxemic animals in this study was significantly increased with cortisol. Endotoxin-treated mice also survived in greater numbers when treated with prednisolone (Weil, 1962). Patient survival following gram-negative shock was doubled with the use of cortisol according to one study (Weil et al., 1964) and it was as high as 85% in another when methylprednisolone was employed (Motsay et al., 1970). Recent animal studies also described a decreased mortality of endotoxin shock following glucocorticoid administration (Hinshaw et al., 1967; Thomas and Brockman, 1968; Kadowitz and Yard, 1970). Other investigators have recommended the use of corticosteroids for treatment of endotoxin shock patients

(Lillehei et al., 1964; Robins, 1966; Lillehei et al., 1967; Oaks and Cohn, 1967; Cavanagh et al., 1968; Rigby and Christy, 1968; Schwarz, 1968; Larson and Abston, 1970; Lefer, 1970).

Corticosteroids have been credited with the ability to prevent and repair the vascular and cardiac damage that endotoxin is thought to cause. In 1958 and 1959, Lillehei and MacLean reported that cortisol prevented the blood pressure drop and the plasma loss they associated with endotoxin administration in dogs. They did find, however, that liver congestion persisted despite corticosteroid treatment. In defense of cortisol therapy in shock patients, Lillehei and co-workers (1964) pointed out that this drug never depressed blood pressure or other vital signs further when given to animals or patients in endotoxic shock and furthermore, that it probably blocked the onset of irreversible shock.

A direct effect on the heart in shock has been described by many investigators as a primary beneficial action of corticosteroids. The administration of corticosteroids was seen to increase cardiac output toward normal levels in shock patients (Melby, 1964; Sambhi et al., 1965; Wilson and Fisher, 1968; Robson, 1971). In agreement with these findings, other investigators have measured a positive inotropic effect of corticosteroids on the normal heart (Franz, 1964), and on the endotoxemic heart in cats (Kadowitz and Yard, 1970).

The reported increase in cardiac output may also be a result of peripheral actions of corticosteroids. Ashford and co-workers (1966) observed various hemodynamic effects including increased central venous pressure, increased mesenteric flow and increased plasma volume in endotoxemic rabbits following dexamethasone. Vasodilation has frequently been

reported as an effect of corticosteroid treatment which restores blood flow and increases tissue perfusion in endotoxin shock (Dietzman et al., 1967; Hinshaw et al., 1967; Dietzman et al., 1969; Motsay et al., 1970 a, b; Rao and Cavanagh, 1970; Margulis et al., 1971). Another important alteration due to endotoxin is the loss of vessel integrity. Using isolated dog forelimb and intestine preparations in endotoxin-treated animals, Motsay and co-workers (1971, 1972) were able to show that methylprednisolone protects capillary and small vessel integrity in endotoxemic animals. They measured the weight and isogravimetric capillary pressure in the isolated structures and concluded that endotoxin leads to an increase in vessel permeability, which is apparently prevented with corticosteroid use.

The damage caused by the release of lysosomal enzymes during endotoxin shock also appears to be prevented by the use of corticosteroids. Several investigators have concluded that corticosteroids may stabilize lysosomal membranes, and in this way protect cells in various tissue from damage (Melby, 1964; Replogle et al., 1966; Rigby and Christy, 1968; Massion and Blumel, 1971).

Carbohydrate metabolism is also seriously affected during endotoxin shock and it appears to respond to corticosteroid treatment. Berry and co-workers (1959) found that endotoxin depleted virtually all carbohydrate from mice but that much of it was preserved when the animals were pretreated with cortisol. In endotoxemic mice, cortisol elevated carbohydrate content significantly above post-endotoxin levels. Cortisol accelerates gluconeogenesis and increases liver glycogen stores while it decreases peripheral glucose utilization (Levenson et al., 1961;

Lecoq et al., 1964; Novak et al., 1970).

The gluconeogenic and glycogenic activities of cortisol are partly explained by its stimulation of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and glycogen synthetase in the liver (Pritham, 1968). Heart enzymatic changes have also been demonstrated. Decreases in rat heart glycogen and glycogen synthetase activity due to endotoxin were prevented by dexamethasone when rats were pretreated with the drug (Daw et al., 1968). Glycogen content and glycogen synthetase activity in rat heart were also restored when dexamethasone was given after endotoxin (Daw et al., 1969).

Effect of Prostaglandins on Endotoxin Shock

Prostaglandins are known to be vasoactive agents. They exert positive chronotropic and inotropic actions on the heart, increase cardiac output, increase pulmonary arterial pressure and increase capillary permeability (Nakano and McCurdy, 1967; Bergström et al., 1968; Nakano and McCurdy, 1968; Nakano and Cole, 1969; Nakano, 1973 a). The prostaglandin E-compounds (PGE_1, PGE_2 and PGE_3) and the prostaglandin A-compounds (PGA_1 and PGA_2) decrease total peripheral resistance and systemic arterial pressure while increasing blood flow in peripheral and coronary vasculature (Bergström et al., 1968; Nakano, 1968; Nakano and McCurdy, 1968; Lee, 1970; Murphy, 1970; Nakano et al., 1971; Barner et al., 1972; Nakano, 1973 a). Prostaglandin F-compounds ($PGF_{1\alpha}$ and $PGF_{2\alpha}$) exert an oposite effect. They produce a mild vasoconstriction, elevating systemic arterial pressure and decreasing blood flow in the periphery. (Nakano, 1968; Nakano and Cole, 1969; McCloy et al., 1973; Nakano, 1973 a).

Other vasoactive agents such as kinins and biogenic amines have

been associated with the development of hemodynamic alteration in endotoxin shock (Mignone, 1962; Shoemaker, 1967; Hinshaw, 1971; Kitzmiller, 1971; Thal, 1971). Recently, prostaglandins have also been implicated in the pathological disorder found in shock. Speculation by several investigators has involved increased biosynthesis and release of prostaglandins in various pathological derangements involving inflammatory and immunological reactions (Greaves et al., 1971: Kaley et al., 1971; Piper and Vane, 1971; Willis et al., 1972). Other workers have studied the potent pharmacological effects of prostaglandins which simulate inflammatory and immunological reactions in various animals (Glenn et al., 1972; Glenn and Rohloff, 1972; Nakano, 1973 a, b).

Prostaglandin levels in the plasma have been shown to increase during endotoxin shock, indicating perhaps that these compounds are intimately involved in the shock syndrome (Kessler et al., 1969; Skarnes and Harper, 1972; Milton, 1972; Anderson et al., 1973; Kessler et al., 1973). These compounds have also been shown to mediate certain manifestations of endotoxin shock such as abortion, diarrhea, fever and pulmonary hypertension (Milton, 1972; Skarnes and Harper, 1972), possibly further implicating them in the genesis of the shock syndrome. Prostaglandin $F_{2\alpha}$ has been shown to induce lysosomal fragility, another common characteristic of endotoxin shock which probably leads to widespread cellular damage (Weiner and Kaley, 1972).

Beneficial effects of prostaglandins on the shock state in animals have also been reported. An anti-inflammatory effect of prostaglandins was observed in recent studies (Glenn et al., 1972; Glenn and Rohloff, 1972). Prostaglandin E_1 inhibited the endotoxin-induced release

of lysosomal enzymes from leukocytes in studies by other investigators (Weissman et al., 1962; Sorrells et al., 1972). Sorrells and his associates also found that PGE_1 blocked the rise in pulmonary vascular resistance associated with endotoxin shock in dogs. However, this effect may be related to the inhibition of lysosomal enzyme release in the lungs. Additional hemodynamic and metabolic improvements and an increase in animal survival due to PGE_1 and $PGF_{2\alpha}$ were found in postoligemic shock (Glenn, 1972; Priano et al., 1972) and in endotoxin shock (Glenn et al., 1971).

Specific Aims

All of the studies incorporated into this dissertation are directly concerned with the use of methylprednisolone or prostaglandin E_1 for the treatment of endotoxin shock. Corticosteroids have been used for the treatment of endotoxin shock in patients and animals, and these drugs are considered by many investigators to be beneficial. In spite of their common therapeutic use, no well-controlled studies in humans have been done to clearly establish the therapeutic efficacy of corticosteroids in septic shock (Robson, 1971). The lack of adequate controls also applies to dog endotoxin shock models. There, the uncertain biological status of the control animals and variability due to the usually small number of animals per study cause an unreliable condition for endotoxin shock survival, mechanism and treatment studies (Fine, 1966).

For toxicological studies, mice are more advantageous than mongrel dogs since there are considerably fewer pharmacogenetic and physiologic variations in mice. Furthermore, mice are less expensive so that relatively large numbers of animals can be used for more meaningful

statistical analysis. Hence, a study was undertaken to establish the lethality of E. coli endotoxin in male adult albino mice, and also to compare the effect of methylprednisolone on the lethality of endotoxin-treated mice to that of control mice.

The metabolic response to E. coli endotoxin has not been clearly defined, and neither has the effect of corticosteroid treatment on metabolic parameters in endotoxemic animals. Carbohydrate and high energy phosphate metabolism is certainly crucial to the survival ability of animals in shock, as indicated by the studies which have been concerned with this problem. For this reason, selected metabolites were measured in the heart, liver and plasma of mice following endotoxin administration and following the use of methylprednisolone in the endotoxemic animals.

As described earlier, the beneficial effect of prostaglandins in endotoxin-treated animals is uncertain. Prostaglandins may possibly promote or prevent pathogenic manifestations of endotoxin shock. In order to clarify the possible beneficial actions of prostaglandins in this disorder, cardiovascular, metabolic and hematological effects of PGE_1 were studied in canine endotoxin shock.

Elevated plasma levels of prostaglandins have been shown to occur in endotoxin shock in animals. Although several investigators have suggested that prostaglandin biosynthesis and release are enhanced during pathological disorders, the possibility exists that in endotoxin shock, where pronounced lung damage is known to occur, the metabolic degradation of prostaglandins may also be impaired. The reported beneficial effects of methylprednisolone on the structure and function of the alveoli and the vasculature in the lung during shock may prevent such a disorder, if

in fact it does occur. This hypothesis was tested by measuring the metabolic degradation of PGE_1 in the lung of rats in lethal endotoxin shock. Furthermore, the ability of methylprednisolone to protect the lung from any apparent alterations was also investigated.

In summary, the specific aims of the studies described in this dissertation were:

1) To determine the lethality of E. coli endotoxin in mice.

2) To study the effect of methylprednisolone on lethality in endotoxin-treated mice.

3) To study the effect of methylprednisolone on the levels of ATP, PCr, lactate, pyruvate and glycogen in the heart and liver, and on the level of blood glucose in endotoxin-treated mice.

4) To study the cardiovascular, metabolic and hematological effects of PGE_1 on endotoxin shock in dogs.

5) To study the effect of E. coli endotoxin on the activity of prostaglandin dehydrogenase (PGDH) in the lung of rats.

6) To study the effect of methylprednisolone on the activity of PGDH in the lung of endotoxin-treated rats.

CHAPTER II

MATERIALS AND METHODS

Materials

Animals

Male adult albino mice were obtained from Arthur Sutter, Springfield, Missouri and from The Mouse House, Marlow, Oklahoma. Male adult albino Holtzman strain rats were obtained from the Oklahoma Medical Research Foundation Animal Wing, Oklahoma City, Oklahoma.

Adult mongrel dogs were obtained from Charles Alexander, Wynnewood, Oklahoma. A total of 533 mice, 12 rats and 28 dogs were used in these studies. All animals were kept in the University of Oklahoma Health Sciences Center animal facility for a period of time ranging from 5-7 days to accustom them to the change in environment and diet before they became experimental subjects.

Drugs and Hormones

E. coli endotoxin (lipopolysaccharide W, batch #209684 and batch #261989) was obtained from Difco Laboratories, Detroit, Michigan. Lipopolysaccharide W, batch #261989 was used only in the study concerning the effects of prostaglandin E_1 on the cardiovascular, hematological and metabolic responses to endotoxin in dogs. Methylprednisolone sodium

succinate (Solu-Medrol^{Tm}) was supplied by The Upjohn Company, Kalamazoo, Michigan. Liquaemin^{Tm} sodium heparin was supplied by Dr. H. A. Strade of Organon, Inc., W. Orange, New Jersey. Pentobarbital sodium was purchased from J. T. Baker Chemical Company, Phillipsburg, New Jersey. The PGE_1 used in these studies was supplied by Dr. J. R. Pike, The Upjohn Company, Kalamazoo, Michigan. PGE_1 was dissolved in pure ethanol (10 mg/ml), stored at 0°C and diluted with 0.9% NaCl solution to make a 100 ng/ml solution. ^{3}H-prostaglandin E_1 (^{3}H-PGE_1) (50 mc/mmole) was purchased from the New England Nuclear Corporation, Boston, Massachusetts.

Enzymes and Co-Enzymes

Yeast hexokinase (140 U/mg), glucose-6-phosphate dehydrogenase (140 U/mg), creatine kinase from rabbit muscle (25 U/mg) and lactate dehydrogenase from rabbit muscle (550 U/mg) were obtained from Boehringer Mannheim Corp., New York, New York. Lactate dehydrogenase from beef heart (125 U/mg) was purchased from Worthington Biochemical Corp., Freehold, New Jersey. β-Nicotinamide adenine-dinucleotide (β-NAD), β-dihydronicotinamide adenine dinucleotide (β-NADH) and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from Sigma Chemical Company, St. Louis, Missouri.

Substrate Standards

Glucose was obtained from Mann Research Laboratories, New York, New York. L-lactate and pyruvate were obtained from Boehringer Mannheim Corporation, New York, New York. Glycogen, adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP) and phosphocreatine (PCr) were purchased from Sigma Chemical Company, St. Louis, Missouri.

Others

Glucostat™ was obtained from Worthington Biochemical Corporation, Freehold, New Jersey. Fisher reducing reagent was purchased from Fisher Scientific Company, Fair Lawn, New Jersey. Bovine Albumin (lot No. 10C-4380) was obtained from Sigma Chemical Company, St. Louis, Missouri. Silica Gel G (acc. Stahl) produced by E. Merck was purchased from Brinkman Instruments, Incorporated, Westbury, New York. Silicic acid (100 mesh) was obtained from Malinckrodt Chemical Works, St. Louis, Missouri. 1, 4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) and 2, 5-diphenyloxazole (PPO) were purchased from Packard Instrument Company, Downers Grove, Illinois. Tris hydrochloride, Tris (base) were obtained from Schwartz/Mann, Orangeburg, New York. Other chemicals used were all reagent grade.

Special Solutions

The free fatty acid determination required the use of the following solutions:

CHM solution contained:

Chloroform	1000 ml
Heptane	750 ml
Methanol	35 ml

Cu-TEA solution (made weekly at pH 8.1) contained:

$Cu(NO_3)_2$	2.42 g
1M Triethanolamine	10.00 ml
1N NaOH	3.50 ml
NaCl	33.00 g
Distilled water to	100.00 ml

DPC solution (made daily) contained:

Diphenylcarbazide		0.4 g
1M Triethanolamine		1.0 ml
96% Ethanol	to	100.0 ml

Buffers

100 mM Tris buffer was used for ATP and PCr determinations and at pH 7.5 it contained per liter:

Tris (base)	9.88 g
Tris HCl	0.68 g

200 mM carbonate buffer was required for lactate determination and at pH 9.7 it contained per liter:

Na_2CO_3	16.10 g
$NaHCO_3$	4.05 g

100 mM phosphate buffer, which was used for pyruvate determination, at pH 7.0 contained per liter:

K_2HPO_4	11.32 g
KH_2PO_4	4.78 g

Survival Studies

Three hundred thirty-nine male adult albino mice weighing between 20 and 50 g, which were fed Purina Rat Chow ad libitum continuously throughout the experiment, were divided into three sections. The First Section contained 129 mice which were divided into three groups. Each group received a different dose of E. coli endotoxin: Group A (10 mice) 100 mg/kg, intraperitonealy (i.p.); Group B (60 mice) 30 mg/kg, i.p. and Group C (59 mice) 10 mg/kg, i.p. The mice were inspected hourly

for 48 hours and the number of survivors recorded. All mice alive at the end of 48 hours after endotoxin were considered to be survivors. The percentage of mice which died in each group was taken as the measure of lethality of that particular dose of E. coli endotoxin.

The Second Section contained 72 mice which were divided into three groups and all animals received E. coli endotoxin (30 mg/kg i.p.). Each group received a different dose of methylprednisolone one hour before endotoxin; Group A (20 mice) 6 mg/kg, subcutaneously (s.c.); Group B (20 mice) 30 mg/kg, s.c. and Group C (32 mice) 150 mg/kg, s.c. All mice were inspected hourly for 48 hours and the number of survivors was recorded. The percentage of survival was calculated for each group and the values were compared to Group B in the First Section which received endotoxin (30 mg/kg, i.p.) alone.

The Third Section contained 138 mice which were divided into three groups and all animals received E. coli endotoxin (10 mg/kg, i.p.) Each group received a different dose of methylprednisolone at various times relative to the injection of endotoxin: Group A (69 mice) 30 mg/kg, s.c. and Group B (69 mice) 150 mg/kg s.c. The methylprednisolone was administered to Groups A and B according to the following schedule: 1 hour before endotoxin (12 mice each group); simultaneous with endotoxin (12 mice each group); 1 hour after endotoxin (12 mice each group); 2 hours after endotoxin (12 mice each group); 4 hours after endotoxin (11 mice each group) and 6 hours after endotoxin (10 mice each group). The mice were inspected hourly for 48 hours and the number of survivors recorded. The percentage of survival was computed for each group and the values were compared to Group C in the First Section, which received endotoxin

(10 mg/kg, i.p.) alone.

