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MECHANISM OF FORELIMB SKIN AND SKELETAL MUSCLE GLUCOSE UPTAKE DURING ESCHERICHIA COLI ENDOTOXIN SHOCK IN THE DOG

By

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ABSTRACT

MECHANISM OF FORELIMB SKIN AND SKELETAL MUSCLE GLUCOSE UPTAKE DURING ESCHERICHIA COLI ENDOTOXIN SHOCK IN THE DOG

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This study was undertaken to test the hypotheses that glucose uptake by forelimb skeletal muscle and skin increases during endotoxin shock in the dog and that the mechanism for this proposed increase in glucose uptake is related to local tissue hypoxia. Mongrel dogs were anesthetized with pentobarbital sodium and heparinized. The isolated, innervated, forelimb preparation perfused at either natural or constant blood flow was used. Shock was induced by i.v. infusion of 2 mg/kg *E. coli* endotoxin. Muscle and skin glucose uptake increased by 30 minutes of endotoxin shock and remained elevated in the natural flow study. Total forelimb blood flow decreased and the limb became severely hypoxic. In the constant flow study, glucose uptake by both muscle and skin was increased at 30 minutes of endotoxin shock but thereafter returned to control. This group was neither ischemic or hypoxic. These data support the above hypotheses that glucose uptake by muscle and skin increases during endotoxin shock and that the mechanism for the increased glucose uptake is related to local tissue hypoxia.

To my wife, Alice,
and to my family, for
their unfailing love
and support

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CHAPTER I

INTRODUCTION

A characteristic feature of endotoxin shock in a variety of animal species, including the dog (15), rat (75), and subhuman primate (67), is the progressive development of severe hypoglycemia. This phenomenon appears to result from the combined effects of decreased glucose production by the liver (75) and an increase in peripheral glucose utilization. Although it is well recognized that there is an increase in peripheral glucose utilization after endotoxin administration, the primary tissues involved in this response have not been completely defined. Hinshaw et al (67) have shown that the heart-lung system is not involved in this response. Raymond and Emerson (99) demonstrated that glucose uptake by the central nervous system decreases during endotoxin shock in the dog. Hinshaw and associates (100) have observed an increase in glucose uptake by leucocytes from dogs given endotoxin, but it is improbable that such a small tissue mass could be totally responsible for the large increase in glucose utilization observed during endotoxin shock. Because skeletal muscle comprises a large percentage of total body mass and can utilize large quantities of glucose via anaerobic glycolysis, as occurs during low flow states, it is a prime candidate for the increase in peripheral glucose utilization.

Recent work by Raymond et al (83) demonstrated that glucose uptake by the naturally perfused gracilis muscle increases during irreversible E. coli endotoxin shock in the dog. The mechanism for this increased glucose uptake was shown to be related to local tissue hypoxia secondary to muscle ischemia (83). Glucose uptake was also reported to increase in the dog hindlimb during mild endotoxemia (102). On the other hand, Furr et al (101) saw no change in glucose metabolism by the isolated forelimb during endotoxin shock in the dog. Even though systemic arterial blood pressure and forelimb blood flow decreased markedly after endotoxin administration, the isolated forelimb never became hypoxic. This paradox, viz. lack of local forelimb hypoxia in the presence of severe forelimb ischemia, would implicate a reduced forelimb metabolic rate such that the severely reduced forelimb blood flow and decreased oxygen delivery to the forelimb was adequate to meet the metabolic needs of the tissue.

Upon review of the protocols of the isolated gracilis muscle (83) and isolated forelimb studies (101), a major difference was that temperature of the isolated forelimb was not maintained at core temperature as it was in the isolated gracilis muscle and glucose uptake by the isolated forelimb could be related to the temperature of the isolated organ (Q10 effect). Furthermore, a reduction in isolated organ temperature could have also blunted the metabolic response of skin to endotoxin. Therefore the hypotheses to be tested are: 1) that glucose uptake by forelimb skeletal muscle and skin increases during endotoxin shock in the dog, and 2) that the mechanism for this proposed increase in glucose uptake is related to local tissue hypoxia and/or tissue ischemia.

CHAPTER II

SURVEY OF THE LITERATURE

Structure of the Endotoxin Molecule

Bacterial endotoxin is a constituent of the outer cell wall of many gram-negative bacteria (1), which includes the common intestinal organism Escherichia coli. In fact E. coli, of the family Enterobacteriaceae, is the most commonly found facultative anaerobe in the fecal flora of many mammals (2). Endotoxins were detected in cell free filtrates of autolyzed gram-negative cultures more than 100 years ago, indicating that these cells release endotoxin into the surrounding medium upon lysis or disintegration (3,4). The chemical composition of endotoxin has been elucidated through the efforts of numerous investigators (59). Specifically, endotoxin exists as a large macromolecular complex composed of polysaccharides, lipids some amines, phosphorous and small quantities of proteins (57).

The chemical structure and molecular arrangement of the various substituents of the cell wall of gram-negative bacteria has recently been described as consisting of three distinct layers: a) an outer membrane; b) a layer of peptidoglycan (murein net); and c) an inner cytoplasmic membrane. Costerton et al (6) and Braun (7) give similar descriptions of the cell wall in that it consists of two lipoprotein bilayer membranes separated by a periplasmic space. Based on chemical structure analysis, Braun (7), devised a model for the supermolecular structure of the rigid layer (murein-lipoprotein complex) of the cell wall of E. coli. According to his data, approximately 250,000 lipoprotein molecules of known amino acid sequence are evenly distributed over the one-layered murein net (10). The murein net is made up of disaccharide units composed of N-acetyl-muramic acid linked by beta-1,4-glycosidic bonds to N-acetyl-glucosamine. These subunits are then cross-linked by short peptide bonds to form a large polysaccharide macromolecule (molecular weight 1-4 X 10^{9} daltons) (7).

The multi-layered cell wall of gram-negative bacteria has been attributed many functions including, protection of the organism in a wide range of environments, conferring rigidity to the microorganism, excluding certain toxic substances, regulating the passage of ions, and binding specific enzymes (6).

The Toxic Factor

For many years investigators have attempted to determine which part of the lipopolysaccharide molecule of gram-negative bacteria was responsible for its vast array of pathophysiological effects. For example, endotoxin has been shown to inactivate complement (11), induce fever (12,13), initiate local and generalized Shwartzman reactions (14), and deplete animals of their carbohydrate reserves (14). Research during the last decade points to the lipid A molecule as being the toxic factor responsible for these pathophysiological alterations (2,16).