Measurement of Selected Metabolites in Endotoxin-Treated Mice

One hundred ninety-four male adult albino mice weighing between 30 and 45 g, which were fed Purina Rat Chow ad libitum continuously throughout the experiment, were divided into 7 sections. The schedule for the injection of endotoxin and methylprednisolone to study the effect of methylprednisolone on metabolism in endotoxemic mice is shown in Figure 1. All injection schedules were begun (0 Hour) between 6 and 7 a.m. The First Section contained 30 mice which were divided into five groups. All mice received 0.9% NaCl (0.5 ml, i.p.). Each group was sacrificed at a different time after the saline injection: Group A, 0 hours; Group B, 2 hours; Group C, 4 hours; Group D, 8 hours; and Group E, 12 hours.

The Second Section contained 42 mice which were divided into four groups. All mice received E. coli endotoxin (10 mg/kg i.p.). Each group was sacrificed at a different time after endotoxin injection: Group A, 2 hours; Group B, 4 hours; Group C, 8 hours; and Group D, 12 hours. Each of the following sections contained three groups of mice which were sacrificed at a specified time following the injection of methylprednisolone: Group A, 2 hours; Group B, 4 hours; and Group C, 8 hours. The Third Section contained 18 mice which received methylprednisolone (30 mg/kg, s.c.) one hour before E. coli endotoxin (10 mg/kg, i.p.). The Fourth Section contained 18 mice which received methylprednisolone (30 mg/kg, s.c.) simultaneously with E. coli endotoxin (10 mg/kg, i.p.). The Fifth Section contained 30 mice which received E. coli endotoxin (10 mg/kg, i.p.) followed by methylprednisolone (30 mg/kg, s.c.)

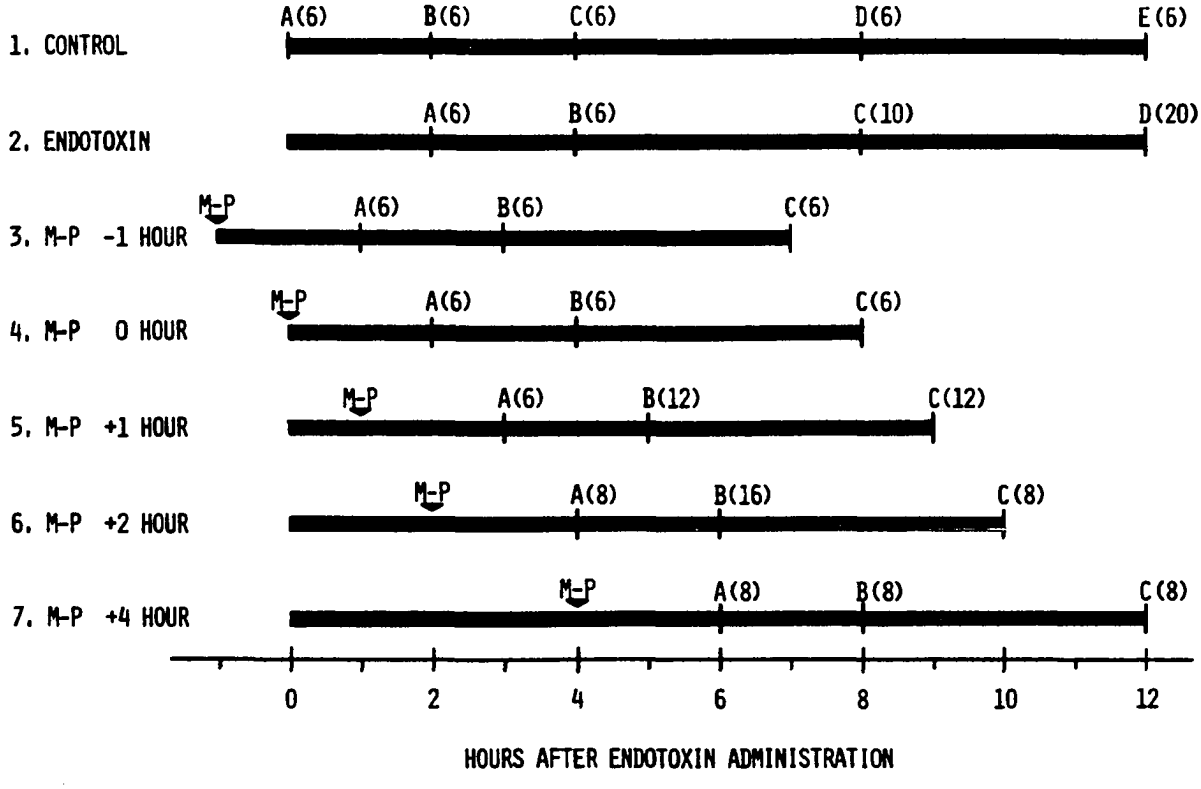


Figure 1. Schedule for injection of E. coli endotoxin (10 mg/kg i.p.) and methylprednisolone (M-P, 30 mg/kg, s.c.) to study effect of methylprednisolone on metabolism in endotoxemic mice. Section 1 (control) received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hours after endotoxin. Sections 2-7 received endotoxin at 0 hour and methylprednisolone as indicated by M-P. Each experimental group was designated by a letter and the number of animals it contained, for example, C(12), at the time it was sacrificed after endotoxin or saline injection.

one hour later. The Sixth Section contained 32 mice which received E. coli endotoxin (10 mg/kg, i.p.) which was followed by methylprednisolone (30 mg/kg, s.c.) 2 hours later. The Seventh Section contained 24 mice which received E. coli endotoxin (10 mg/kg, i.p.) followed by methylprednisolone (30 mg/kg, s.c.) 4 hours later.

Each mouse was lightly anesthetized with 60 mg/ml sodium pentobarbital (0.05 ml, s.c.) 10 minutes before it was sacrificed. Then, the abdomen and chest were rapidly opened with scissors, and the heart and liver were excised directly into a beaker containing acetone and dry ice. The temperature of this mixture was -80°C. The frozen tissues were wrapped in aluminum foil and stored at -80°C. Blood from the open chest cavity was collected in cold centrifuge tubes which were then spun in an International CL Centrifuge at 3000 r.p.m. for 10 minutes. An aliquot (0.2 ml) of the supernatant plasma was analyzed for glucose content.

Blood glucose content was determined by an enzymatic method. The blood sample was diluted 10 x in water, mixed, and 2% $ZnSO_4$ 7·H_2O (1 ml) and 1.8% $Ba(OH)_2$ 8·H_2O (1 ml) were added in order to deproteinate the sample. The mixture was centrifuged (International CL) at 3000 r.p.m. for 10 minutes. An aliquot (500 µl) of the supernatant, a glucose standard and a water blank were each mixed with Glucostat^{Tm} reagent (2 ml). The reaction was stopped after exactly 10 minutes with the addition of 1 drop of 2 N HCl, and 5 minutes later the optical density of each sample was read at 400 nm in a spectrophotometer (Hitachi, type S_{12}-B). The concentration of the unknown glucose sample was calculated as follows: Cu = Cs x Au/As, where A is absorbancy, C is concentration of glucose,

u is unknown and s is standard.

The tissue was next prepared for the measurement of metabolites by the method of Lowry and Passonneau (1972). The frozen hearts and livers were powdered in a small metal mortar and pestle which was cooled to -80°C. This powdered tissue was then weighed to approximately 100 mg on a Roller-Smith torsion balance in a room maintained at -20°C. At all other times the tissue was kept at -80°C or under dry ice. The weighed tissue powder was transferred directly to an 8 ml glass tube containing 0.3 ml 3M $HClO_4$ which was frozen. This combination was mixed in an alcohol bath (25% ETOH and dry ice) at -8° to -10°C until the acid penetrated the tissue particles (5-15 min). Then, 1 ml distilled water was added to each tube. The contents were mixed at 4°C for 5-10 min and centrifuged (3000 r.p.m., 10 min) in an International CL centrifuge. The supernatant was transferred and neutralized immediately with 2M $KHCO_3$ (0.33 ml per ml distilled water added). The neutralized supernatant was frozen and stored at -80°C until it could be assayed for ATP, PCr, lactate and pyruvate. The precipitate was assayed for glycogen.

Heart and liver tissue glycogen content were determined via the method described by Walaas and Walaas (1950). The precipitate from the perchloric acid extraction procedure described above was subjected to digestion by 30% KOH (2 ml) for 15 min at 100°C. After the tubes were at room temperature again, 4.5 ml 96% ETOH and 2 drops saturated Na_2SO_4 were added and the contents were mixed. This mixture was slowly brought to a boil and slowly allowed to cool. Once the tubes were at room temperature, they were centrifuged (International UV) at 2000 r.p.m. for 15 min. The supernatant was decanted and the precipitate was washed

with 4 ml 96% ETOH. The centrifugation was then repeated. The supernatant was again decanted and the precipitate was dried by immersing the tubes in hot water. Glucose was released from the glycogen by heating the precipitate with 1 ml 2N H_2SO_4 in a boiling water bath (Precision Scientific Company) for 1 hour. This solution was neutralized with approximately 1 ml 2N NaOH. An aliquot (2 ml) of Glucostat^{Tm} reagent was added to 500 µl of the sample, a glucose standard and a water blank. The reaction was stopped after exactly 10 minutes with the addition of 1 drop of 2N HCl, and 5 minutes later the optical density of each sample was read at 400 nm in a spectrophotometer (Hitachi, type S_{12}-B). The concentration of the unknown glucose sample was calculated as follows: Cu = Cs x Au/As, where A is absorbancy, C is concentration of glucose, u is unknown and s is standard. The glycogen content of the tissue was expressed as mg of glucose measured per g of tissue from which glycogen was extracted.

The following metabolite determinations were accomplished by the method of Lowry and Passonneau (1972). Adenosine-5'-triphosphate (ATP) and phosphocreatine (PCr) were measured on the same sample. The analysis was conducted with 1 ml of reagent in fluorometer tubes (10 x 75 mm) plus 50 µl neutralized $HClO_4$ extract, which was equivalent to 3 mg of tissue. The reagent was 100 mM Tris, pH 7.5, with 1 mM glucose, 5 mM $MgCl_2$ and 0.25 mM $NADP^+$. An initial fluorometer (Farrand, A-3) reading was made before yeast hexokinase (5 µg/ml) and glucose-6-phosphate dehydrogenase (2 µg/ml) were added to initiate the reaction. After 10 min the second reading was taken. The difference between these readings was directly related to the amount of ATP present. To prevent error caused by ATP

contamination of adenosine-5'-diphosphate (ADP), 10 µl 10 mM ADP was added to each tube and a reading was made after 10 minutes. ADP is necessary for the measurement of PCr and erroneously high PCr values could have been recorded if allowance was not made for contamination by ATP. Following the third reading, creatine Kinase (80 µg/ml) was added and the last reading was made 25 min later. The difference between the third and fourth readings was directly related to the amount of PCr present. The enzyme blank was a tube containing reagent, but no tissue extract, which was treated as a sample in every other way. The blank readings for each enzyme addition were subtracted from the appropriate substrate readings. A standard NADH reading was made to determine the number of micromoles represented by each division on the fluorometer scale. This value (µmoles NADH/div.) was incorporated into the calculation for µmoles/g of ATP and PCr; µmoles/g = Δ x µmoles NADH/div x TV/SV x DIL/TW, where Δ is the difference in initial and final fluorometer readings (in div.) minus the enzyme blank, where TV is total reaction mixture volume, where SV is the volume of the added extract, where DIL is the dilution of the tissue in the extract and where TW is the weight of the tissue in gms.

Lactate analysis was also conducted with 1 ml of reagent in fluorometer tubes plus 50 µl neutralized $HClO_4$ extract. The reagent was 200 mM Na_2CO_3-$NaHCO_3$, pH 9.7 with 1 mM NAD^+ and 100 mM hydrazine. An initial fluorometer reading was made, lactic dehydrogenase (75 µg/ml) was added and 10 min later the final reading was made. The calculations were the same as those described for ATP and PCr.

Pyruvate analysis was conducted with 1 ml of reagent in fluorometer tubes plus 100 µl neutralized $HClO_4$ extract, which was equivalent

to 6 mg of tissue. The reagent was 100 mM K_2HPO_4-KH_2PO_4, pH 7.0 and 0.001 mM NADH. A fluorometer reading was made, lactic dehydrogenase (0.5 µg/ml) was added and 6 min later, the final reading was made. The calculations were the same as those described for ATP and PCr.

Cardiovascular, Hematological and Metabolic Studies Concerning the Effect of Prostaglandin E_1 on Endotoxemic Dogs

Twenty-eight dogs weighing between 10 and 27 kg were fed ad libitum with Purina Dog Chow through the day before the experiment, and fasted overnight. The dogs were anesthetized with an intravenous administration of 30 mg/kg sodium pentobarbital. The cardiovascular techniques used in this study were described previously (Nakano and McCurdy, 1967). In all experiments, the trachea was exposed through a mid-line incision of the neck and a Y-shaped tube was inserted in the trachea. Then, the left hemithorax was opened under artificial respiration (Palmer respirator pump). The pericardium was incised and the heart was suspended in a pericardial cradle. Before cannulation of blood vessels, sodium heparin (2.5 mg/kg) was administered intravenously and this dose was repeated every half hour. Catheters were placed in the brachiocephalic artery via the left internal mammary artery, in the root of the pulmonary artery via a branch, in the right atrium via a branch of the right external jugular vein and in the left atrium via a pulmonary vein, they were connected to Statham pressure transducers (P23AA) which measured systemic and pulmonary arterial pressures, and right and left atrial pressures, respectively. Heart rate was measured continuously using an Electronics for Medicine tachometer (TDC 1). A Grass polygraph (Model 7) was used to

record all hemodynamic parameters except heart rate.

A control group (6 dogs) underwent all experimental procedures but received no endotoxin and no PGE_1. In 12 dogs an LD_{80} (0.35 mg/kg) of E. coli endotoxin was injected into a femoral vein, and in an additional 10 dogs, the same dose of E. coli endotoxin was given after the continuous i.v. infusion of PGE_1 (1 µg/kg/min) was started into the contralateral femoral vein with a Harvard infusion pump (Model 600-900). Blood samples were taken from the femoral artery 20 and 10 min before endotoxin injection and 0, 5, 10, 20, 40, 60, 90, 120, 180 and 240 min after endotoxin for hematocrit, leucocyte, platelet and pH measurements. Blood samples taken at 0, 20, 60 and 120 min after endotoxin were also analyzed for glucose, lactate, free fatty acid and phospholipid content.

Hematocrit capillary tubes were spun on an International M B centrifuge and they were read in an International Micro-Capillary Reader. Leucocyte and platelet counts were performed in duplicate in a hemocytometer (Improved Neubauer). Blood pH was measured with an Instrument Laboratories meter.

Blood glucose was measured by the method described earlier in the section: Measurement of Selected Metabolites in Endotoxin-Treated Mice.

Blood lactate was measured according to the method of Bergmeyer (1966). Blood (1 ml) was mixed with 6% perchloric acid (1 ml) and centrifuged (International UV) at 2000 x g for 15 min to deproteinate the sample. Glycine-hydrazine buffer (2 ml), sample supernatant (0.1 ml), 0.05 M NAD^+ (0.2 ml) and 5 mg/ml LDH (25 µl)were mixed and incubated for 1 hour at room temperature. Optical density of the sample mixture was then measured at 340 nm in a spectrophotometer (Hitachi, type S_{12}-B).

Reagent blank readings were made on an identical mixture, except 6% perchloric acid (0.1 ml) was substituted for the sample. Plasma lactate concentration was calculated in the following way:

$(E_S - E_B) \times F =$ mg/ml in blood, where E_S is the optical density reading of the sample reagent, E_B is the optical density reading of the blank reagent and F is the standard factor.

Plasma free fatty acid concentration was determined by the method of Laurell and Tibbling (1967). An aliquot (200 µl) of plasma was pipetted into a 16 x 125 mm glass tube in duplicate. CHM solution (8 ml) and activated (120°C overnight) silicic acid (250 mg) were added to the tubes and the contents were mixed and centrifuged (International UV) at 2000 x g for 5 min. An aliquot (5 ml) of the supernatant was transferred to 20 x 125 mm glass tubes and Cu-TEA solution (2 ml) was added. The tubes were shaken for 10 min and 3 ml from the upper layer was transferred to 15 x 85 mm tubes. DPC solution (0.5 ml) was added and mixed. After 15 min incubation at room temperature, the sample optical density was read at 550 nm in a spectrophotometer (Hitachi, type S_{12}-B). Standard free fatty acid solutions (1.5, 1.0 and 0.5 mEq/L) and distilled water blank solutions were also treated in the same way. Plasma free fatty acid concentration was calculated in the following way:

$(E_S - E_B) \times \text{Std.} / (E_{Std} - E_B) =$ mEq/L free fatty acids, where E_S is the optical density of the sample-containing solution, E_B is the optical density of the distilled water blank solution, Std. is the concentration in mEq/L of the free fatty acid standard and E_{Std} is the optical density of the free fatty acid standard solution.

Prior to the phosphorous determination, plasma lipids were extracted

according to the method of Folch et al. (1957). Plasma (1 ml) was mixed with an aliquot (19 ml) of Folch solution (chloroform-methanol, 2:1). After standing for 30 min., this mixture was filtered with lipid-free Whatman #1 paper into a 20 x 125 mm glass tube, to which was also added 0.5% NaCl solution (5 ml). The mixture was shaken for 10 min and centrifuged (International UV) at 2000 x g for 15 min. The upper (aqueous) layer was discarded and the remainder was evaporated with a stream of nitrogen while the tubes were heated (70°C) in a water bath. Chloroform (0.5 ml) was added to the plasma extract. This plasma lipid solution was then analyzed for inorganic phosphorous according to the method of Gerlach and Deuticke (1963). Aliquots (100 µl) of the sample were pipetted into Pyrex (No. 9820) test tubes and 0.7 ml of ashing reagent (H_2SO_4 - 60% perchloric acid, 1:1) was added. The tubes were heated on a hot plate for 1.5-2 hours or until the samples were charred. Then, 0.2 ml Fisher reducing reagent (100 g in 100 ml H_2O mix and filter with Whatman #3 paper) and 1% ammonium molybdate solution (4 ml) were added and the tubes were placed in boiling water for 5 min. The samples were next centrifuged at 2000 x g for 30 min. The supernatant (2.5 ml) was pipetted into the cuvette and the optical density was read at 660 nm in a spectrophotometer (Hitachi, type S_{12}-B). Water was used as a blank and a phosphorous stock solution (1 mg Pi/ml) containing 4.394 g KH_2HPO_4 per liter was used as a standard. Plasma phospholipid was calculated as follows: $(E_S - E_B) \times Std/(E_{Std} - E_B) \times H = \mu g/ml$, where E_S is the optical density of the sample solution, E_B is the optical density of the blank, where Std is the concentration of the standard solution, E_{Std} is the optical density of the standard solution, and where H is the dilution factor.