Listed above are a few of the biological responses used to determine the relative potencies of different lipopolysaccharides. For example, it has long been known that lipopolysaccharides can interact with complement in vitro and cause its inactivation (11). This inactivation of complement by endotoxin has been shown to be a good qualititive measure of the endotoxicity of different lipopolysaccharide preparations (8,17). Bioassays such as this are routinely used to differentiate between active and inactive lipopolysaccharides.

The two major constituents of endotoxin were first discovered by Bovin, Mesrobeanu, and Mesrobeanu (18,19) in 1933. Extraction of endotoxin with trichloroacetic acid resulted in the isolation of a phosphorous-containing lipid and a degraded polysaccharide. Although the polysaccharide was neither immunogenic nor toxic, the lipid fraction exhibited residual toxicity (18). This finding suggested that specific components within the endotoxin molecule are responsible for its toxic effects.

Twelve years later, in 1945, Brinkley, Goebel, and Perlman attempted to identify a substructure within the endotoxin complex as being the toxic element (20). They split the endotoxin complex with alkaline and acidic hydrolysis. Treatment with dilute acid led to a so-called, non-toxic polysaccharide and a toxic lipoprotein, while treatment with alcholic alkali gave rise to a nontoxic protein and toxic lipopolysaccharide (20). The authors concluded from these experiments the existence of a toxic "T" factor which was neither protein nor polysaccharide. At the time, the authors did not realize the existence of lipid A in the complex.

In the early fifties, Westphal and colleagues (21) claimed that lipid A was the biologically active component of the endotoxin molecule. Using mild acid hydrolysis, lipid A was precipitated from the parent endotoxin molecule and its biological properties investigated (21). The lipid A fraction exhibited one-tenth of the pyrogenic activity of the parent endotoxin molecule. Since the lipid A fraction no longer contained the polysaccharide carrier which makes it water soluble, it was assumed that the reduced solubility of the isolated lipid A was responsible for its reduced pyrogenicity (8,22). However at the time, Westphal and colleagues could not present conclusive evidence in support of their theory i.e. that the lipid component is responsible for the endotoxic activities of lipopolysaccharides.

Meanwhile, Ribi, Landy, and coworkers (23) disagreed with Westphal's (21) theory that the lipid component is responsible for the endotoxic activities of lipopolysaccharides. Ribi et al (23) reasoned that since lipopolysaccharide preparations having minimal lipid A content were able to elicit potent endotoxic activity, the endotoxic properties of lipopolysaccharides could not reside within the lipid A complex. However, conclusive proof could not be presented since an entirely lipid-free endotoxin preparation with full endotoxin activities could not be produced. Such a preparation still does not exist.

The question as to whether lipid A was primarily responsible for the toxicity of the endotoxin molecule remained unanswered for nearly a decade until the discovery of so-called rough mutants that are defective in the synthesis of complete lipopolysaccharides. Subsequent investigations using Salmonella minnesota R595, which contains only lipid A and a trisaccharide, 2-keto-3-deoxyoctonate (KDO), made the final answer possible (16). This lipid A-KDO complex was found to be as toxic as lipopolysaccharides from gram-negative bacteria (5). This finding suggested that the polysaccharide component of the lipopolysaccharide molecule is not essential for endotoxicity (16,24). Succinylation did not diminish the toxicity of the lipid A-KDO complex (16-24). Finally, the lipid A-KDO complex was split with dilute acid to liberate free KDO units and water insoluble lipid A. In 1972, it was found that lipid A could be made soluble by complexing it with bovine serum albumin (24).

Subsequent investigations with complexes of lipid A and bovine serum albumin revealed enhanced endotoxicity (25-27). These experiments conclusively demonstrate that lipid A is the toxic component of the endotoxin complex. They also demonstrated that the polysaccharide component is not specifically involved in endotoxicity. However, polysaccharides are important by acting as solubilizing agents allowing lipid A to interact with the host (8).

General Systemic Hemodynamic Effects of Endotoxin

Gram-negative septic shock is a serious clinical problem with a high mortality rate in spite of significant advances in antimicrobial therapeutics (28). Recent reports have estimated between 70,000 to 100,000 persons a year die from septic shock in the United states (29). The effects of endotoxin on the host are extensive and extremely complex, often leading to severe hemodynamic dysfunction and death.

Circulatory shock of any etiology is characterized by lack of adequate tissue perfusion, which in itself causes tissue damage leading to further insufficiencies, setting up a vicious positive-feedback cycle resulting in complete circulatory deterioration and death. A decrease in tissue perfusion precipitates the development of tissue hypoxia which results in a variety of metabolic disorders at the cellular level (30). This aspect of circulatory shock will be discussed in a later section (see Metabolism during Shock).

Cardiovascular function during endotoxin shock has been extensively studied in a variety of animal species, of which the canine is most popular. Hence, the largest body of knowledge about cardiovascular function during endotoxin shock comes from the canine model on which the following discussion will be focused, with any exceptions noted.

The overall hemodynamic response of the dog during endotoxin shock is characterized by severe hypotension, hepatic portal hypertension, bradycardia, and an increased total peripheral resistance early in shock followed by a gradual decline towards or below control later in shock. The mechanism responsible for the paradoxical decrease in heart rate in the presence of severe hypotension has not been clearly established. It has been suggested by Trank and Visscher (31) and others (32,33) that this paradoxical cardiac response may be due in part to a resetting of arterial baroreceptors.

A recent review by Hess et al (34) suggested that the second fall in arterial blood pressure during endotoxin shock is due to myocardial dysfunction. Whether myocardial dysfunction is due to a circulating myocardial depressant factor or a decrease in coronary perfusion is still controversial (35,36). It has also been postulated that the second fall in arterial blood pressure that occurs during the later phases of shock could be due to the progressive sequestration of blood in capacitance vessels and/or transcapillary movement of fluid from the microvasculature into the surrounding tissues (37). This hypothesis will be discussed in a later section (see Transcapillary Fluid Fluxes during Shock).

Endotoxin administered intravenously as a rapid bolus injection in the dog causes a decrease in systemic arterial blood pressure from approximately 150 mmHg to 50 mmHg within 2 to 5 minutes of injection. The initial fall in arterial blood pressure is followed by a compensatory rise in pressure which approaches near normal levels and lasts for approximately 20 to 60 minutes. Arterial pressure then declines progressively over a 4 to 6 hour period until death of the animal (38). The initial decline in arterial pressure has been attributed to a decrease in cardiac output subsequent to a decrease in venous return due to hepatosplanchnic pooling of blood (39,40).