Studies Involving the Metabolic Degradation of PGE_1 in the Lung of Endotoxemic Rats

Twelve male Holtzman rats weighing between 200 and 250 g, which previously had been fed *ad libitum*, were fasted overnight. A LD80 dose (10 mg/kg) of *E. coli* endotoxin was given intraperitoneally to 4 rats, the same dose of endotoxin was given to an additional 4 rats 1 hour after injection of methylprednisolone (50 mg/kg, s.c.). In yet another group of 4 rats, which served as controls, 0.9% NaCl solution was injected intraperitoneally in a volume equivalent to an endotoxin injection. Eight hours after the injection of either endotoxin or 0.9% NaCl solution, the rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and systemic arterial pressure was measured in a femoral artery cannulated with PE 90 polyethelene tubing which was connected to a Statham pressure transducer (P23AA). The recording was made on a Grass polygraph (Model 7). Systemic arterial pressure of control rats was 121 ± 4 and of endotoxemic rats, 52 ± 6 mm Hg. The lungs were then excised and cooled immediately, and homogenized at 40°C in 9 volumes of Bücher medium (20 mM KH_2PO_4, 70 mM K_2HPO_4, 27.6 mM nicotinamide, 3.6 mM $MgCl_2$, pH 7.4) with a Potter-Elvehjem tissue grinder. The homogenates were centrifuged (Sorvall RC 2) at 10,000 x g for 20 min. The protein concentration of the supernatant was determined by the method described by Lowry *et al.* (1951) and averaged 12.1 ± 0.2 mg/ml. After 25 mμc/ml of ^3H-PGE_1 (28 c/mmole), 50 ng/ml of PGE_1 and 2 mM of NAD^+ were added, the supernatant was incubated at 37°C in a Dubnoff temperature-controlled water bath shaker. Before, and 5 and 15 min after the incubation was started, an aliquot (5 ml) of the incubation media was pipetted into tubes

containing 0.5 ml of 2 N HCl solution to terminate the reaction and to acidify the mixture to pH 3.0. ^{3}H-PGE_1 and its metabolites were extracted twice with ethyl acetate. The extract was filtered through a Whatman No. 3 paper and washed with distilled water. After evaporation at 40°C under reduced pressure, the extract was dissolved in 1 ml of 95% ethanol. An aliquot (100 µl) of the extract solution and 10 µl of PGE_1 standard solution (5 mg/ml) were applied on an activated (100°C for 1 hr) Silica gel G thin layer chromatography plate as shown in Figure 2. ^{3}H-PGE_1 and its metabolites were separated at room temperature using a solvent system (upper layer) of ethyl acetate-isooctane-acetic acid-water (100:20:10:110, v/v). After drying at room temperature, a PGE_1 spot was identified by brief exposure to iodine vapor, and scraped into a counting vial. After dissolving with 2 ml of methanol, 15 ml of counting solution containing POPOP, PPO and toluene (0.1:4:1000) was added. Counts per minute of the samples were measured with a Packard Tricarb liquid scintillation spectrometer (3000 series).

Data

The data in these studies were evaluated statistically, employing the t test (Snedecor, 1956). P values of less than 0.05 were accepted as indicating a significant difference between compared values.

In Figures 6 through 11, all of the data points in the M-P Groups which did not fall on 2, 4, 8 or 12 hours after endotoxin administration were compared to a data point 1-3 hours before or after it in the Endotoxin Group. In every case, the difference between the compared values was the same or less than that between the particular M-P Group value and the corresponding point on the Endotoxin Group curve.

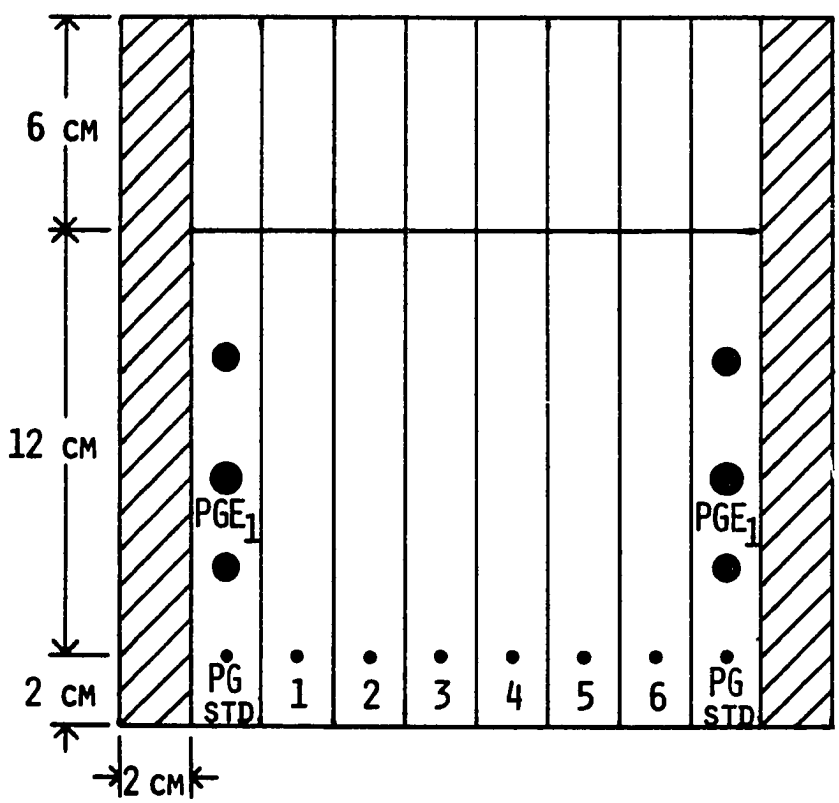


Figure 2. Diagram of Silica Gel G-coated thin layer chromatography plate, showing location of reference prostaglandin standard (PG std) and sample applications (1-6). Reference standard PGE_1, PGA (distal to PGE_1) and $PGF_{2\alpha}$ (proximal to PGE_1), as seen after development, are shown above the PG std application. The solvent front limit during plate development is the line 14 cm above the base of the plate. RF values are: PGA_1, 0.62; PGE_1, 0.32; $PGF_{2\alpha}$, 0.25.

CHAPTER III

RESULTS

Effect of Methylprednisolone on the Lethality of Endotoxemic Mice

The lethality of various doses of *E. coli* endotoxin in mice is shown in Fig. 3. The highest dose of endotoxin, 100 mg/kg, resulted in the death of 90% of the mice within 8 hours. The 10 mg/kg dose resulted in 58% lethality at 24 hours, while the 30 mg/kg dose accounted for 78% lethality at that time. Forty-eight hours after endotoxin, 60% of the mice had died in the 10 mg/kg group (LD_{60}) and 87% had died in the 30 mg/kg group. The 10 mg/kg and 30 mg/kg doses of endotoxin were used in subsequent studies because of their relatively well controlled lethality.

As shown in Fig. 4, mice which received methylprednisolone 1 hour before *E. coli* endotoxin (30 mg/kg, i.p.) survived in greater numbers than those which received only endotoxin. Twenty-four hours after endotoxin all methylprednisolone-treated groups had a higher survival percentage than the endotoxin alone group. Forty-eight hours after endotoxin the endotoxin alone group had 13% survival, while a 6 mg/kg dose of methylprednisolone resulted in 29.5% survival. The 150 mg/kg dose resulted in 56.2% survival. Thirty mg/kg methylprednisolone was therefore considered a good protection against the lethality of endotoxin in mice.

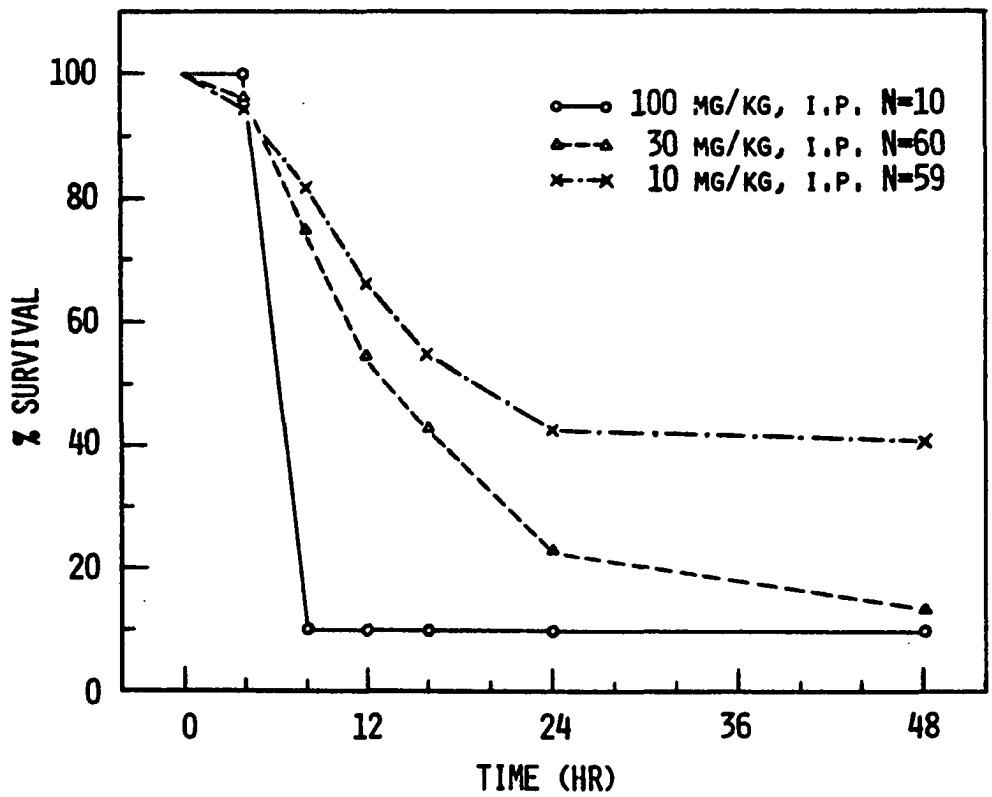


Figure 3. Lethality in mice produced by administration of E. coli endotoxin in varying doses. Each point describes the percentage of the N value alive at that time after injection. N = total number of mice in each group.

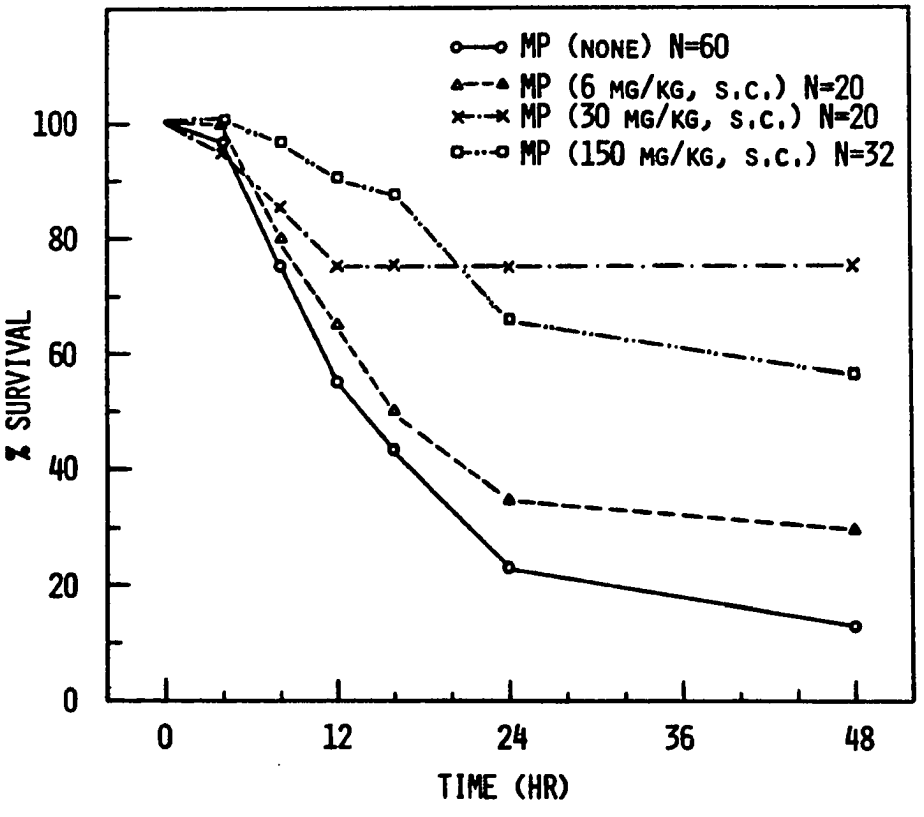


Figure 4. Effect of methylprednisolone (MP) on survival of mice given E. coli endotoxin (30 mg/kg, i.p.). Group MP (none) received only endotoxin, all other groups received methylprednisolone 1 hour before endotoxin. Each point describes the percentage of the N value alive at that time after injection. N = total number of mice in each group.

Methylprednisolone was also shown in Fig. 5 to enhance the survival of mice when administered before, simultaneously with or after a 10 mg/kg dose of E. coli endotoxin. The dark area represents the percentage of mice which survived in a group which received endotoxin alone. All other groups received endotoxin at 0 hour and methylprednisolone at 0 hour or some other time as indicated. The 150 mg/kg dose of methylprednisolone excerted beneficial effects when given 1 hour before endotoxin or at the same time as endotoxin. However, there appeared to be no beneficial effect of the high dose of methylprednisolone when it was given after endotoxin. The 30 mg/kg dose of methylprednisolone apparently had a beneficial effect before or after endotoxin. When this dose of methylprednisolone was injected into the mice one hour before endotoxin or two hours after endotoxin, survival was increased 24% over the endotoxin alone group. When given one hour or four hours after endotoxin, the drug increased survival 40% as compared to the endotoxin alone group. A X^2 one-way classification was used to evaluate this data. The groups which received 30 mg/kg of methylprednisolone 1 hour, 2 hours and 4 hours after endotoxin had survival values which were significantly ($p<0.05$) higher than that of the endotoxin alone group.

Effects of Methylprednisolone on Metabolic Responses to Endotoxin in Mice

Blood Glucose

The effect of methylprednisolone on blood glucose concentration in endotoxemic mice is shown in Fig. 6. Blood glucose values in endotoxin-treated mice were significantly lower than in control mice throughout the

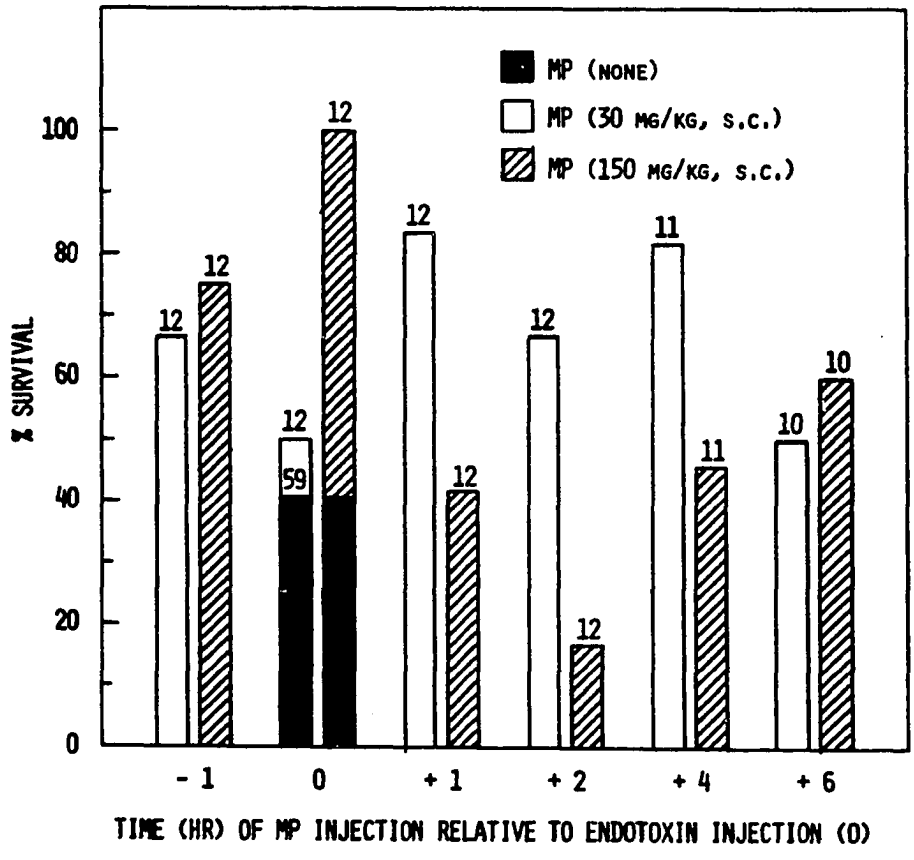


Figure 5. Effect of methylprednisolone (MP) on survival of mice when given before, simultaneously with, and after E. coli endotoxin (10 mg/kg, i.p.). Group MP (none) received only endotoxin, and the other group received the appropriate dose of methylprednisolone in addition to endotoxin at the times relative to endotoxin injection shown. The N for each group is shown on each bar.