The classical studies of MacLean, Weil, Spink and Visscher (39,40) clearly demonstrated that the initial fall in arterial pressure after endotoxin administration in the dog is due to a reduced cardiac output subsequent to a decrease in venous return. Because arterial pressure is directly determined by cardiac output and total peripheral resistance, experiments were conducted to determine whether the initial drop in arterial pressure was due to a decrease in cardiac output or total peripheral resistance, or a combination of the two. This was tested by experiments in which the total venous return of the dog was diverted into a reservoir from which it was pumped at a constant rate back to the right atrium (40). Since cardiac output is maintained constant in this preparation, except for brief transient changes due to accumulation or release of blood by

the lungs, a change in arterial pressure can occur only if total peripheral resistance changes. With this preparation, no significant decline in arterial pressure was produced by endotoxin for approximately 30 minutes, indicating that the initial fall in arterial pressure was not due to a decrease in total peripheral resistance. If total peripheral resistance had fallen, arterial pressure would have also declined since cardiac output was maintained constant. However, this was not the case, therefore the initial fall in arterial pressure was due to a reduced cardiac output. This statement is supported by the fact that after administration of endotoxin, the volume of blood in the reservoir rapidly fell. Changes in the volume of blood in the reservoir reflect changes in the inflow rate or venous return because the volume of blood in the reservoir is pumped out at a constant rate. Therefore, since cardiac output is actually a reflection of the total venous return, it was concluded that the initial fall in arterial pressure was due to a reduction in cardiac output subsequent to a decrease in venous return, as evidenced by a decrease in the volume of blood in the reservoir (40).

MacLean and Weil (39) and others (37,40,41) have ably demonstrated that the decrease in venous return in endotoxin shock in the dog is caused primarily by hepatosplanchnic pooling. MacLean and Weil (39) were also the first to note that after administration of endotoxin there was an immediate precipitious drop in arterial pressure and a simultaneous rise in portal venous pressure. Extensive hemorrhagic congestion and edema of the liver and intestines were also noted. The fact that portal vein pressure was elevated allowed them to hypothesize that there must be an increase in resistance to outflow through the liver which would cause stasis and a loss of circulating blood volume. To answer this question, dogs were eviscerated and given endotoxin while arterial blood pressure was monitored (39). Numerous investigators have reported that when the hepatosplanchnic organs are removed or isolated from the circulation there is no immediate fall in arterial blood pressure or venous return (37,39). However, it should be pointed out that even though the initial drop in systemic arterial blood pressure can be prevented by removing the hepatosplanchnic organs (evisceration), a slow decline in arterial pressure is observed approximately 30 minutes after endotoxin administration, reaching lethal levels within 2.5 hours. Hinshaw et al (37) have also demonstrated that when cardiac output is held constant in either eviscerated or non-eviscerated dogs, a slow decline in systemic arterial blood pressure occurs. Hinshaw et al (37) indicated that the progressive fall in systemic arterial blood pressure observed in both eviscerated and non-eviscerated dogs in the presence of constant cardiac filling was due to a gradual decline in total peripheral resistance. These studies demonstrated that pooling of blood in the hepatosplanchnic organs is responsible for the decrease in venous return and subsequent drop in cardiac output and arterial pressure after endotoxin administration.

In summary, the initial fall in arterial blood pressure following endotoxin administration is due to a reduction in cardiac output subsequent to a decrease in venous return which is due to pooling of blood in the hepatosplanchnic organs.

Transcapillary Fluid Fluxes during Shock

As previously indicated, the initial fall in arterial pressure in the dog is due to a decrease in venous return subsequent to hepatosplanchnic pooling of blood (39). The possibility also exists that intravascular pooling of blood and/or extravascular pooling of fluid due to transcapillary fluid movement in sites other than the hepatosplanchnic organs may contribute to the decreasing cardiac output seen during both the early and late stages of endotoxin shock. The literature contains inconsistancies on the direction and location of transcapillary fluid movement during shock. Mellander and Lewis (42) reported a decrease in the pre- to postcapillary resistance ratio in muscle during hemorrhagic shock in the cat, resulting in a net movement of capillary fluid into muscle. Plasma volume, as determined by dye dilution techniques, has been shown to decrease during endotoxin shock in the dog, indicating net capillary fluid filtration (43). However, measurement of plasma volume during shock may be in error due to inadequate mixing of dye (44).

The studies of Weidner et al (45) and Hinshaw and Owens (46) demonstrated an increase in the pre- to postcapillary resistance ratio and a decrease in limb weight during endotoxin shock in the naturally perfused canine forelimb. The initial decrease in limb weight was largely attributed to a decrease in vascular volume subsequent to vasoconstriction, while the prolonged steady weight loss was due to extravascular fluid reabsorption subsequent to a fall in capillary hydrostatic pressure. Capillary hydrostatic pressure would decrease during shock if the rise is postcapillary resistance was overwhelmed by the rise in precapillary resistance and fall in aortic pressure. These studies demonstrated that transcapillary fluid filtration or sequestration of blood in skin and/or skeletal muscle do not contribute to the decreasing venous return during endotoxin shock in the dog.

The Role of Histamine in Endotoxin Shock

Various vasoactive substances such as catecholamines (47), serotonin (48), histamine (49), kinins (50), prostaglandins (51), proteolytic enzymes (52) and beta-endorphins (53) are released during endotoxin shock. Of these histamine appears to play a dominant role in the early phase of endotoxin shock in the dog. Weil and Spink (41) recognized the similarities of the early effects of endotoxin to those seen in anaphylaxis. Their observation suggested the possibility that histamine, which is known to be released during anaphylaxis, may also participate in endotoxin