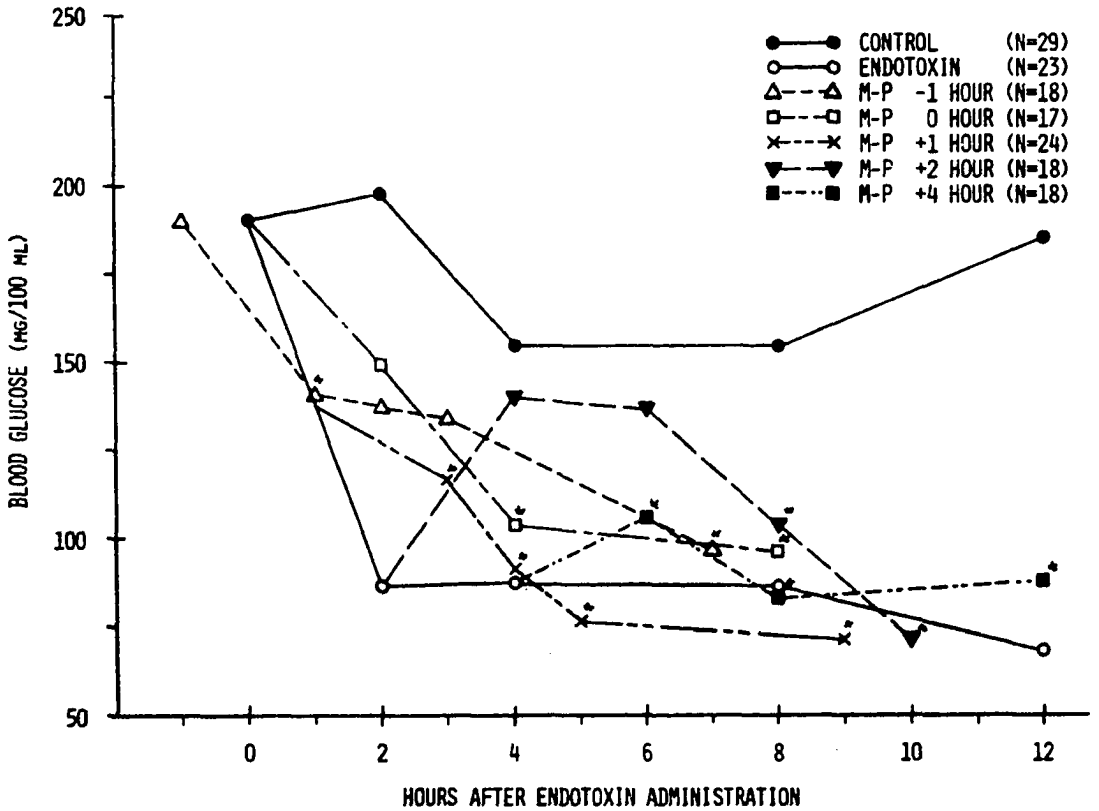


Figure 6. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on blood glucose concentration in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. Glucose concentration was described as mg glucose/100 ml plasma. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals in each group.

*All values except those marked with an asterisk are significantly ($p<0.05$) different from the Endotoxin group values.

12 hour experimental period. When methylprednisolone was given one hour before or simultaneously with endotoxin, blood glucose levels remained significantly higher than those of the endotoxin group for 2 hours after endotoxin injection. A dramatic elevation of blood glucose content was seen when methylprednisolone was administered to mice which had received endotoxin two hours earlier. In this group, blood glucose rose to 141.4 ± 8.00 mg/100 ml as compared to 87.6 ± 6.20 mg/100 ml in the endotoxin group. Two remaining groups did not show significant changes in blood glucose content following methylprednisolone, and ultimately the blood glucose in all groups except control gradually declined to the endotoxin group level.

Heart and Liver Glycogen

Heart glycogen content in endotoxemic mice (Fig. 7) was significantly below control levels at all times over the 12 hours, except 8 hours after endotoxin administration, when the control and endotoxin values overlapped. In each methylprednisolone group, the drug caused an increase in myocardial glycogen content which was significantly above the level of the group which received endotoxin only. This effect lasted from 4 to 8 hours after the injection of methylprednisolone. The greatest effect was seen in mice injected with methylprednisolone 1 hour before endotoxin, where myocardial glycogen at 3 hours after endotoxin rose to 3.165 ± 0.149 mg/g as compared to 2.329 ± 0.074 mg/g in control and 1.286 ± 0.180 mg/g in endotoxin alone groups. Eight hours after the injection of methylprednisolone, all groups except the +2 hour group had myocardial glycogen values which were significantly elevated above the

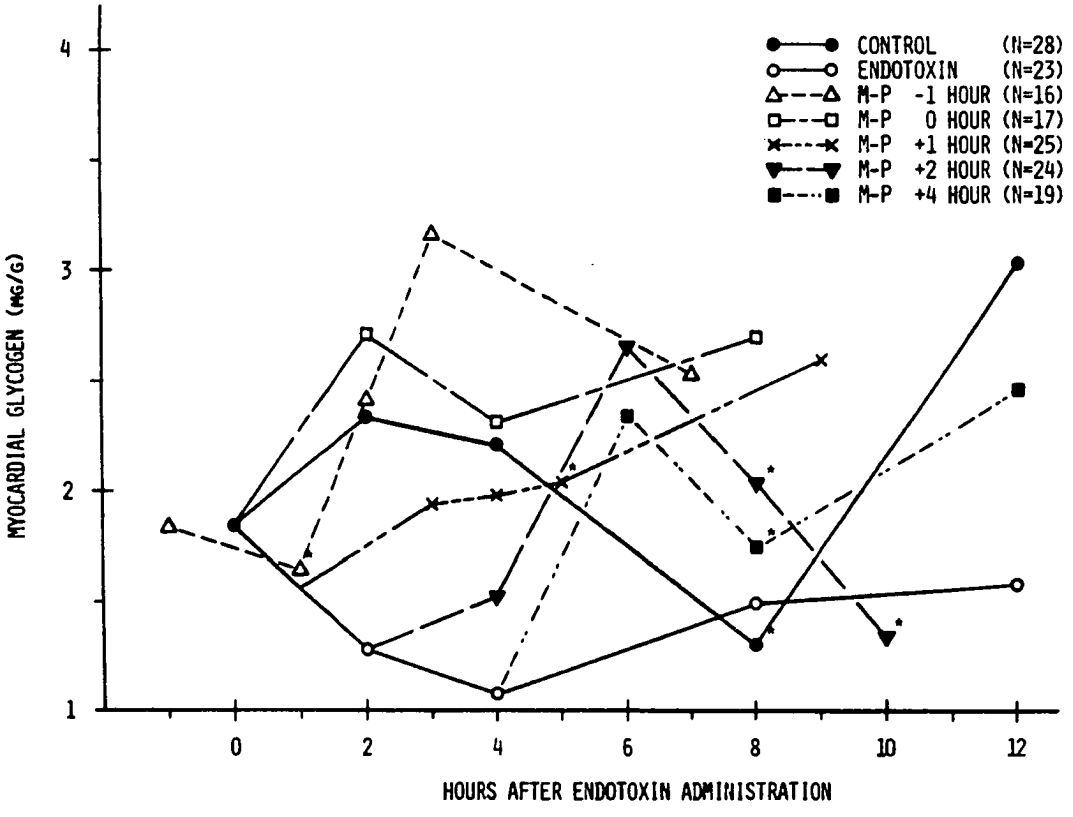


Figure 7. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on myocardial glycogen content in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. Glycogen content was described as mg glucose recovered from glycogen/g tissue. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 Hour. All other groups received endotoxin at 0 Hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals represented in each group.

*All values except those marked with an asterisk are significantly ($p<0.05$) different from the Endotoxin group values.

endotoxin group values. Myocardial glycogen content in the group which received methylprednisolone 2 hours after endotoxin gradually declined to endotoxin group values 8 hours after endotoxin.

As seen in Fig. 8, liver glycogen content in endotoxemic mice fell significantly below that of control animals and remained there for 12 hours. When methylprednisolone was given 1 hour before, simultaneously with, or 1, 2 or 4 hours after endotoxin, liver glycogen values rose significantly above endotoxin group levels for 2-8 hours. In the groups which received methylprednisolone after endotoxin, the liver glycogen values returned to endotoxin group levels 4-8 hours after the injection of the drug. Methylprednisolone had the greatest effect on liver glycogen content when it was given 1 hour before or simultaneously with endotoxin. Liver glycogen content rose to 9.311 ± 0.787 mg/g in the -1 hour group and to 8.568 ± 0.239mg/g in the 0 hour group as compared to 3.101 ± 0.285 mg/g in the endotoxin group. Furthermore, methylprednisolone maintained the liver glycogen values in both groups significantly above endotoxin group levels 8 hours after injection of the drug.

Heart and Liver ATP and Heart PCr

The effect of methylprednisolone on myocardial ATP in endotoxin-treated mice is shown in Fig. 9. Myocardial ATP in control animals fluctuated between 3.067 ± 0.393 µmoles/g and 4.108 ± 0.154 µmoles/g throughout the experimental period. Endotoxin reduced ATP content in the heart to values which were apparently below the sensitivity of the measuring system. When methylprednisolone was given to different groups of mice 1 hour before, simultaneously with or 1, 2 or 4 hours after endotoxin,

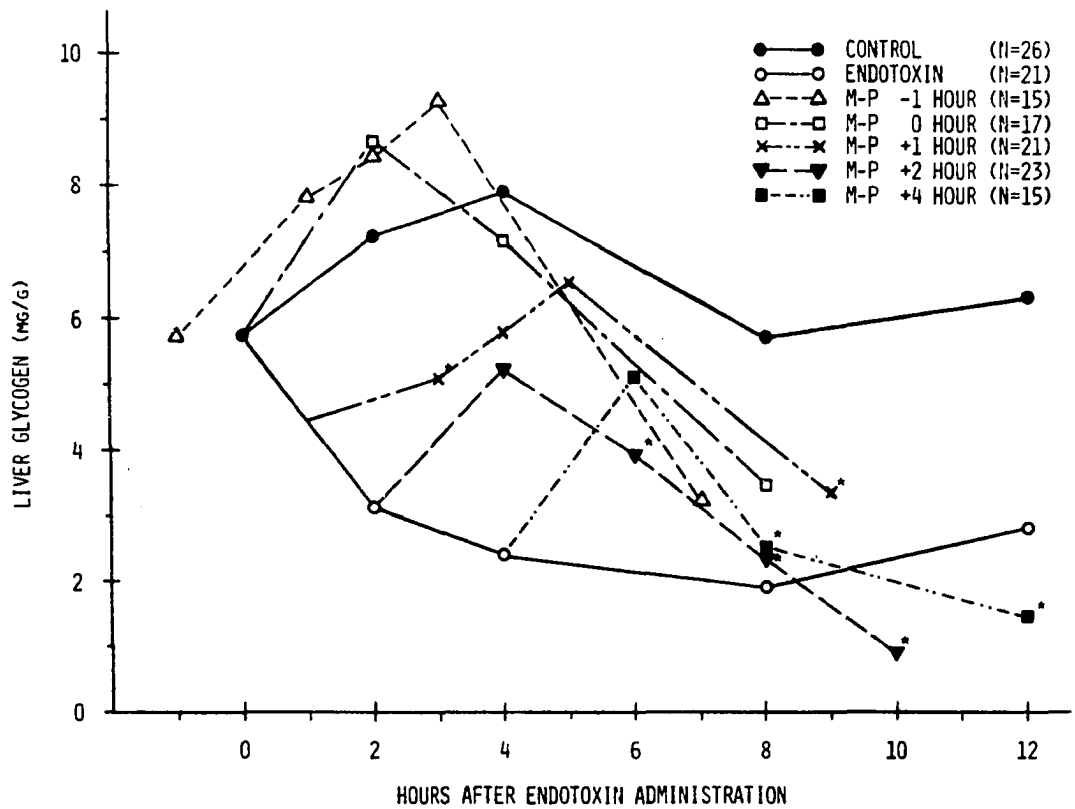


Figure 8. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on liver glycogen content in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. Glycogen content was described as mg glucose recovered from glycogen/g tissue. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals represented by each group.

*All values except those marked with an asterisk are significantly ($p<0.05$) different from the Endotoxin group values.

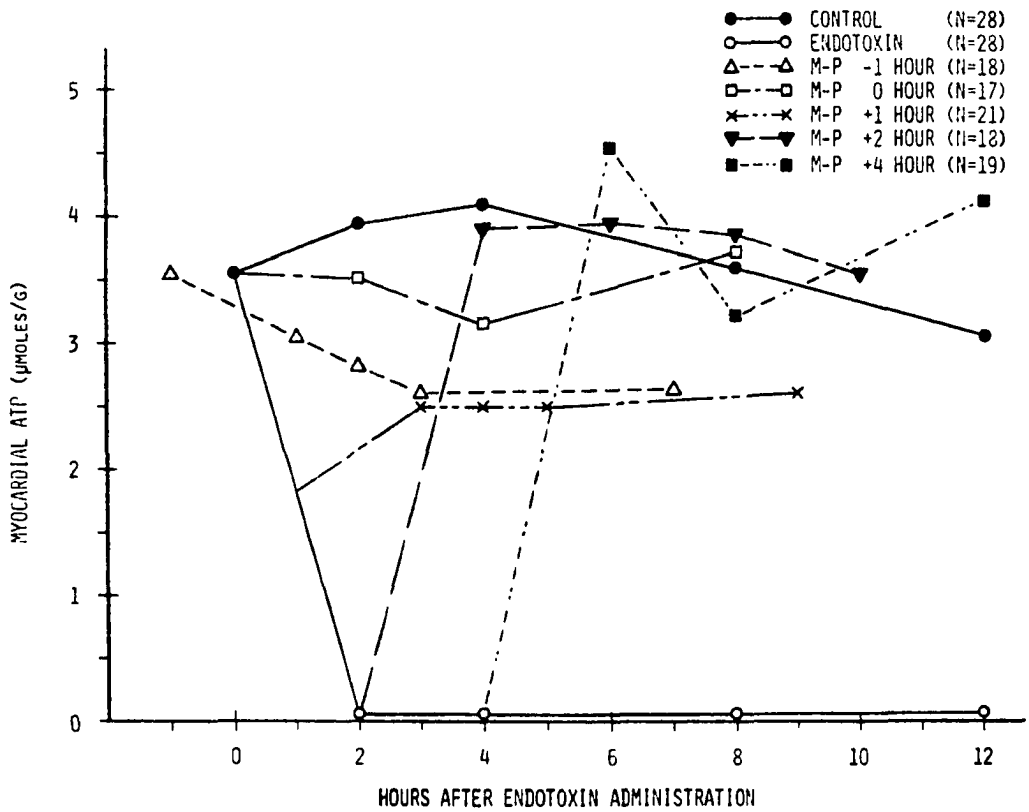


Figure 9. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on myocardial ATP content in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. ATP content was described as μmoles of ATP/g tissue. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals represented by each group. All values which appear 2-12 hours after endotoxin are significantly ($p<0.05$) different from the Endotoxin group values.

it restored myocardial ATP to control levels or to a level which was significantly above the endotoxin level.

Endotoxin also decreased ATP content in the liver (Fig. 10) to values apparently below the sensitivity of the measuring system. The control group values varied between 1.771 ± 0.201 μmoles/g and 2.486 ± 0.200 μmoles/g. As in the myocardium, liver ATP was elevated to the control group level or to a level significantly above that found in the endotoxin group mice, when methylprednisolone was administered before, simultaneously with or after endotoxin.

Fig. 11 shows the effect of methylprednisolone on myocardial PCr content in mice treated with endotoxin. Endotoxin caused myocardial PCr values to drop significantly below those found in the control group (1.542 ± 0.575 μmoles/g to 3.527 ± 0.238 μmoles/g). When methylprednisolone was given before, simultaneously with, or after endotoxin, myocardial PCr values rose significantly above those found in the endotoxin group and these values remained elevated 8 hours after the administration of methylprednisolone in each group.

Heart and Liver Lactate/Pyruvate

The effect of methylprednisolone on the lactate/pyruvate ratio in the heart of endotoxin-treated mice is shown in Fig. 12. During the first four hours after endotoxin, the endotoxin-treated group lactate/pyruvate ratio was higher than that of the control group. This relationship was reversed, however, during the last four hours. When methylprednisolone was given 1 hour before, simultaneously with, or 1, 2 or 4 hours after endotoxin, the lactate/pyruvate ratio decreased 7.8-18.0 units below the endotoxin group level. This change was a result of a decrease in the

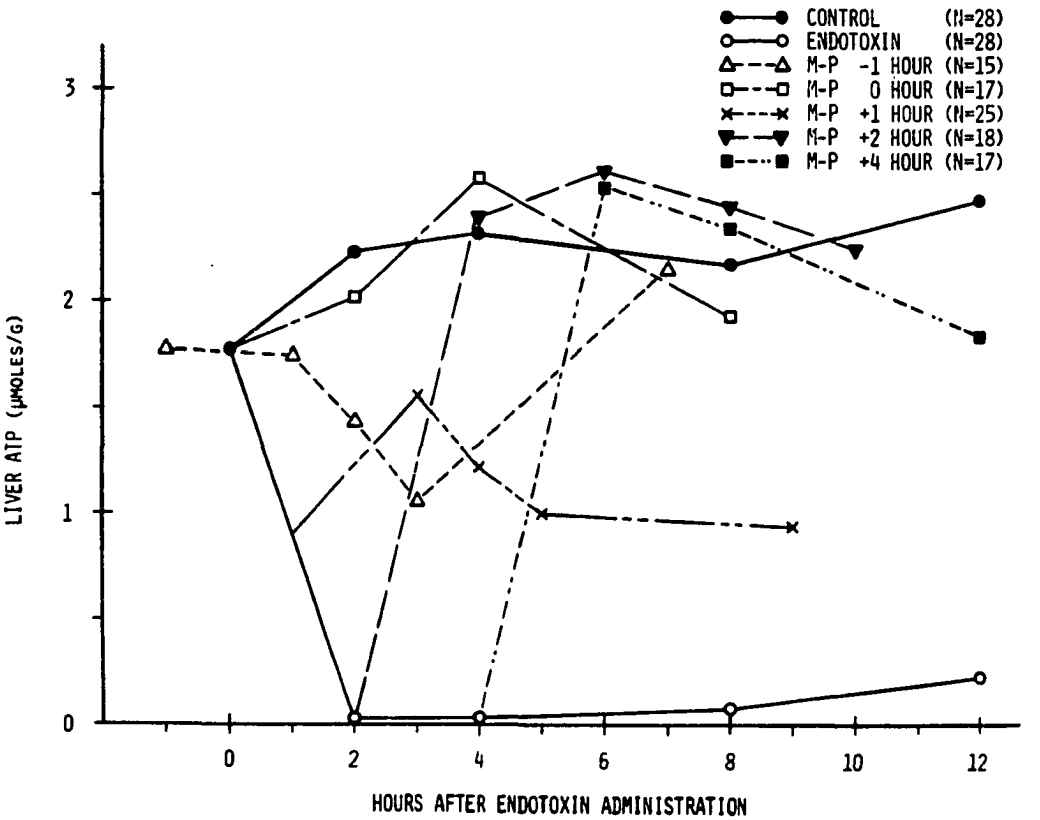


Figure 10. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on liver ATP content in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. ATP content was described as μmoles of ATP/g tissue. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals represented by each group.