shock. Plasma samples from the inferior vena cava upstream from the hepatic veins contained appreciable quantities of histamine (41). Kobald et al (54) demonstrated that histamine and other vasoactive polypeptides are present in high concentration in the venous blood draining the portal circulation of dogs shocked with endotoxin. Hinshaw et al (49) demonstrated a rise in unbound histamine and a decrease in the bound form of histamine in whole blood within two minutes after endotoxin administration. Changes in whole blood and plasma histamine levels were explained on the basis that histamine is released from the bound form in whole blood (mast cells and basophils) and other tissue to the unbound form in plasma. Shayer (55) reported that endotoxin increased histidine carboxylase activity, thus accelerating the rate of histamine synthesis. Subsequently, Hinshaw et al (56) demonstrated an accelerated conversion of histidine to histamine following endotoxin administration. Bauer et al (57) have shown that the hepatic veins contain smooth muscle sphincters, which are believed to be species-specific to the dog (58). Because appreciable quantities of histamine were found in the venous blood draining the liver immediately after endotoxin administration (41), combined with the fact that histamine acts primarily on smooth muscle and therefore on smooth muscle spincters located in the hepatic veins, it was suggested that histamine might be responsible for the rise in portal venous pressure and hepatosplanchnic pooling. Hinshaw et al (59) demonstrated that pretreatment with the histamine-releasing agent 48/80 alters the typical response of endotoxin, viz. portal vein pressure changed little and the rapid drop in systemic arterial pressure did not occur after endotoxin administration. It should to noted that injection of histamine or endotoxin causes an elevation of portal vein pressure and a substantial decrease in arterial pressure (59). Pretreatment with phenoxybenzamine, an alpha-blocker, also prevented the typical response to endotoxin (60). However, depending on the concentration, phenoxybenzamine has variable blocking properties on many agents such as histamine, epinephrine, norepinephrine, serotonin, and acetylcholine (61).

To demonstrate that catecholamines were not responsible for the early rise in portal vein pressure, splanchnic neurotomy and/or adrenalectomy were performed on dogs (62). These interventions did not prevent the portal venous hypertension or systemic hypotension after endotoxin, suggesting that the early vascular response to endotoxin is not mediated by catecholamines. Furthermore, it has been demonstrated that catecholamines are released subsequent to the development of hypotension indicating that these agents probably do not play a role in the initial phase of endotoxin shock (62).

In summary, histamine induces a constriction of the hepatic veins with resultant venous pooling in the hepatosplanchnic organs, thus resulting in a decrease in venous return, a reduced cardiac output, and a fall in systemic arterial blood pressure.

Metabolism during Shock

It has been known for many years that carbohydrate homeostasis is altered during circulatory shock in a variety of animal species. As early as 1877, Claude Bernard reported increased blood glucose levels in dogs subjected to experimental hemorrhagic shock and also in man after accidental injury (63). In 1924, Menten and Manning (64) demonstrated that rabbits given endotoxins from gram-negative bacteria developed a transient hyperglycemia followed by a progressive decline in arterial blood glucose concentration to hypoglycemic levels. The subsequent literature is replete with studies indicating that carbohydrate homeostasis is altered in a variety of pathogenic states including hemorrhagic (65), cardiogenic (66), bacteremic (67) and endotoxin shock (15).

In 1946, Engel (68) described a variety of metabolic defects in various tissues due to hemorrhagic shock, and stated that "from a biochemical standpoint there does not appear to be a great deal of difference between the various types of shock, for the common denominator in all is tissue anoxia." In 1972, a paper published by Lundsgaard-Hansen et al (69) supported Engel's basic premise; they stated that the decisive factor impairing cellular metabolism during endotoxin shock was the state of shock which it produces. A recent review on cellular metabolism during shock by Schumer et al (30) clearly indicates that inadequate tissue perfusion and the ensueing tissue hypoxia is the common denominator in all forms of shock. It is clear from the foregoing examples that a reduction in blood flow to the tissues has profound effects on the host by producing disturbances in the normal aerobic metabolic pathways.

It is the purpose of this thesis to review the literature specifically dealing with carbohydrate metabolism during endotoxin shock and its pathophysiological consequences. However, since many of the metabolic consequences of endotoxin shock are similiar to various other forms of shock, studies done in these shock states will also be reviewed when the observations are applicable to endotoxin shock.

Alterations in Blood Glucose during Endotoxin Shock

Reviews by both Hinshaw (15) and Berry (70) on carbohydrate metabolism during circulatory shock stress a basic theme of hyperglycemia followed by a progressively developing hypoglycemia. In 1970, Berk and associates (71) reported in an exhaustive study that a majority of dogs receiving various doses of *E. coli* endotoxin an early and transient hyperglycemia lasting for approximately one hour was observed, which was followed by a gradual decline in blood glucose levels. In the remainder of the dogs receiving endotoxin, blood glucose levels declined immediately and remained low. They also noted that dogs having a more rapid and greater fall in blood glucose concentration tended to die earlier than dogs exhibiting a gradual decline in blood glucose concentration.

Filkins et al (72) demonstrated that rats subjected to endotoxin or traumatic shock developed low levels of blood glucose within 5 hours. Hypoglycemia has also been reported in humans during septic (71) and cardiogenic shock (66). Groves et al (73) reported hypoglycemia during live _E. coli_ bacteremic shock in the dog. Wolfe et al (74) reported that low doses of _E. coli_ endotoxin administered to conscious dogs induced a transient hyperglycemia reaching a peak within 15 minutes which was followed by a decrease in blood glucose concentration over the ensueing 4 hours of shock. Several studies have indicated that the dog seems to be more prone to developing hypoglycemia at a faster rate than primates, which generally exhibit hyperglycemia with hypoglycemia only occuring as a terminal event (67,71). However, Hinshaw's group (67) in recent studies observed an initial period of hyperglycemia followed by 4 to 15 hours of progressively developing hypoglycemia in baboons shocked with live _E. coli_ bacteria.

In summary, the net result of an intravenous bolus injection of purified endotoxin or administration of live _E. coli_ bacteria in a variety of animal species, including the rat (75), rabbit (76), dog (15), and subhuman primate (67), is an early increase in blood glucose levels followed by a depletion of the animal's carbohydrate reserves and eventual hypoglycemia.

Hyperglycemia during Endotoxin Shock

During the early phase of endotoxin shock blood glucose levels are elevated because of an increased mobilization of glucose by the liver via gluconeogenesis and glycogenolysis (75). The kidney is also theoretically capable of elevating blood glucose levels via gluconeogenesis, however, Archer et al (77) reported the absence of a gluconeogenic role of the kidney during endotoxin shock.

Activation of the sympathoadrenal system in shock subsequent to a drop in systemic arterial pressure (62) causes the release of a variety of glucoregulatory hormones including epinephrine (47), glucagon (78), and the glucocorticoids (79). It should be noted that secretion of insulin is relatively depressed during the hyperglycemic phase of shock because of sympathetic inhibition (80). Adrenalectomy (81) or alpha-adrenergic blockade (82) will prevent the decrease in insulin secretion. Depressed plasma insulin levels during the early phase of endotoxin shock is important because insulin is a diabetogenic hormone i.e. it reduces blood glucose concentration. Consequently, there is no hormonal mechanism for counteracting the early rise in blood glucose levels after endotoxin administration. However, blood glucose levels do decrease during endotoxin shock and this decrease has been attributed to non-hormonal factors, viz. local tissue hypoxia (83) and the insulin-like activity of the endotoxin molecule (84) combined with a decrease in glucose production by the liver (75) and

kidney (77). These factors will be discussed in a later section (see Hypoglycemia during Endotoxin Shock).