All values which appear 2-12 hours after endotoxin are significantly ($p<0.05$) different from the Endotoxin group values.

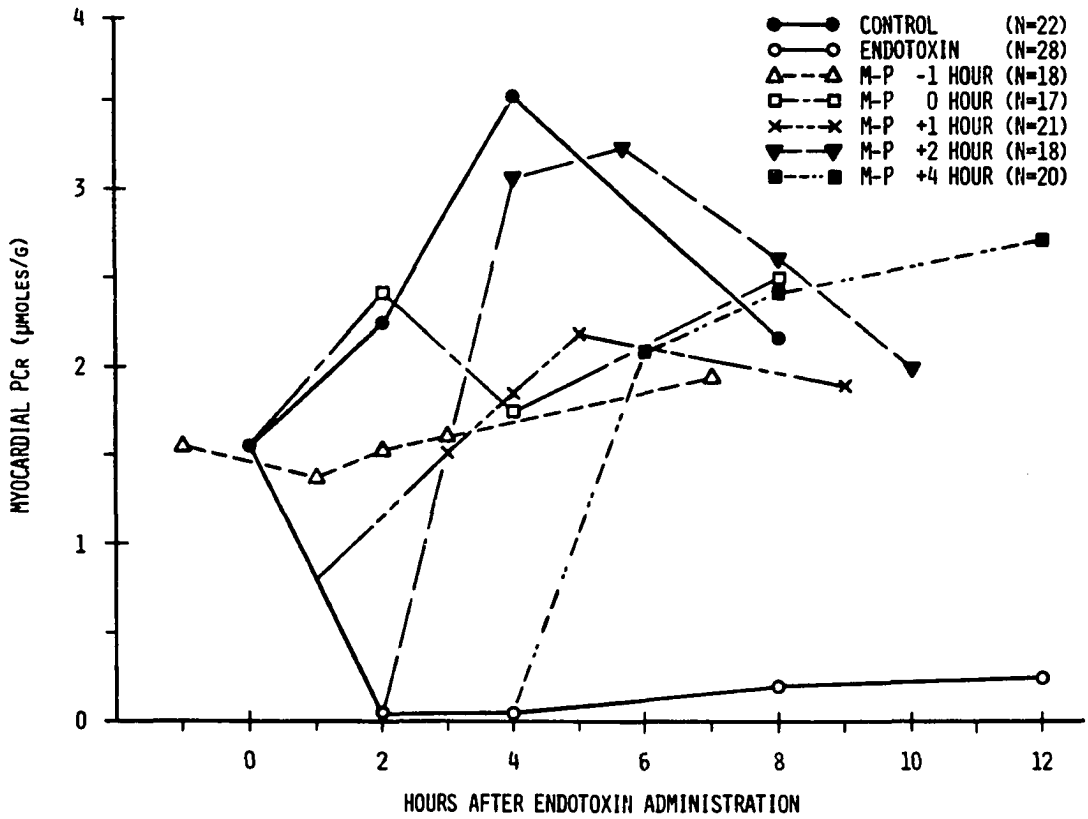


Figure 11. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on myocardial phosphocreatine (PCr) content in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. PCr content was described as µmoles of PCr/g tissue. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. N values equal the total number of animals represented by each group.

All values which appear 2-12 hours after endotoxin are significantly ($p<0.05$) different from the Endotoxin group values.

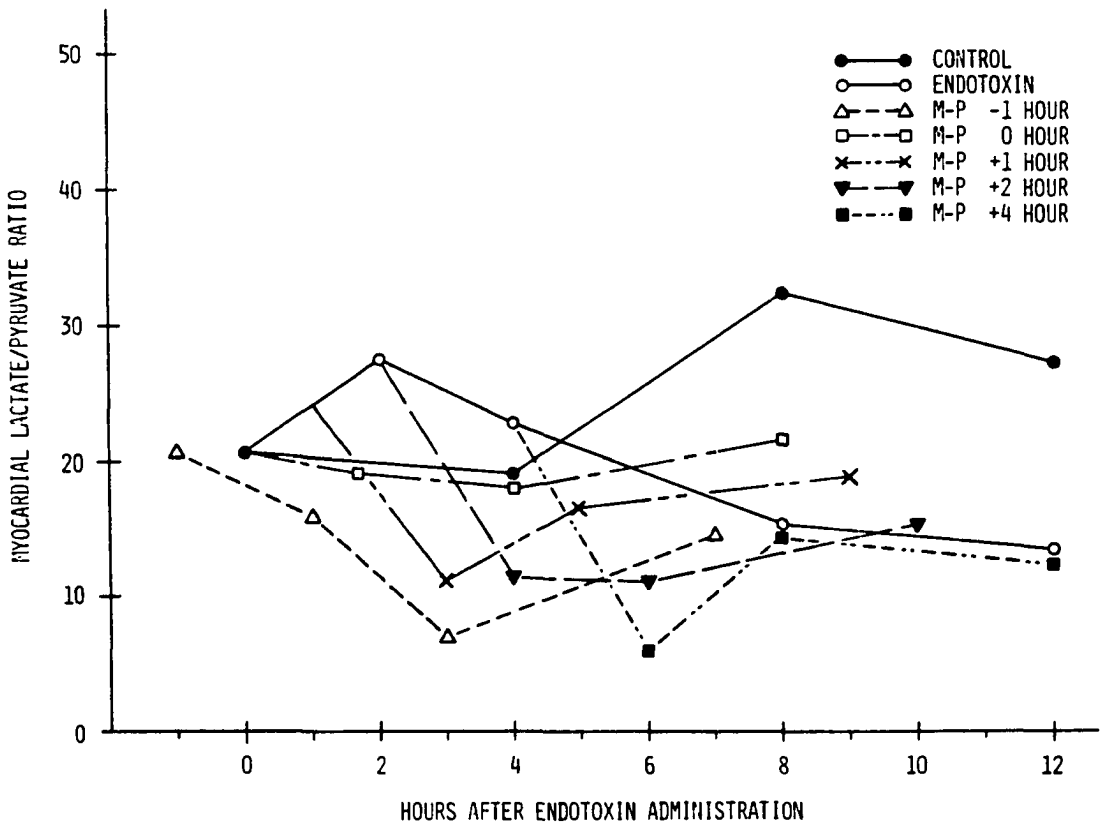


Figure 12, Effect of methylprednisolone (M-P, 30 mg, s.c.) on myocardial lactate/pyruvate ratio in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. Each value represents 5-14 animals.

lactate content of the myocardium after methylprednisolone was given.

In the liver (Fig. 13), a more pronounced change due to endotoxin was seen. The lactate/pyruvate ratio 2 hours after endotoxin was highly elevated in the endotoxin group (39.54) when compared to the control group (7.65). Four hours after endotoxin there was little difference between the two groups and they paralleled each other over the remaining 8 hours. In the group in which methylprednisolone was given 1 hour before endotoxin, the lactate/pyruvate ratio dropped from 27.52 to 6.26 at 3 hours after endotoxin. The lactate/pyruvate ratio decreased from 39.54 to 20.25 in the 0 hour group at 2 hours after endotoxin and from 27.52 to 10.38 in the +1 hour group at 3 hours after endotoxin. The +2 and +4 hour groups demonstrated a smaller decrease in the lactate/pyruvate ratio following methylprednisolone. Lactate/pyruvate ratio in all groups eventually rose to values of 12.06 to 23.30 8 hours after methylprednisolone was given. Control group and endotoxin group lactate/pyruvate ratios, after 12 hours, were 23.36 and 22.13, respectively.

Effects of Prostaglandin E_1 on the Cardiovascular, Hematological and Metabolic Responses to Endotoxin in Dogs

Cardiovascular and Hematological Responses

As seen in Fig. 14, there was no significant effect of PGE_1 on the cardiovascular response to endotoxin at the end of 2 hours. The initial drop in systemic arterial pressure and the rise in pulmonary arterial pressure seen in the PGE_1 + endotoxin group was due to the direct effect of PGE_1 on the vasculature before endotoxin was given. The early rise in heart rate was a reflex change associated with the PGE_1-

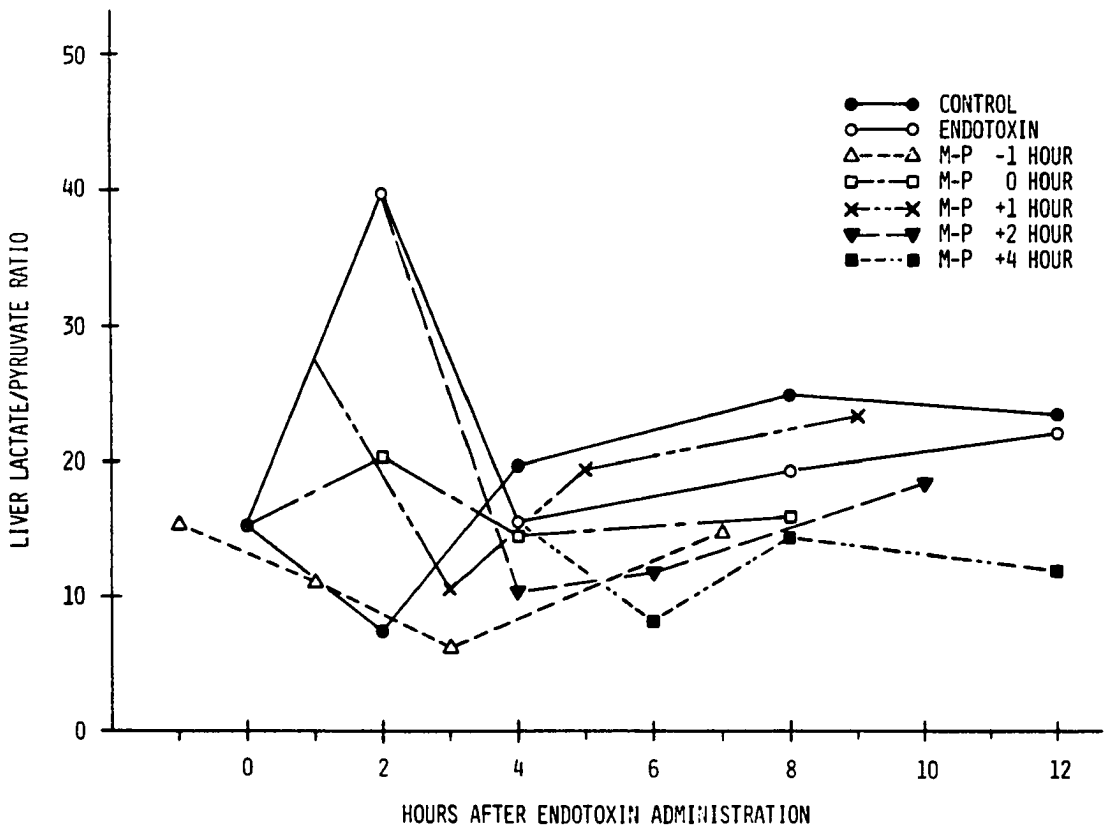


Figure 13. Effect of methylprednisolone (M-P, 30 mg/kg, s.c.) on liver lactate/pyruvate ratio in E. coli endotoxin (10 mg/kg, i.p.)-treated mice. Control groups received only 0.9% NaCl solution (0.5 ml, i.p.) at 0 hour. All other groups received endotoxin at 0 hour, and in addition, M-P groups received methylprednisolone at the designated hour relative to endotoxin injection. Each value represents 5-14 animals.

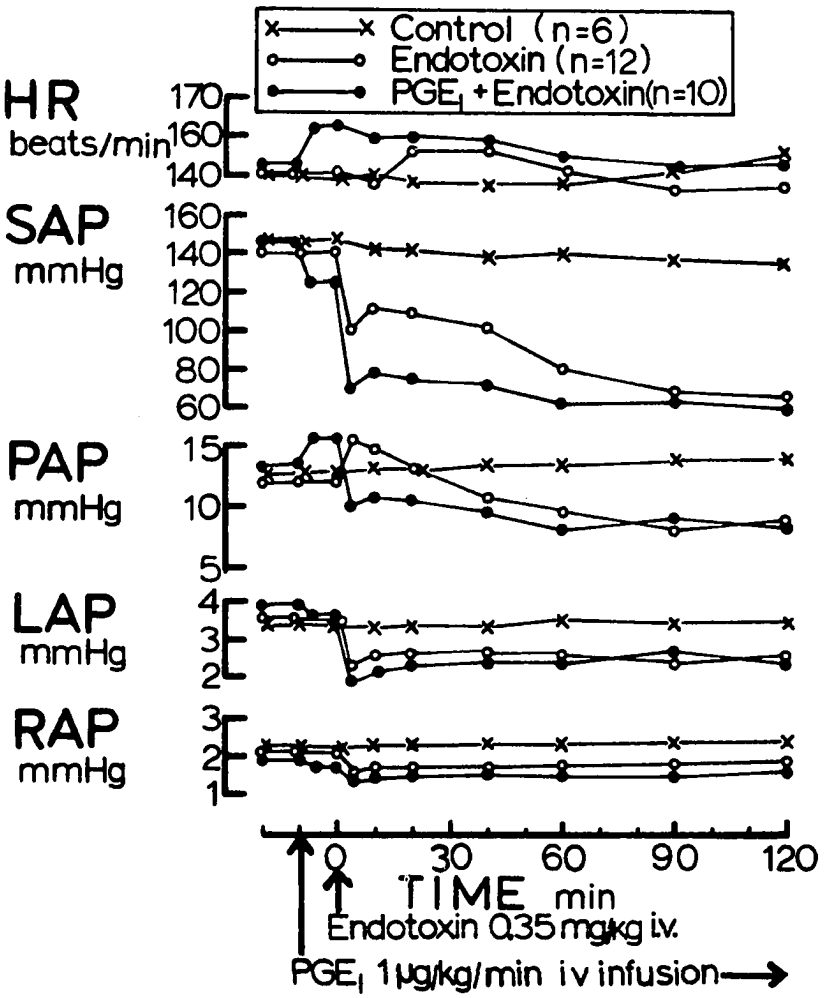


Figure 14. Effect of prostaglandin E_1 on cardiovascular responses to E. coli endotoxin in dogs. The parameters studied were heart rate (HR), systemic arterial pressure (SAP), pulmonary arterial pressure (PAP), left atrial pressure (LAP) and right atrial pressure (RAP). N values equal the total number of animals represented by each experimental group.

induced drop in systemic arterial pressure. Pulmonary arterial pressure increased sharply over control values following endotoxin and then gradually declined to a level below control. PGE_1, however, caused an elevated pulmonary arterial pressure. When endotoxin was given, the pressure dropped below control resulting in an reaction opposite to that seen with endotoxin alone. After 40 min, the PGE_1 + endotoxin and the endotoxin groups were at the same level below control.

PGE_1 infusion had no effect on the hematocrit, platelet count or blood plasma pH in dogs 4 hours after they were treated with endotoxin (Fig. 15). A significant difference was seen in the white blood cell count, however, between the endotoxin-treated animals and those which also received PGE_1.

Metabolic Responses

Blood glucose levels returned to control values in the PGE_1-treated animals 2 hours after endotoxin (Fig. 16). As shown in the same figure, the plasma lactate concentration in prostaglandin E_1-treated dogs was unchanged from the endotoxin group values at the end of 2 hours. Plasma concentration of free fatty acids (Fig. 17) decreased significantly below the control group level 1 hour after endotoxin in the endotoxin group, but there was no change in the concentration of phospholipids in any of the groups throughout the two hours after endotoxin injections.

Effect of Methylprednisolone on the Metabolic Degradation of Prostaglandin E_1 in the Lung of Endotoxemic Rats

In the control animals, which received no endotoxin or drug, 81%

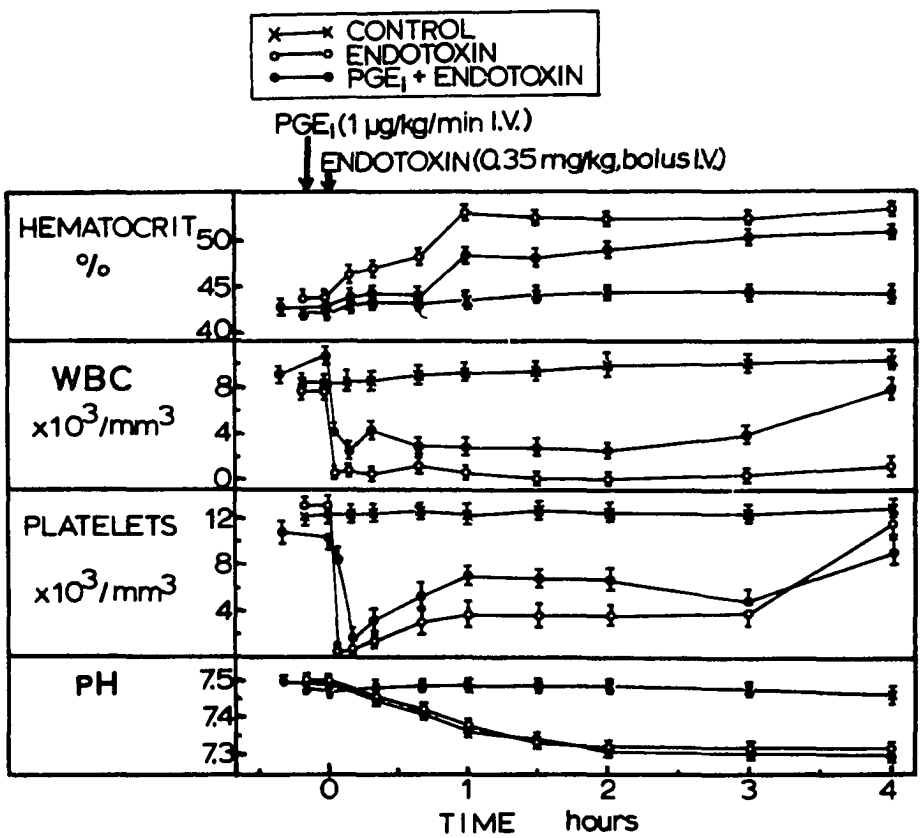


Figure 15. Effect of prostaglandin E1 (PGE_1) on hematological responses to E. coli endotoxin in dogs. White blood cell count is abbreviated WBC. Control N = 6, Endotoxin N = 12 and PGE_1 + Endotoxin N = 10.

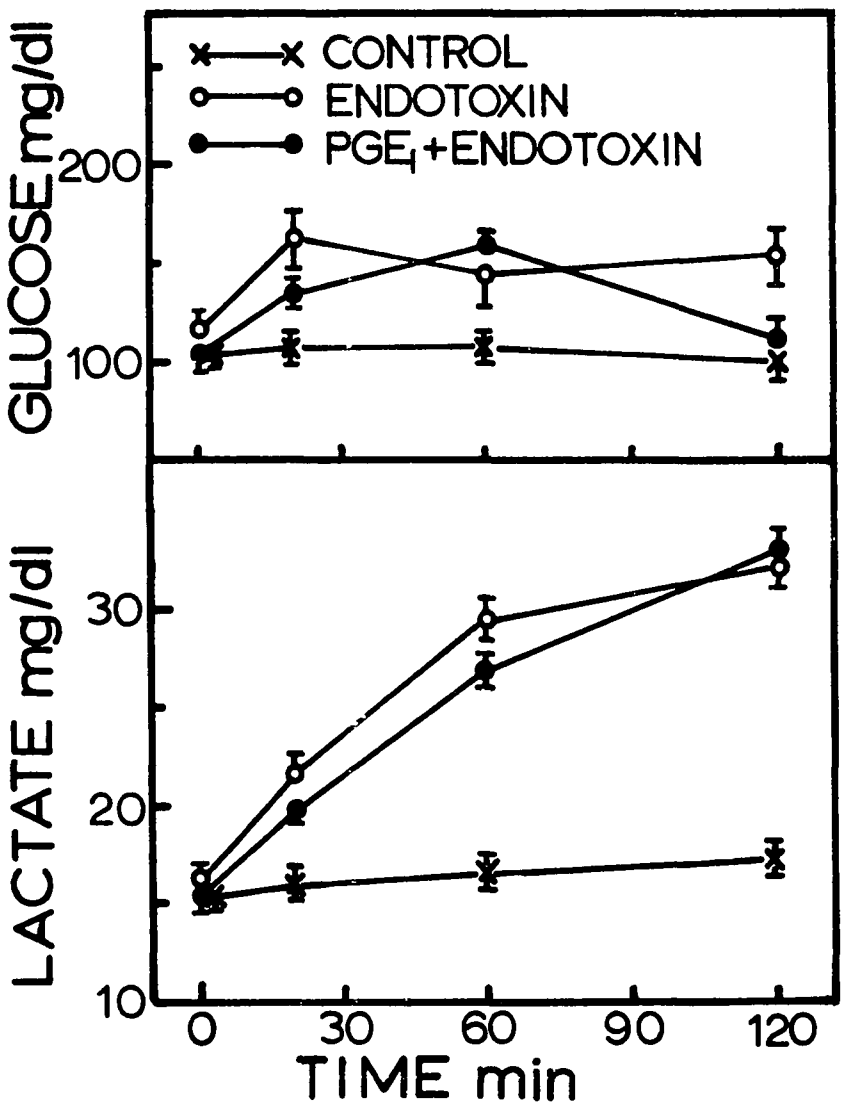


Figure 16. Effect of prostaglandin E_1 (PGE_1) infusion (1 µg/kg/min., i.v.) on blood lactate and glucose concentration in dogs treated with *E. coli* endotoxin (0.35 mg/kg, bolus, I.V.) 20 min after PGE_1 infusion was started. Control N = 6, Endotoxin N = 12 Endotoxin + PGE_1 N = 10. Lactate and glucose concentrations were described as mg lactate or glucose/100 ml blood.

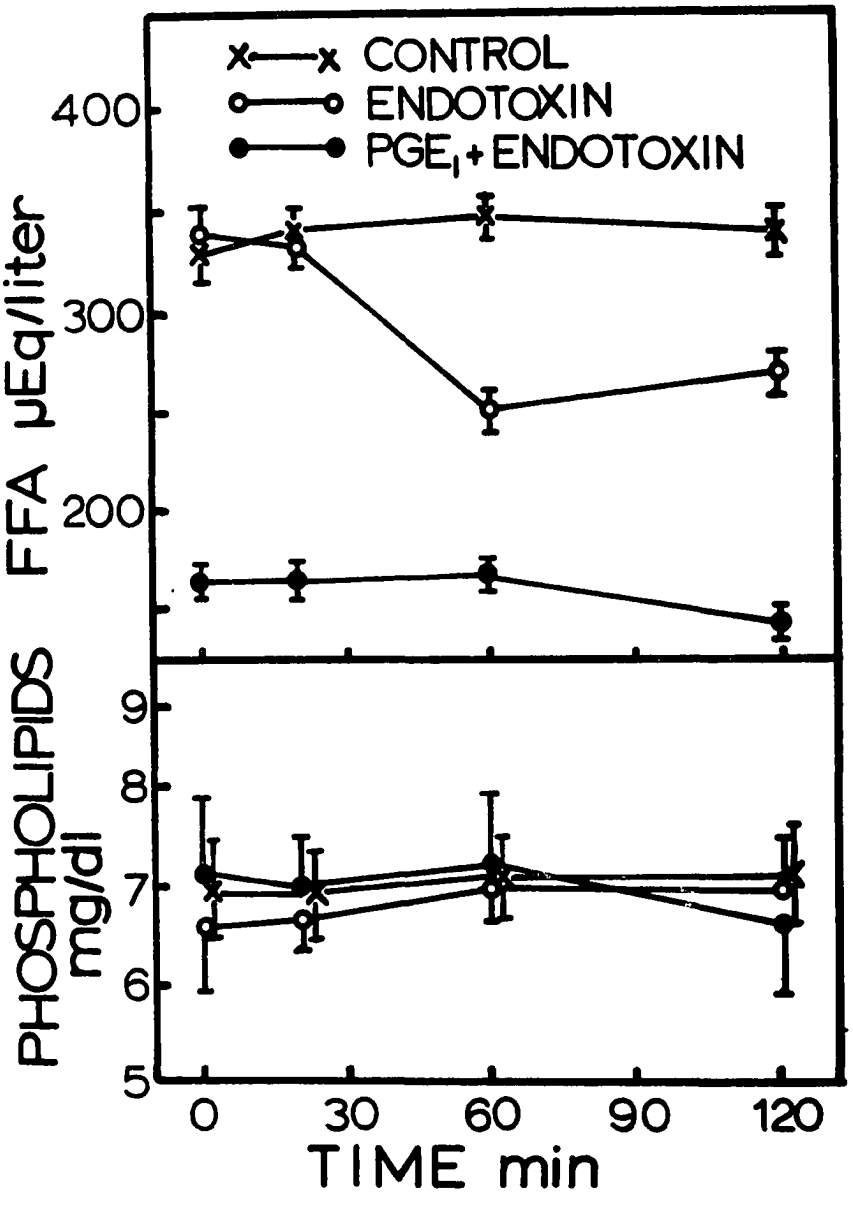


Figure 17. Effect of prostaglandin E_1 (PGE_1) infusion (1 µg/kg/min., i.v.) on blood free fatty acid (FFA) and phospholipid concentration in dogs treated with *E. coli* endotoxin (0.35 mg/kg, bolus, i.v.) 20 min after PGE_1 infusion was started. Control N = 6, Endotoxin N = 12 and Endotoxin + PGE_1 N = 10. Phospholipid concentration was described as mg phospholipid/10 ml blood and FFA concentration was described as µequivalents fatty acids/liter blood.

of the ^{3}H-PGE$_{1}$ applied to the incubation medium was destroyed by prostaglandin dehydrogenase within 5 min and 88% was destroyed within 15 min (Fig. 18). In animals treated with endotoxin, this process was impaired and only 20% of the ^{3}H-PGE$_{1}$ was metabolized in 5 min and 26% was metabolized by 15 min. When methylprednisolone was given to rats 1 hour before endotoxin, the amount of ^{3}H-PGE$_{1}$ inactivated in the lung homogenate increased significantly to 50% at 5 min and to 63% at 15 min.

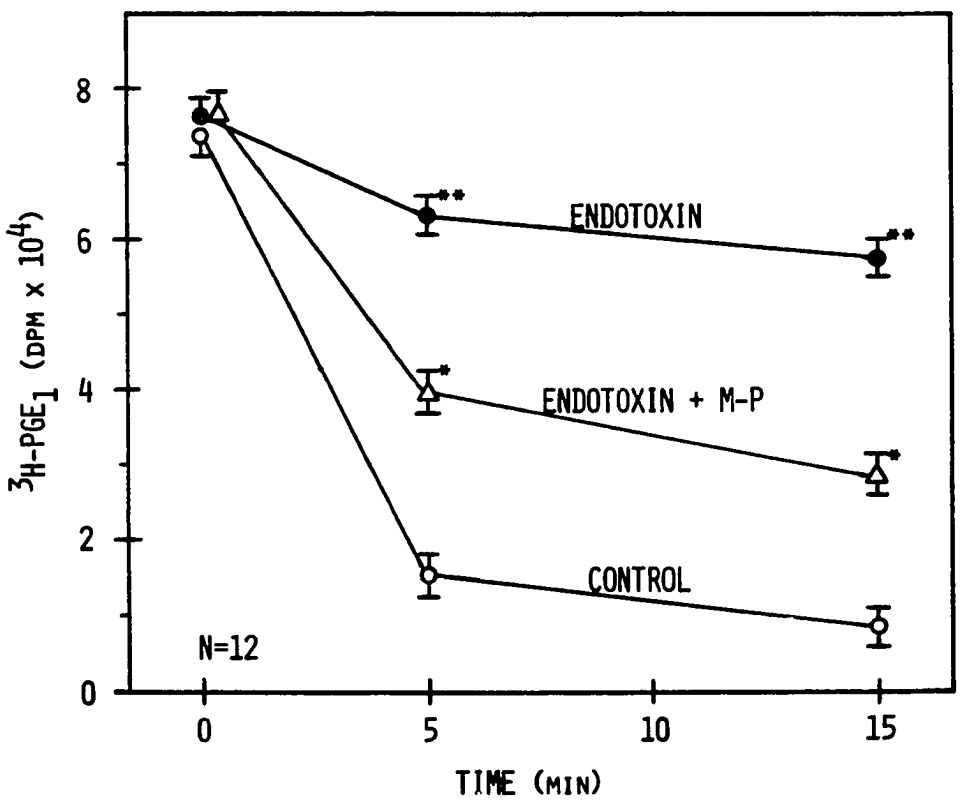


Figure 18. Effect of methylprednisolone (50 mg/kg, s.c.) on degradation of ^{3}H prostaglandin E$_1$ (^{3}H-PGE$_1$) in lungs *in vitro* of *E. coli* endotoxin (10 mg/kg, i.p.)-treated rats. Amount of ^{3}H-PGE$_1$ was measured before incubation was begun and 5 and 15 min after its start. Each group represented 4 rats.

*$p<0.01$

**$p<0.001$

CHAPTER IV

DISCUSSION

The studies reported in this dissertation were undertaken to examine the capabilities of methylprednisolone sodium succinate and prostaglandin E_1 to prevent the onset of endotoxin shock and to change its course. A new endotoxin shock animal model, the mouse, was developed to study the effects of methylprednisolone on lethality and on blood and tissue levels of selected metabolites after endotoxin. It was necessary initially to establish the lethality of the E. coli endotoxin in the mice. In order to evaluate the pharmacological efficacy of methylprednisolone, survival studies were conducted wherein the drug was administered at various times before and after endotoxin. Closer evaluation of these effects was accomplished by measuring selected metabolites during the course of endotoxin shock and its treatment. The tissue treatment procedures and the metabolite assays were standard methods adapted from the literature. The effect of prostaglandin E_1 on endotoxin shock was tested on the classical hemodynamic and metabolic responses of the dog to endotoxin. A closer look at the interactions of prostaglandin E_1 and endotoxin was accomplished by indirectly measuring the relative prostaglandin dehydrogenase (PGDH) activity in the lung following endotoxin and methylprednisolone administration.

Effect of Methylprednisolone on the Lethality of Endotoxemic Mice

A consistent percentage of lethality was found among a large number of mice when E. coli endotoxin was administered (Fig. 3). Endotoxin (10 mg/kg, i.p.) produced a 60% lethality (LD_{60}) in which most animals died between 6 and 24 hours after endotoxin administration. This dose of endotoxin in mice will provide a useful control for studying the protective and therapeutic value of drugs such as methylprednisolone (Fig. 5). Further, it will provide a good model for studying the mechanism of irreversible endotoxin shock as well as the reversal of such shock states. A higher dose of endotoxin (30 mg/kg, i.p.) produced a greater lethality in mice (87%), as seen in Fig. 3. However, pretreatment with methylprednisolone (30 mg/kg, s.c. and 150 mg/kg, s.c.) resulted in a dramatic protection from the lethal effects of this dose of endotoxin (Fig. 4). A 100 mg/kg dose of endotoxin appeared to be too large a dose to be useful in therapeutic studies.

Methylprednisolone had a marked protective effect on mice when given 1 hour before E. coli endotoxin or simultaneously with E. coli endotoxin (Fig. 4 and 5). After endotoxin, the smaller dose of methylprednisolone (30 mg/kg, s.c.) improved the survival rate of endotoxemic mice. The larger dose of methylprednisolone (150 mg/kg, s.c.), however, had no effect on survival when given 1 and 4 hours after endotoxin, and it actually decreased survival when given 2 hours after endotoxin. The reason for this effect is unclear, but it seems to be related to the stage of shock during which the drug is administered.

Previously, several workers reported the protective effect of glucocorticoids in septic shock. In earlier work with dogs, pretreatment

with cortisol in large doses had favorable effects on mortality of the animals (Lillehei and MacLean, 1959). Similar results were also reported with cats and mice (Kadowitz and Yard, 1970; Berry et al., 1959). Hinshaw and co-workers (1967) found a slightly higher percentage of survival in dogs when methylprednisolone (20 mg/kg, i.v.) was given 15 min after an LD_{80} dose of endotoxin. Thomas and Brockman (1968) also reported a protective effect of methylprednisolone in endotoxin-treated dogs. They did not find enhanced survival when methylprednisolone treatment was started after endotoxin, however. The result of pretreatment with methylprednisolone on the mortality of endotoxemic mice as it is reported in the present study is in agreement with earlier work by other investigators. Since the results of the current study also showed a marked therapeutic effect of methylprednisolone on endotoxemic mice, this work also adds support to reports by other investigators of a significant therapeutic effect of prednisolone in mice (Weil, 1962) of hydrocortisone in patients (Weil et al., 1964; Rigby and Christy, 1968) and especially of methylprednisolone in patients (Motsay et al., 1970, 1972). However, precise evaluation of the therapeutic effects of methylprednisolone cannot be carried out in patients.

Usually, adult mongrel dogs or primates are employed for determining the pharmacological efficacy of a drug in endotoxin shock, but a problem exists there too. Dogs, especially, have been found to be an unreliable animal model for endotoxin shock studies as reported by Fine (1966). In the studies he reported, which involved several groups of investigators working on related problems, the survival of 9 groups of endotoxin-treated dogs ranged from 0% to 50%. Despite careful control

studies, it was difficult to define LD_{50} or LD_{80} doses of a single batch of endotoxin due to the variability of individual dogs and of different breeds of dogs. In order to extensively study a problem, it is necessary to use animals which exhibit a consistent response to the same condition. In the present study, mice appear to be considerably more consistent in their response to endotoxin than dogs and, therefore, they may become a valuable experimental shock model for survival and metabolic studies.