It is a well known fact that the breakdown of glycogen is initiated by the action of the enzyme of phosphorylase "a", which is specific for the breakdown of glycogen to yield glucose-1-phosphate (85). However, for this to occur phosphorylase "a" must be converted from its active form phosphorylase "b". The conversion of phosphorylase "b" to phosphorylase "a" occurs as a result of stimulation of the enzyme adenylate cyclase by either epinephrine or glucagon. Through a series of enzymatic steps, adenylate cyclase eventually causes the conversion of phosphorylase "b" to phosphorylase "a" and the breakdown of glycogen. It has also been shown by Bitensky and associates (86) that endotoxin stimulates the enzyme adenylate cyclase in mouse liver, which initiates the enzymatic steps necessary to cause activation of phosphorylase "a" and glycogenolysis. McCallum and Berry (87) demonstrated that endotoxin selectively inhibits glycogen synthase, the enzyme responsible for glycogen synthesis. These studies, plus the fact that hepatic glycogen stores are exhausted after endotoxin administration (73), lend strong support to the hypothesis that the initial phase of hyperglycemia is due, at least in part, to increased glycogenolysis.

The formation of new glucose via gluconeogenesis has been shown to be affected biphasically during endotoxin shock (75). Characteristically, during the early phases of irreversible endotoxin shock this process and the formation of new glucose is increased, however during the later stages of shock gluconeogenesis is depressed (72,75). The release of glucocorticoids (79), combined with the increase in gluconeogenic substrates such as lactate and alanine (72), have been shown to be responsible, at least in part, for the increase in gluconeogenesis during endotoxin shock. The glucocorticoids stimulate gluconeogenesis by stimulating several enzymes in the liver to cause accelerated conversion of gluconeogenic substrates into glucose (85). The factors responsible for the depression of hepatic gluconeogenesis during the later stages of endotoxin shock appear to be highly diversified and will be discussed in the following section (see Hypoglycemia during Endotoxin Shock).

Filkins et al (116) examined the relationship between fasted and fed rats and how enhanced insulin secretion is deleterious in endotoxin shock. Overnight fasting has been shown to markedly depress insulin levels as compared to the fed state. It was subsequentially shown that fed rats exhibited a greater sensitivity to endotoxin than overnight fasted rats. The fact that elevated insulin levels were sensitizing rats to endotoxin lead the authors to conclude that hyperinsulinemia was affecting the liver by preventing it from converting from a glycogenolytic organ to a gluconeogenic organ. Whereas in the fasted state the liver is already a gluconeogenic organ and is not as susceptible to gluconeogenic depression.

In summary, the increase in blood glucose levels during the early phases of endotoxin shock is caused by an increased mobilization of glucose by the liver via gluconeogenesis and glycogenolysis (75).

Hypoglycemia during Endotoxin Shock

Profound hypoglycemia during the intermediate and later stages of endotoxin shock is a well documented fact in a variety of animal species. Hinshaw (15) recently reviewed the role of glucose in endotoxin shock and stated that "hypoglycemia is not merely a terminal event but is correlated with the pathogenesis of shock." Therefore, the mechanisms responsible for the shift from hyperglycemia to hypoglycemia during endotoxin shock are fundamentally important to our understanding of its pathogenesis.

A depletion of blood glucose levels during endotoxin shock could occur as the result of one or any combination of the following: 1) a loss of glucose through excretion, 2) a decrease in glucose production, and 3) an increase in glucose utilization. In shock, there is no evidence that glucosuria occurs (88). In cardiovascular collapse of any etiology renal shutdown is a feared complication. As to the second factor, decreased glucose production, Filkins et al (89) demonstrated a depression of gluconeogenesis as determined by *in vivo* and *in vitro* studies of the rat liver. They suggested that the depression appeared to be caused by a mediated effect rather than a direct one, because hepatocytes incubated with endotoxin *in vitro* showed no change in capacity for gluconeogenesis. Groves et al (73) reported impaired gluconeogenesis during live *E. coli* bacteremic shock in the dog and suggested that there appears to be a metabolic block between pyruvate and the formation of glucose in the gluconeogenic pathway. A study by Lanoue et al (90) demonstrated that the overall rate of gluconeogenesis in rat liver was impaired by endotoxemia and indicated that a decrease in glucose-6-phosphatase was the cause. Williamson's group (91) also demonstrated impaired gluconeogenesis during endotoxin shock in the rat and suggested that there is a defect in the enzymatic step between fructose-1,6-diphosphate and fructose-6-phosphate, as evidenced by an accumulation of fructose-1,6-diphosphate.

Sufficient evidence therefore exists to document that in endotoxin shock glucose production is decreased and that this decrease is due essentially to a shutdown of gluconeogenesis. However, the mechanisms responsible for the failure of gluconeogenesis appear to be highly diversified.

The third factor which can cause a decrease in blood glucose levels during shock is an increase in glucose utilization. Several investigators have established the contribution of the peripheral tissues to the development of hypoglycemia during shock by eliminating the liver, spleen, pancreas and the entire gastrointestinal tract from

the circulation (65,77,92). As early as 1944, Russel et al (65) reported rapid and profound hypoglycemia in eviscerated rats during hemorrhagic shock, which they attributed to inefficient metabolism of glucose via anaerobic glycolysis due to peripheral anoxia. More recently, Peyton et al (92) demonstrated similiar results during endotoxin shock in eviscerated dogs and attributed the decline in blood glucose levels to a predominance of anaerobic over aerobic metabolism of glucose. These studies (65,92) have demonstrated that peripheral carbohydrate utilization increases during shock and that the mechanism responsible for this increase is primarily due to the ineffecient metabolism of glucose via anaerobic glycolysis.

A prominent feature of many pathological processes including sepsis is an increase in body temperature, which is often associated with an increased metabolic rate because of the influence of temperature on chemical reactions (93). Dubois (94) pointed out that for every degree fahrenheit increase in temperature there is a corresponding 7.2 percent increase in caloric expenditure. However, Roe and Kinney (95,96) demonstrated that the increased metabolic rate of septic patients was in excess to that predicted for an elevated temperature alone. Furthermore, a case study by Halmagyi et al (93) pointed out that some normothermic septic patients have elevated metabolic rates. Long et al (97) reported that the rate of glucose oxidation was more that doubled in septic patients. Hinshaw et al (98) demonstrated that large quantities of glucose were needed to maintain constant blood glucose levels in endotoxin-shocked dogs and that exogenously administered glucose prevented death. It should be evident from the preceeding studies that there is an elevated metabolic rate associated with sepsis and that fever alone cannot account for the increase in energy requirements.