Effects of Methylprednisolone on Metabolic Responses To Endotoxin in Mice

A sharp decrease in blood glucose concentration following endotoxin in mice was shown in this study (Fig. 6). Early work with hemorrhagic shock (Bernard, 1877) revealed a hyperglycemia as did experiments with bacterial suspensions to produce shock in rabbits (Menten and Manning, 1924 and Zeckwer and Goodell, 1925). Delafield (1932), however, also administering bacterial suspensions to rabbits, reported a prompt fall of blood glucose to hypoglycemic levels. Finally, Wiggers (1950) stressed the importance of identifying the phases or stages an animal passes through in every instance of fatal shock. This is important in order to relate the hemodynamic and biochemical changes which occur as shock progresses. Recent work has shown a hyperglycemic response to endotoxin (Rangel *et al.*, 1970), hemorrhage (Coran *et al.*, 1971) and trauma (Carey *et al.*, 1970). However, when Wiggers' considerations are applied, it is apparent that the hyperglycemia in each case is either mild or temporary. Other investigators have described more clearly the initial hyperglycemia followed by a profound hypoglycemia which characterizes

endotoxin shock (Berk et al., 1970; Williamson et al., 1970), hemorrhagic shock (Cowley et al., 1960; Halmagyi et al., 1966; Moffat et al., 1968; Bauer et al., 1969, b; Beck et al., 1970) and tourniquet shock (Stoner and Threfall, 1960). The present work agrees with this general picture of hypoglycemia in the advanced stage of shock.

The initial increase in blood glucose concentration has been primarily attributed to hepatic glycogenolysis caused by circulating epinephrine which was released reflexly from the adrenal medulla (Ellis et al., 1956; Stoner and Threfall, 1960; Levensen et al., 1961; Bauer et al., 1969, a). Insulin does not respond to a rise in blood glucose during elevated catecholamines in the circulation (Halmagyi et al., 1966) or during hypoxia (Baum and Porte, 1972) insuring the maintenance of hyperglycemia. Other factors which can be considered to affect blood glucose via epinephrine are a decrease in glucose delivery to the periphery caused by vasoconstriction, a direct inhibition of cellular uptake of glucose and an inhibition of insulin production by pancreatic β cells (Kinney et al., 1970). Hyperglycemia was not seen in this study, because blood samples were not taken until 2 hours after endotoxin, at which time the transition from hyperglycemia to hypoglycemia may have already occurred (Halmagyi et al., 1966; Berk et al., 1970; Williamson et al., 1970). The onset of hypoglycemia reflects the early depletion of hepatic glycogen stored by catecholamine release and the subsequent uptake of glucose by ischemic tissue (Bauer et al., 1969; Vigas et al., 1971; Filkins, 1973). A defect in hepatic gluconeogenesis is probably responsible for the persistent hypoglycemia which leads to death (LaNoue, Mason and Beckel, 1968; LaNoue, Mason and Daniels, 1968; Shands et al., 1969). Williamson and

co-workers (1970), using a perfused rat liver preparation from hypoglycemic, endotoxemic rats found elevated fructose-1,6-diphosphate gluconeogenic levels, which will effectively block gluconeogenesis. They also found depressed fructose-6-phosphate and glucose-6-phosphate levels. Decreased concentration of these indicates activation of phosphofructokinase. Furthermore, decreased glucose-6-phosphate results in activation of hexokinase, thereby utilizing glucose (Kubler and Spieckermann, 1970).

Methylprednisolone protected the mice which were pretreated with this drug from the endotoxin-induced drop in blood glucose. In the group which received methylprednisolone 2 hours after endotoxin, blood glucose increased to control levels following administration of the drug. The observed blood glucose increase was probably due to an inhibition of peripheral utilization of glucose and to a promotion of gluconeogenesis (Britton and Silvette, 1931; Berry et al., 1959; Levenson et al., 1961; Novak et al., 1970). Pritham (1968) reported that cortisol promotes deamination of amino acids to glucose and increases the activities of gluconeogenic and glycogenic enzymes in the liver, i.e., phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and glycogen synthetase. Ashmore and Weber (1968) also saw increased glucose-6-phosphatase activity as well as increased fructose-1,6-diphosphatase in the liver following glucocorticoids. The blood glucose concentration in two experimental groups (M-P +1 and M-P +4) did not respond to methylprednisolone treatment, and blood glucose concentrations in all methylprednisolone-treated mice eventually declined to Endotoxin group levels. Continued tissue ischemia and high levels of glucose uptake, accelerated by insulin release once catecholamines were depleted, may have contributed to the continued hypoglycemia. Also, since

high levels of lactate were observed, the subsequent metabolic acidosis may have impaired hepatic metabolism.

It is evident in this study that glycogen content in the heart and liver fell sharply following endotoxin injection in mice (Fig. 7, 8). This finding agrees with those of other investigators who found a glycogen depletion following endotoxin administration (Delafield, 1932; Berry et al., 1959; Hamosh and Shapiro, 1960; Lundsgaard-Hansen et al., 1972). Bauer and co-workers (1969), studying experimental hemorrhagic shock reported similar findings, as did several other investigators who examined ultrastructural changes in the liver following endotoxin (Boler and Bibighaus, 1967; Stewart, 1970). The control group glycogen content values varied over the 12-hour experimental period. This may be accounted for by the normal diurnal fluctuation of glycogen content in the heart and liver in nocturnal animals such as mice. Thomas and Meyer (1961) found diurnal peaks at 12:00 noon and 12:00 midnight when measuring cardiac glycogen in the mouse and their low values fell at 8:00 a.m. and 4:00 p.m. It would appear that one heart and liver glycogen peak in this study occurred at 8-10 a.m. (The control mice were injected with 0.9% saline at 6 a.m.) Furthermore, a definite low appears at 2 p.m. The sampling procedure used in this study is not compatible with an earnest appraisal of the diurnal fluctuations in tissue glycogen content and, therefore, no conclusions can be made in this regard. The general pattern of glycogen flux, however, does fit the description reported by Thomas and Meyer.

The depletion of glycogen has been attributed to glycogenolysis stimulated by the release of epinephrine from the adrenal medulla early

in endotoxin shock (Ellis, 1956; Levenson et al., 1961; Bauer et al., 1969, a; Kinney et al., 1970). Hamosh and Shapiro (1960) reported an increase in liver and muscle phosphorylase kinase activity and phosphorylase a activity, which accounted for the elevated glycogenolysis in muscle and liver of endotoxemic rats. In their study, the endotoxin produced a conversion of practically all phosphorylase to the active form. Under normal conditions, almost no phosphorylase is in the active form (Wollenberger and Krause, 1963). In shock syndromes the reaction for the activation of phosphorylase begins with adenyl cyclase stimulation mostly by epinephrine. ATP is converted to adenosine 3',5'-phosphate, which stimulates the conversion of non-activated phosphorylase b kinase to activated phosphorylase b kinase. The activated kinase, then promotes the activation of the phosphorylase, itself, from the b form to the active a form. Phosphorylase a catalyzes the reaction: Glycogen + Pi → Glucose-1-Phosphate (Green and Cori, 1943; Krebs et al., 1966; Bowness, 1966).

The precise biochemical mechanism responsible for the metabolic alterations caused by endotoxin remains unknown. However, it is known that endotoxin causes the release of lysosomal enzymes from leukocytes and platelets localized in the lung (Massion et al., 1972; Wilson et al., 1972, a,b) and from the liver (Janoff et al., 1962; Weissman and Thomas, 1962). The massive release of these enzymes into the general circulation may be directly responsible for cellular damage which could impair metabolic function (Wilson et al., 1972 a, b). In addition, the lysosomal enzymes released by endotoxin may stimulate the release of kinins, causing hypotension and capillary leakage, thereby producing a tissue hypoxia which could also impair metabolic processes. Lundsgaard-Hansen and co-workers

(1972), working with endotoxin-treated rabbits, evaluated the metabolic changes in the heart and liver as they were related to the progress of endotoxin shock. They concluded that the typical depression in glycogen and glucose, elevation in lactate and lactate/pyruvate ratio along with other changes seen in endotoxin shock are probably a reflection of progressive hypoxia. Other investigators have reported a rapid decline of cardiac glycogen levels during anoxia (Conn et al., 1959; Michal et al., 1959; Klarwein et al., 1961). Glycogen, because of its profuse distribution, constitutes by far the largest reservoir for anaerobic myocardial energy and is rapidly utilized to replenish stores of ATP (Wollenberger et al., 1967). This rapid depletion of glycogen is only known to occur in hearts which are not well-oxygenated and therefore, an abnormal glycogenolytic response is seen in shock (Williams and Mayer, 1966; Williamson, 1966). Wollenberger and Krause (1963) reported a rapid activation of glycogen phosphorylase following the abrupt arrest of blood flow in perfused dog heart. They found an increase in the percentage of the total phosphorylase which was in the a configuration from 0% to 60% within 17 sec. Other investigators have also reported that ischemia and anoxia shomehow cause activation of phosphorylase in heart muscle (Klarwein et al., 1963; Morgan and Parmeggiani, 1964; Wollenberger et al., 1967).

Methylprednisolone appears to exert a glycogenetic effect in the heart and liver of mice treated with endotoxin. The permanence of this effect seems to be greater in the heart (Fig. 7) since most groups maintained control-level or elevated glycogen values 8 hours after methylprednisolone. The increase of glycogen in the liver (Fig. 8), however, was

temporary in the groups which received methylprednisolone after endotoxin. The two pre-treated groups (M-P -1, M-P 0) maintained glycogen values significantly above Endotoxin group levels 8 hours after methylprednisolone. Despite the relapse of glycogen content in the liver to Endotoxin group levels, all groups showed a profound increase in liver glycogen following methylprednisolone. Synthesis of glycogen is important for survival during shock as shown by Strawitz and Hift (1960). In their studies, only the rats which were able to synthesize glycogen in the liver survived hemorrhagic shock. The rats which died showed no evidence that they had been synthesizing glycogen. Cortisone, alone, greatly increases the glycogen content of the liver, and when used with endotoxin, it maintains glycogen at a significantly higher level than in animals treated with endotoxin alone (Berry et al., 1959). Other related work also points to a glycogenetic effect of glucocorticoids. Lone and coworkers (1940) found that cortisol elevated liver glycogen and maintained normal blood glucose in adrenalectomized animals. Berry and his associates (1968) demonstrated the ability of cortisone to counter the inhibition of acclimatization by endotoxin in high altitude mice. Increased glycogen synthesis and storage was part of this adaptive change. In other investigations, cardiac glycogen content and α-1,4-glucan α-4-glucosyltransferase activity, which decreased following adrenalectomy in rats, were restored to normal levels by dexamethasone (Daw et al., 1968, 1969). Pritham (1968) also reported elevated glycogen synthetase activity in liver due to cortisol.

A rapid depletion of adenosine 5'-triphosphate (ATP) from the heart and liver and of phosphocreatine (PCr) from the heart, was observed

in mice treated with endotoxin in the present study (Fig. 9, 10, 11). Pappova and his co-workers (1971) and Lundsgaard-Hansen and his associates (1972) also found depressed ATP levels and furthermore, increased ADP and AMP levels, following endotoxin administration in rabbits. Delafield (1932) reported increased levels of circulating inorganic phosphate, which indicates a breakdown of phosphorylated compounds. Although there was no decrease of ATP in the heart, Staples and his co-workers (1969) found a drop in liver, brain and kidney ATP levels in rats following injection of endotoxin. The present results are essentially in agreement with those of the investigators cited above.

Although Lundsgaard-Hansen and his co-workers found a response to endotoxin, they concluded that the metabolic derangements were due to a progressive tissue hypoxia rather than to a direct effect of endotoxin. Indeed, several investigators have reported a diminished coronary blood flow and lowered myocardial oxygen consumption during the normovolemic phase of hemorrhagic shock (Wiggers and Werle, 1942; Opdyke and Foreman, 1947; Edwards et al., 1954; Crowell and Guyton, 1961). Other investigators have described endotoxin shock as reversible ischemic shock, which includes: elevated catecholamines and total peripheral resistance, and lowered cardiac output, venous return, circulating blood volume and organ perfusion (Lillehei et al., 1964 a). This condition culminates in irreversible shock characterized by a stagnant anoxia, which eventually causes depressed organ function. In either case, it was emphasized that wide-spread hypoxia and eventually anoxia are the main effects and characteristics of shock.

Myocarial high energy phosphate stores have been studied by

other investigators to define the metabolic defects which occur in ischemic and hypoxic heart failure. The observed changes reported in this study are similar to those described earlier in ischemic and hypoxic heart failure. Some investigators found no change in cardiac ATP stores in response to acute hypoxia (Pool et al., 1966; Covell et al., 1967; Lochner et al., 1968). They did observe a depression of PCr, however, and it is possible that the degradation of PCr may account for a combination of its high energy phosphate with ADP to form ATP, thereby maintaining the ATP stores. Scheuer and Stezoski (1967) found normal ATP and PCr levels during perfused rat heart ischemia. They concluded, however, that the key to myocardial energetics is subtle changes in high energy compounds, not the over all changes. Since only 1/5 - 1/4 of total cardiac ATP equilibrates with PCr and is concerned with contraction (Fleckstein et al., 1967), perhaps the performance-related changes in myocardial high energy compounds cannot always be seen. Several investigators have reported a decline in cardiac ATP during anoxia (Greiner, 1952; Furchgott and deGubareff, 1958; Regen et al., 1964). Others observed a decrease in both ATP and PCr (Michal et al., 1958; Olson and Platnek, 1959; Benson et al., 1961; Williamson, 1966; Fleckstein et al., 1967; Lamprecht, 1967).

Olson (1967) concluded that low ATP and PCr levels in failing hearts are due to an insufficient rate of oxidative phosphorylation. This hypothesis was recently tested in isolated mitochondria from endotoxemic rat liver, and the results showed decreased oxidation of succinate, α-ketoglutarate and palmitylcarnitine (DePalma et al., 1970). Due to this relative blockade of the electron transport system and inhibition of

oxidative phosphorylation, high energy phosphate stores decline and release the controls for glycolysis. Lowered ATP allows an increase in phosphofructokinase activity, which drives glycolysis unchecked (Passoneau and Lowry, 1962; Regen et al., 1964; Williamson, 1966; Williamson et al., 1970). In this way, glycogen stores are depleted via phosphorylase and via glycolysis to pyruvate and lactate. Glycolytic ATP production does not compensate for high energy utilization during ischemia (Scheuer, 1967).

Methylprednisolone is clearly effective in maintaining and restoring high energy compounds at normal levels in the heart and liver of mice which have received endotoxin. Because of the close association of carbohydrate metabolism and regulation with high energy phosphate functions and stores, this recovery of ATP and PCr to normal levels is probably due to the gluconeogenic and glycogenetic characteristics of methylprednisolone. Glucose and glycogen serve as anaerobic energy reservoirs and are catabolized to replenish stores of ATP (Wollenberger et al., 1967).

The lactate/pyruvate ratio of both heart and liver in mice given endotoxin was higher than that seen in the control group over the first 4 hours after injection. An elevated lactate/pyruvate ratio has been reported by other investigators during septic shock in humans (Blair et al., 1969) and during experimental endotoxin shock in animals (Cavanagh, 1969; Pappova et al., 1971; Lundsgaard-Hansen et al., 1972). The value for the endotoxin group did not remain elevated compared to the control group throughout the course of the experiment, because the pyruvate content of the heart and liver in the mice of the Endotoxin group became progressively elevated, while the tissue lactate content declined slightly.

In the presence of hypoxia, there is an increase of NADH which shifts the equation: pyruvate + NADH $\overset{LDH}{\leftrightarrow}$ lactate + NAD^+, to the right, resulting in production of lactate (Bing, 1965). This sequence of events takes place in the heart when it becomes anoxic (Evans, 1936; Conn et al., 1959; Bing and Ramos, 1961; Lamprecht, 1967), as well as in the liver (Makin, 1971).

During the period of high lactate levels in the heart and liver of endotoxin-treated mice (0-8 hours), methylprednisolone decreased the lactate/pyruvate ratio. Eventually, there was no difference between endotoxin-treated, methylprednisolone-treated and control animals in their tissue lactate/pyruvate ratios, except for the myocardial control group. The early decrease of lactate/pyruvate ratio by methylprednisolone in endotoxin-treated mice was probably due to rapid utilization of lactate as a substrate for gluconeogenesis and glycogenesis.

Whether all of these metabolic changes in response to endotoxin shock are the result of a direct action of endotoxin or the result of an accompanying anoxia, the changes are nevertheless a severeand usually fatal blow to any organism and they usually result in death. It cannot be determined from these studies if endotoxin directly affects the metabolic mechanisms. However, it is certain that methylprednisolone exerts a protective and therapeutic effect upon the metabolic status of the heart and liver in endotoxin-treated mice. Methylprednisolone not only protects the mouse against glucose, glycogen and high energy compound depletion, it restores these substances to normal levels or levels significantly higher than those seen in animals receiving endotoxin alone. Great importance is placed on these metabolites in their relation to heart

and liver function, as well as to the function of cells throughout the body which depend upon liver metabolism. It is, therefore, concluded that the capability of methylprednisolone to maintain and restore glucose, glycogen, ATP and PCr levels in various tissues of the body is a primary factor in its life-saving effect during E. coli endotoxin in mice.

Effects of Prostaglandin E$_1$ on the Cardiovascular, Hematological and Metabolic Responses to Endotoxin in Dogs

As seen in Fig. 14, endotoxin injection in dogs resulted in a severe drop in systemic arterial pressure (SAP) followed by a reflex rise in heart rate (HR). SAP remained depressed and progressively declined over 2 hours, while HR returned to control values 60 min after endotoxin injection. Pulmonary arterial pressure (PAP) exhibited a sharp rise, initially, followed by a steady decline. Left atrial pressure (LAP) and right atrial pressure (RAP) dropped immediately and remained low. The hemodynamic changes, as well as the hematocrit and pH changes (Fig. 15) reported here, agree closely with those of earlier investigators studying the hemodynamic response to endotoxin shock (Hinshaw et al., 1958; Lillehei and MacLean, 1958; Lillehei et al., 1964; Hinshaw et al., 1966; Hinshaw et al., 1970; Hinshaw et al., 1971). White blood cell (WBC) and platelet responses to endotoxin shock, similar to those reported here, have also been described by other workers (Ratliff et al., 1971; Wilson, 1972 a, b). The increase in blood glucose during the first hour of shock, which declines to control levels at 2 hours, as seen in Fig. 16, has been clearly documented as a temporary state,

which eventually progresses to severe hypoglycemic levels (Rangel et al., 1970; Bert et al., 1970; Williamson et al., 1970). Also, the progressive elevation of lactate in response to endotoxin administration, as observed in the present study (Fig. 16), has been reported to be a product of the metabolic derangements associated with this shock state (Bing and Ramos, 1961; Blair et al., 1969; Vitek and Cowley, 1971: Lundsgaard-Hansen et al., 1972). Phospholipids remained unchanged following endotoxin (Fig. 17). Free fatty acids (FFA) decreased sharply one hour after endotoxin, as shown in the same figure. In contrast, other investigators have reported a hyperlipidemia following E. coli endotoxin in animals (Graves et al., 1970; Horowitz, 1972). The hypolipidemia seen in this study may be a consequence of elevated lipogenesis in response to the altered metabolic picture seen during endotoxin shock. Hirsch and coworkers (1964) observed fatty liver following endotoxin in rabbits. Glycolysis is accelerated in the heart and liver during endotoxin shock nad may provide substrate for lipogenesis (Conn et al., 1959; Levenson et al., 1961; Bauer et al., 1969). Pretreatment of dogs with PGE_1 resulted in lowered FFA levels in the blood, because of the antilipolytic effect of prostaglandins (Nakano and Koss, 1973).

Prostaglandin E_1 (PGE_1) appeared to exert a beneficial effect only on WBC levels during the third and fourth hours of shock. PGE_1 elevated WBC counts to the control range from the depressed endotoxin group level. This action may have involved a stabilization of lysosomal membranes, leukocyte preservation and inhibition of platelet aggregation (Kloeze, 1967; Emmons et al., 1967; Marquis et al., 1969). The exaggerated depression of SAP by PGE_1 when given with endotoxin, compared to

endotoxin alone, was probably due to the vasodilator activity of the prostaglandin, itself. The early elevation of PAP due to PGE_1 alone, was probably caused by an increase in venous return due to peripheral effects which are uncertain at this time. The addition of endotoxin resulted in a sharp decreased in SAP, and consequently, the PAP could not be maintained in the face of a greatly lowered venous return. Endotoxin alone, however, caused a sharp rise in PAP. This effect was probably due to rapid platelet aggregation and mechanical blockage (Wilson, 1972) and possibly due to localized vasocontriction following catecholamine release.

Prostaglandin E_1 did not appear to be beneficial in the treatment of endotoxin shock in the dog. In some instances, it actually seemed to aggravate the shock state (SAP, FFA), and only in the maintenance of WBC counts did PGE_1 exert a positive effect on one of the pathophysiological characteristics of endotoxin shock. This work is not in agreement with studies which reported hemodynamic and metabolic improvements and an increase in survival following PGE_1 and $PGF_{2\alpha}$ administration to animals in hemorrhagic shock (Glenn, 1972; Priano et al., 1972) and in endotoxin shock (Glenn et al., 1971). These reports may be of doubtful significance because other investigators have recently described elevated prostaglandin levels during endotoxic shock, which may indicate that prostaglandins actually mediate some of the manifestations of this syndrome (Kessler et al., 1969; Skarnes and Harper, 1972; Anderson et al., 1973; Kessler et al., 1973).

Effect of Methylprednisolone on the Metabolic Degradation of Prostaglandin E_1 in the Lung of Endotoxemic Rats

In the normal lung, most of the prostaglandin added (^{3}H-PGE_1) was rapidly metabolized within 15 min (Fig. 18). This observation closely agrees with findings of earlier investigations on healthy rat lung and kidney (Änggård and Samuelsson, 1964; Nakano et al., 1969; Nakano, 1970; Änggård and Larsson, 1971; Samuelsson et al., 1971). As shown in Fig. 19, this rapid and almost complete metabolism has been defined as a conversion of PGE_1 via NAD^+ - dependent prostaglandin dehydrogenase (PGDH) to 15-keto-PGE_1 (Änggård and Samuelsson, 1964; Nakano et al., 1969; Nakano, 1970; Samuelsson et al., 1971). Further metabolism of 15-keto-PGE_1 is mediated by Δ^{13}-prostaglandin reductase in the lung and by non-specific beta- and omega-oxidation in the liver (Samuelsson et al., 1971). Although these metabolites were not measured in this study, it is certain that the disappearance of PGE_1 accounts for its conversion to 15-keto-PGE_1 by PGDH (Änggård and Samuelsson, 1964; Nakano, et al., 1969; Nakano, 1970; Änggård and Larsson, 1971).

E. coli endotoxin, administered to rats 8 hours before the lungs were removed, caused almost complete impairment of PGE_1 degradation. It is likely that this effect is associated with the cellular damage seen in the lungs following endotoxin (McKay et al., 1967; Coalson et al., 1970; Massion et al., 1972). The elevation of circulating prostaglandin levels in animals during endotoxin shock has recently been reported by other investigators (Skarnes and Harper, 1972; Anderson et al., 1973; Kessler et al., 1973). This rise in prostaglandins during shock may be a result

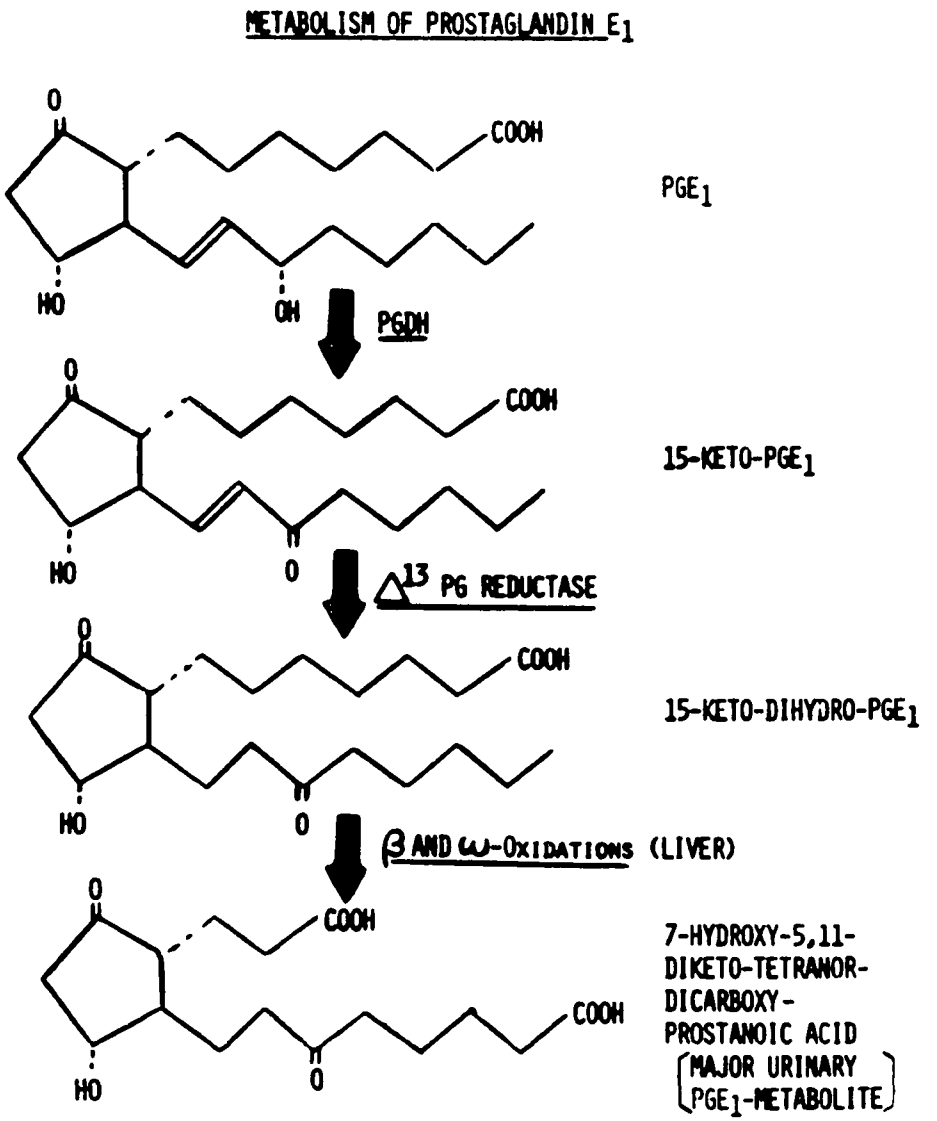


Figure 19. Metabolism of prostaglandin E_1.

of biosynthesis and release from various tissues and organs (Greaves et al., 1971; Piper and Vane, 1971). However, 95% of the newly synthesized prostaglandin would be cleared from the circulation in one pass through the lungs by normal PGDH-mediated degradation (Ferreira and Vane, 1967; Horton and Jones, 1969). Therefore, it is more reasonable to assume that a mechanism such as the one described in the results of this study, namely, impairment of PGDH activity following endotoxin, is responsible for elevated prostaglandin in shock. Perhaps other catalysts, Δ^{13} prostaglandin reductase and beta- and omega-oxidation enzymes are also inhibited by endotoxin. The scheme for regulation of the metabolism of prostaglandins in the lung shown in Fig. 20 summarizes these findings. Under normal conditions, most of the prostaglandins released in the peripheral tissues are degraded during the first pass through the lungs. More prostaglandins are released in cases of inflammation, but the same high percentage of these compounds is destroyed in the lung. Endotoxin shock, however, incapacitates this prostaglandin metabolism system in the lung and the result is recirculation of large amounts of prostaglandin released in the periphery as an inflammatory response.

Methylprednisolone, when administered 1 hour before endotoxin, maintained the major metabolic activity of PGDH in the lung. As mentioned earlier, endotoxin is known to exert structural and biochemical damage in the pulmonary vascular bed (McKay et al., 1967; Coalson et al., 1970; Massion et al., 1972). Methylprednisolone has been shown to be effective in preventing this type of damage in lungs of endotoxin-treated animals (Massion et al., 1972; Wilson, 1972 a, b). The observed enzymatic defect in prostaglandin metabolism may be related to the endotoxin-

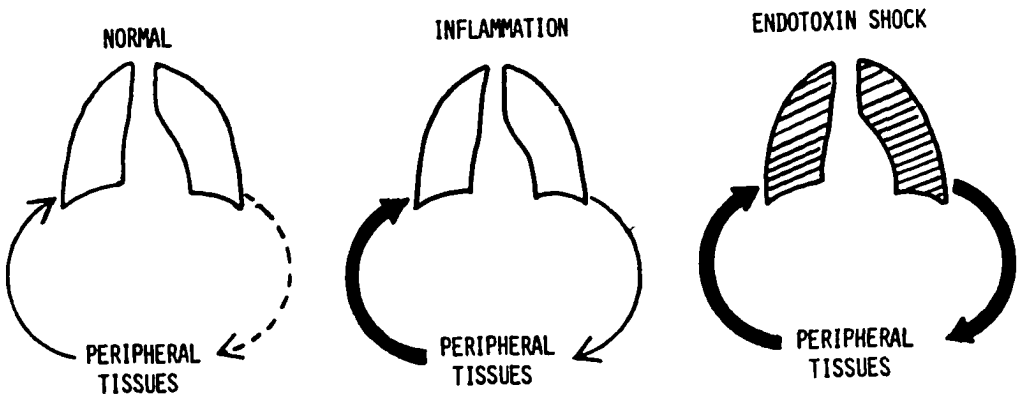


Figure 20. Scheme of relative amount of prostaglandin metabolism by prostaglandin dehydrogenase (PGDH) in lungs under normal, inflammation and endotoxin shock conditions.

induced pulmonary damage reported by the investigators cited here. If they are related, then the likelihood is great that methylprednisolone preserves prostaglandin metabolism by preventing structural and biochemical changes caused by endotoxin.

CHAPTER V

SUMMARY

Survival studies were performed on 349 male adult albino mice, first to establish the lethality of E. coli endotoxin, and second to determine the life-saving effect of methylprednisolone on endotoxemic mice. Three doses of endotoxin (10 mg/kg, i.p.; 30 mg/kg, i.p.; 100 mg/kg, i.p.) were used in the first study and one (10 mg/kg, i.p.) was used in the second. Mice in the second study also received methylprednisolone (30 mg/kg, s.c.; 150 mg/kg, s.c.) before, simultaneously with and after endotoxin. Survival for 48 hours was set as the criterion for survivors.

Selected metabolites (glucose, glycogen, ATP, PCr, lactate and pyruvate) were measured in heart, liver and plasma of another group of 194 male adult albino mice treated with endotoxin (10 mg/kg, i.p.) and methylprednisolone (30 mg/kg, s.c.). Methylprednisolone was injected 1 hour before endotoxin, simultaneously with endotoxin, or 1, 2 or 4 hours after endotoxin. Mice were sacrificed at 2, 4 or 8 hours after injection of the drug. The metabolites were measured by various macro- and micro-enzymatic methods.

Cardiovascular, hematological and metabolic studies were carried out on 28 dogs which were treated with endotoxin (0.35 mg/kg, i.v.) and PGE_1 infusion (1 µg/kg/min, i.v.) started 15 min before endotoxin.

Initially, each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and artificially ventilated. The left hemithorax was then opened, and the heart was suspended in a pericardial cradle. Catheters were placed in the brachiocephalic artery, the pulmonary artery, the right atrium and the left atrium to measure systemic and pulmonary pressures, right and left atrial pressures, respectively. Blood samples were taken periodically for hematocrit, leukocyte, platelet, pH, glucose, lactate, free fatty acid and phospholipid measurements.

The metabolic degradation of PGE_1 was studied in the lung of 12 male Holtzman strain rats. Four rats received no drug (0.9% saline, i.p.), 4 rats received endotoxin (10 mg/kg, i.p.) and the final 4 rats received methylprednisolone (50 mg/kg, s.c.) 1 hour before endotoxin. All rats were killed 8 hours after endotoxin or saline injection and the lungs were excised and homogenized. The homogenates were incubated with 25 mμc/ml of ^3H-PGE_1 (28 c/mmoles), 50 ng/ml of PGE_1, and 2 mM of NAD^+ at 37°C for 0, 5 and 15 min. ^3H-PGE_1 was extracted from the mixture with ethyl acetate, separated from the other prostaglandin-compounds by thin-layer chromatography and counted in a liquid scintillation spectrometer.

The conclusions reached for these studies were:

1. A dose of 10 mg/kg i.p. of E. coli endotoxin produced an intermediate lethality in mice (LD_{60}). Higher doses of endotoxin (30 mg/kg; 100 mg/kg) were highly lethal in mice, 87% and 90%, respectively.

2. Methylprednisolone (30 mg/kg, s.c.) exerted a significant life-saving effect on endotoxemic mice when given 1 hour before endotoxin, or 1, 2 or 4 hours after endotoxin. The higher dose of methylprednisolone (150 mg/kg, s.c.) was beneficial only when given 1 hour before endotoxin

and simultaneously with endotoxin.

3. Endotoxin significantly depressed blood glucose levels within 2 hours after the toxin was injected. The glucose level remained low and eventually declined further during the 12 hour experiment. Methylprednisolone maintained blood glucose at levels significantly above those measured in the endotoxin group for two hours after endotoxin administration in those groups which received the drug before or simultaneously with endotoxin. Methylprednisolone was also able to raise the blood glucose concentration to control levels in one group when given 2 hours after endotoxin. The remaining groups (1, 4 hours after endotoxin) were unchanged from the endotoxin group.

Tissue glycogen content also was decreased following endotoxin. Myocardial glycogen content dropped to levels significantly below control after endotoxin and remained low throughout most of the 12 hour experiment. When methylprednisolone was given, all groups showed a significant increase in cardiac glycogen content to control levels or higher.

Liver glycogen content was markedly reduced following endotoxin and it remained low for 12 hours. All groups which received methylprednisolone experienced a sharp rise in liver glycogen levels which lasted 2-8 hours after drug injection. Only the groups which received the drug 1 hour before endotoxin or simultaneously with endotoxin had liver glycogen levels which were significantly elevated above the endotoxin group 8 hours after methylprednisolone.

Heart and liver ATP and heart PCr were completely depleted within two hours after endotoxin injection. The substrate levels remained exhausted for 12 hours. In every case, when methylprednisolone was

administered, ATP and PCr levels rose to control levels or to levels significantly above endotoxin group levels and they remained there.

Heart and liver lactate/pyruvate ratio rose above control after endotoxin administration and within 4 hours it was the same as control. Methylprednisolone decreased the ratio, especially when given before endotoxin or within 2 hours after endotoxin administration.

4. Prostaglandin E_1 had no beneficial effect on the cardiovascular, the metabolic and most of the hematological parameters measured in endotoxemic dogs. Only the white blood cell count was improved following PGE_1 infusion.

5. Prostaglandin dehydrogenase (PGDH) activity in rat lung was apparently depressed by endotoxin administration. Lung from endotoxin-treated rats only degraded 26% of the ^3H-PGE_1 presented to it as compared to control lungs (88%).

6. Methylprednisolone enhanced the ability of the endotoxemic rat lung to metabolize ^3H-PGE_1 from 26% to 63%.

In conclusion, the mouse endotoxin shock model was found to be a responsive and reliable experimental model which will undoubtedly become valuable in the study of survival and of metabolic processes in endotoxin shock. Methylprednisolone was shown to exert beneficial actions on mouse survival and metabolism. The beneficial metabolic alterations due to methylprednisolone found in endotoxemic mice probably contributed to a great extent to their ability to survive. Prostaglandin E_1 did not appear to be beneficial in canine endotoxin shock. Its degradation was depressed following endotoxin, indicating a possible explanation for elevated circulating levels of prostaglandins during endotoxin shock. Methylpredni-

solone to a degree was found to protect rats against the endotoxin-related loss of PGDH activity in the lung.

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