Although it is well recognized that there is an increase in peripheral glucose utilization during endotoxin shock, the primary organs responsible for this increase have not been completely defined. Hinshaw et al (67) have demonstrated that neither the myocardium nor the lungs are sites of increased glucose utilization during endotoxin shock. Raymond and Emerson (99) demonstrated that glucose uptake by the central nervous system decreases during endotoxin shock in the dog. Hinshaw and coworkers (100) demonstrated that glucose uptake by the leucocyte (WBC) mass increases during endotoxin shock in the dog. However, it is improbable that such a small tissue mass (WBC) could be totally responsible for the large increase in glucose utilization observed during endotoxin shock.

Skeletal muscle has been considered a prime candidate for the increase in glucose utilization during endotoxin shock because it can utilize large quantities of glucose via anaerobic glycolysis, as occurs during low flow states. However, in 1978 a study by Furr et al (101) showed no change in glucose uptake by the isolated dog forelimb during E. coli endotoxin shock. On the other hand, recent work by Raymond et al (83) demonstrated that glucose uptake by the naturally perfused gracilis muscle increased during endotoxin shock in the dog. The mechanism for this increased glucose uptake was shown to be related to local tissue hypoxia secondary to muscle ischemia (83). At about the same time, Romanosky et al (102) reported that glucose uptake increased in the dog hindlimb during mild endotoxemia and that there was an increase in lactate and alanine concentration in femoral blood draining the non-isolated hindlimb during moderate endotoxin shock. However this study should be viewed with skepticism since the hindlimb muscle was not vascularly isolated which makes it difficult to know the percent contribution of pure skeletal muscle to the observed changes. It is also difficult to explain the changes in calculated vascular resistance in this hindlimb preparation, which did not change during the first 30 minutes of shock, and then decreased substantially during the entire 4 hour shock period. This is surprising since both active and passive forces which accompany systemic hypotension would dictate an increase in vascular resistance in the hindlimb. Indeed, vascular resistance has been reported to increase substantially during endotoxin shock under conditions of natural and constant blood flow in dog forelimb (46,103), in skeletal muscle and skin of the dog forelimb (104), and in the gracilis muscle (hindlimb) (83,84).

This conclusion is not in agreement with work by Raymond et al (83) where it was reported that glucose uptake by the isolated gracilis muscle did not increase during endotoxin shock when blood flow to the muscle was maintained constant, preventing the development of local tissue hypoxia. The lack of an increase in glucose uptake by gracilis muscle during endotoxin shock was attributed to the fact that local tissue hypoxia was prevented and that following an intravenous bolus injection of endotoxin the endotoxin molecules are rapidly cleared from the circulation by the reticuloendothelial system (105). It was also shown in a separate study, as indicated previously, that glucose uptake by the naturally perfused gracilis muscle increases during _E. coli_ endotoxin shock and that the mechanism for this increased glucose uptake was related to local tissue hypoxia. However, while glucose uptake by the constant flow perfused gracilis muscle does not increase during endotoxin shock, gracilis muscle glucose uptake does increase in the constant flow perfused muscle during live _E. coli_ bacteremic shock (84). This was believed to occur because the endotoxin molecules are released slowly as the live bacteria are killed and the reticuloendothelial system is unable to adequately clear the continually released endotoxin.

Concerning the research of this thesis, we were somewhat puzzled by the study of Furr et al (101), which showed no increase in glucose uptake by the naturally perfused dog forelimb during endotoxin shock. Since the forelimb contains a substantial amount of skeletal muscle there is no reason to suspect that muscle in the forelimb is any different in its response to metabolic stimuli than skeletal muscle from the hindlimb. This apparent discrepancy between glucose uptake by the isolated gracilis muscle and glucose uptake by the isolated forelimb preparation is the subject of this thesis research.

Although systemic arterial blood pressure and forelimb blood flow decreased markedly after endotoxin administration, the isolated forelimb never became hypoxic in the Furr et al (101) study. This paradox, viz. lack of local forelimb hypoxia in the presence of severe forelimb ischemia, would implicated a reduced forelimb metabolic rate such that the severely reduced forelimb blood flow and decreased oxygen delivery to the forelimb was adequate to meet the metabolic needs of the tissue.

Upon review of the protocols of the isolated gracilis muscle (83,84) and isolated forelimb studies (101), a major difference was that temperature of the isolated forelimb was not maintained at core temperature as it was in the isolated gracilis muscle. Therefore, glucose uptake by the isolated forelimb could be related to the temperature of the isolated organ (Q_{10} effect). Furthermore, a reduction in isolated organ temperature could have also blunted the metabolic response of skin to endotoxin. Therefore the hypotheses to be tested are: 1) that glucose uptake by forelimb skeletal muscle and skin increases during endotoxin shock in the dog; and 2) that the mechanism for this proposed increase in glucose uptake is related to local tissue hypoxia and/or tissue ischemia.

CHAPTER III

METHODS

Experimental Animals

Mongrel dogs of either sex weighing 20±2 kg were fasted for 16 to 24 hours but allowed water *ad libitum* prior to use in this study. The animals were anesthetized with an intravenous infusion of pentobarbital sodium (30 mg/kg) and maintained at a surgical level of anesthesia with supplemental doses of anesthetic (1 ml of a 50 mg/ml solution). Surgical anesthesia was indicated by the loss of the wink reflex in response to touching the conjuctiva.

The anesthetized animal was placed on a surgical table on its right side. The trachea was intubated with a cuffed endotrachial tube, and the animal ventilated with room air using a Harvard constant volume respirator. Positive end-expiratory pressure was employed to maintain acceptable arterial oxygen tension by submerging the end of the expiratory line approximately 3 centimeters under water. Heparin sodium (10,000 U.S.P. units) was administered intravenously before the extracorporeal system was established to prevent clotting. Supplemental doses of heparin sodium (200 U.S.P. units) were given hourly.

Forelimb Preparation

The isolated, innervated canine forelimb was employed as the test organ. The brachial artery, brachial vein, cephalic vein and forelimb nerves were isolated and kept intact after circumferentially sectioning the skin approximately 3 centimeters above the elbow using a thermocautery. The remaining muscles and connective tissue were isolated and sectioned using a thermocautery. The humerus was cut with a bone saw and the ends of the marrow cavity packed with bone wax and capped with latex to prevent the exposed ends of the humerus from bleeding.

The brachial and cephalic veins were retrogradely cannulated with polyethylene (PE-320) tubing and their outflows allowed to drain by gravity into a 600 ml glass reservoir. The reservoir was primed with either physiological saline or high molecular weight Dextran (79,000 daltons) prior to starting the extracorporeal circuit. Dextran, a volume expander, was used when arterial blood pressure was declining due to excessive surgical set-up time or blood loss. The level of blood in the reservoir was maintained constant using an electronic-liquid-level controller to prevent changes in total body blood volume during the course of the experiment. Blood was returned to the cannulated (PE-320) left external jugular vein by means of a Masterflex blood pump (model no. 7520-00). The reservoir was placed on a combination stirrer-warmer to prevent formed elements from accumulating on the bottom and to maintain blood temperature at core temperature, respectively. The forelimb stump was then bathed with mineral oil and covered with plastic wrap to prevent drying.

After surgically isolating the forelimb, blood entered the forelimb only through the brachial artery and exited only through the brachial and cephalic veins. The median cubital vein, which represents the major anastomotic channel between the brachial and cephalic veins, was ligated to ensure that brachial venous outflow was predominately from skeletal muscle and that cephalic venous outflow was predominately from skin (106).

Mean systemic arterial blood pressure was measured from the cannulated (PE-240) right femoral artery. The right femoral vein was cannulated (PE-240) for administration of endotoxin and/or saline and drugs. Pressures were recorded using Hewlett-Packard pressure transducers (model no. 1280) coupled to a 4-channel Hewlett-Packard direct writing recorder (model no.77543).

Temperature of the isolated forelimb, contralateral (intact) forelimb, and the animals core temperature was monitored with subcutaneously placed thermistor probes and a rectal probe, respectively. Temperatures were recorded using a Yellow Springs Tele-Thermometer (model no. 43TF). An electric heating pad was placed under the animal to maintain core temperature at control.

Experimental Procedures

Group 1: Controlled Forelimb Temperature-Natural Flow

The surgical preparation of the isolated forelimb was as described above and provisions for temperature regulation were made. This was accomplished by wrapping the isolated forelimb in a temperature regulated water jacket and covering it with 4 towels and plastic wrap. Isolated forelimb temperature was then maintained at the same temperature as the contralateral (intact) forelimb, which was equal to the animals core temperature. This preparation was completed in 10 experimental animals and 6 control animals.

Group 2: Uncontrolled Forelimb Temperature-Natural Flow

After surgically isolating the forelimb as described above, it was placed on the surgical table and exposed to ambient room temperature (23 degrees celcius) with no provision for maintaining forelimb temperature at contralateral (intact) forelimb temperature. This preparation was completed in 8 experimental and 6 control animals.

Group 3: Controlled Forelimb Temperature-Constant Flow

After surgically isolating the forelimb and equipping the forelimb for temperature maintenance as described above (Group 1), a Masterflex blood pump was interposed between the cannulated left femoral (PE-320) and right brachial artery (PE-320) for constant flow perfusion. Total forelimb blood flow was set by adjusting the perfusion pump so that forelimb perfusion pressure approximated mean systemic

arterial pressure. Forelimb perfusion pressure was measured from a needle tipped PE-90 catheter inserted into the perfusion tubing distal to the perfusion pump. Pressures were recorded as described previously. This preparation was completed in 4 experimental and 4 control animals.

Chemical Analyses

Simultaneous blood samples were collected anaerobically from the femoral artery pressure catheter and the brachial and cephalic venous outflow tubing. The following analyses were completed: 1) PO_2, PCO_2, and pH with a Radiometer Acid-Base analyzer; 2) glucose concentration with a Yellow Springs Glucose Analyzer; 3) hematocrit by microcentrifugation.

Control systemic arterial blood pressure, muscle and skin blood flows and metabolic variables listed above were determined following a 15 to 30 minute stabilization period. In the temperature controlled forelimb groups this stabilization period allowed the temperature of the isolated forelimb to equilibrate with contralateral (intact) forelimb temperature. Shock was induced by a 5 minute intravenous infusion of 2 mg/kg purified *E. coli* endotoxin (LD100; Lipopolysaccharide B:8 127; Difco Co.) suspended in 20 ml of physiological saline. Hemodynamic and metabolic measurements were made before and at 30 minute intervals for 3.5 to 4 hours after shock induction or until death of the animal. Control animals received 20 ml of physiological saline containing no endotoxin infused intravenously over a 5 minute period. Otherwise, these animals were treated identically to the experimental animals.

Calculations

Forelimb skeletal muscle (MBF) and skin blood flows (SBF) were determined by timed collections of the brachial and cephalic venous outflows, respectively, using a graduated cylinder and stopwatch. Total forelimb blood flow (FBF) was obtained by adding muscle and skin blood flow. At the end of each experiment, muscle and skin were dissected from the forelimb and weighed so blood flow could be expressed on a weight basis.

1. MBF (ml/min/100gm) = MBF (ml/min)/muscle wt. (gm) X 100
2. SBF (ml/min/100gm) = SBF (ml/min)/skin wt. (gm) X 100
3. FBF (ml/min/100gm) = (MBF (ml/min) + SBF (ml/min)) /forelimb wt. (gm) X 100

Forelimb skeletal muscle (MVR), skin (SVR), and total forelimb vascular resistances (FVR) were calculated by dividing forelimb perfusion pressure (FPP) by the appropriate blood flow.

4. MVR (mmHg/ml/min/100gm) = FPP/MBF (ml/min/100gm)
5. SVR (mmHg/ml/min/100gm) = FPP/SBF (ml/min/100gm)
6. FVR (mmHg/ml/min/100gm) = FPP/FBF (ml/min/100gm)

Forelimb skeletal muscle (MGU) and skin glucose uptakes (SGU) were calculated as the product of the arterio-venous glucose difference and appropriate blood flow. Total forelimb glucose uptake (FGU) was obtained by adding muscle and skin values.

7. MGU (mg/min/100gm) = MBF (ml/min/100gm) X arterio-venous glucose difference (mg/ml)

8. SGU (mg/min/100gm) = SBF (ml/min/100gm) X arterio-venous glucose difference (mg/ml)

Total forelimb venous PO_2, PCO_2 and pH was calculated by averaging the appropriate brachial and cephalic venous PO_2, PCO_2 and pH at each time period.

Details of Whole Blood Glucose Determination

Whole blood glucose concentration was determined with a Yellow Springs glucose analyzer (YSI model no. 23A). The YSI model 23A uses an oxidase enzyme hydrogen peroxide sensor which is highly specific for glucose. Glucose concentration is determined with a sample of only 25 microliters, with no sample modification required. Glucose concentration can be detected in the range of zero to 500 mg/dl with a sensitivity of 1 mg/dl.

Principles of Operation

The conversion of glucose and oxygen in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide (Reaction 1) is the first reaction for the determination of glucose concentration. The platinum anode, which is part of the sensor probe, oxidizes a constant portion of the hydrogen peroxide (Reaction 2), formed in reaction 1. The current produced from reaction 2 is directly proportional to the glucose concentration in the sample. To complete the circuit, oxygen is reduced to water at the silver cathode (Reaction 3).

Reaction 1:

$\text{D-glucose} + \text{oxygen} \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + \text{hydrogen peroxide}$

Reaction 2:

$\text{hydrogen peroxide} \longrightarrow 2\ \text{hydrogens} + 2\ \text{electrons}$

Reaction 3:

$4\ \text{hydrogens} + \text{oxygen} \longrightarrow 2\ \text{waters} + 4\ \text{electrons}$

Statistical Analyses

Data were analyzed using one- and two-way analysis of variance. One-way analysis of variance was used when comparing uneven sample sizes, otherwise two-way analysis of variance was used. Uneven sample sizes were the result of animals dying during the course of the experiment. Means were compared using Duncans and Student-Newman-Keuls (SNK) tests. Duncans test, which is computationally identical to the SNK test except for the use of another table of critical values, was used when the SNK test was unable to detect any significant difference between the means (107). The "Students T" test modified for unpaired replicates was used compare control, non-shocked animals to shocked animals of groups 1 and 3. A "P" value less than or equal to 0.05 was considered significant.

CHAPTER IV

RESULTS

Hemodynamic changes and arterial glucose concentration of both the controlled and uncontrolled forelimb temperature shock groups are graphically illustrated in Figure 1. The numbers in parentheses at the top of Figures 1-12 and the numbers in the first line of Tables 1-6 represent the number of animals at each data point; the smaller numbers represent animals dying during the course of the experiment. In both groups, as shown in Figure 1, arterial blood pressure decreased to approximately 55 mmHg by 30 minutes of shock ($P<0.05$) and remained below control for the duration of the experiment ($P<0.05$). Muscle, skin and total forelimb blood flows in both groups decreased to approximately the same level at 30 minutes of shock ($P<0.05$) and remained below control for the duration of the experiment ($P<0.05$). Muscle, skin and total forelimb vascular resistances were significantly increased throughout the shock period in both groups of animals ($P<0.05$). In both groups of animals, arterial glucose concentration increased at 30 minutes following shock induction ($P<0.05$), then progressively decreased for the duration of the experiment ($P<0.05$). This figure illustrates that the degree of systemic hypotension,

Figure 1. Muscle, skin and total forelimb hemodynamics and arterial glucose concentration in the controlled and uncontrolled forelimb temperature groups during *E. coli* endotoxin shock. (n)=number of animals; ABP=mean systemic arterial blood pressure; BF=blood flow; R=vascular resistances; PRU=peripheral resistance units expressed in mmHg/ml/min/100gm; Art. Glc. Conc.=Arterial blood glucose concentration; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

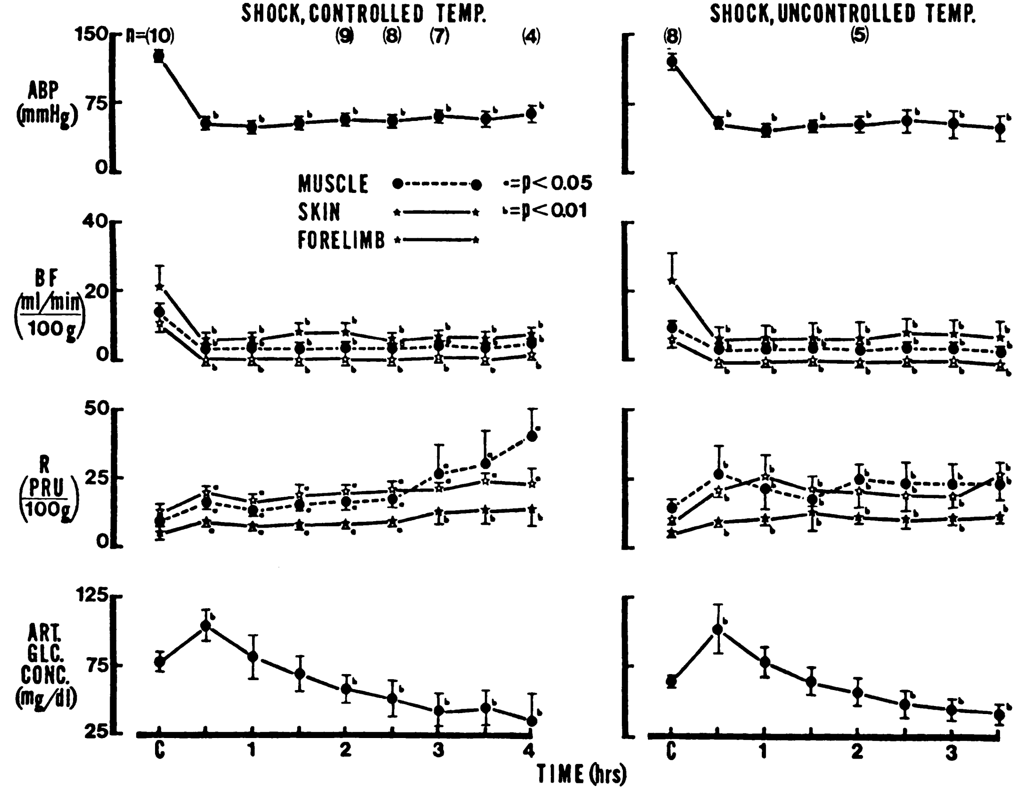
SHOCK, CONTROLLED TEMP.
SHOCK, UNCONTROLLED TEMP.
n=(10)
(9)
(8)
(7)
(4)
(8)
(5)
ABP (mmHg)
150
75
0
MUSCLE
SKIN
FORELIMB
a=p<0.05
b=p<0.01
BF (ml/min / 100 g)
40
20
0
R (PRU / 100g)
50
25
0
ART. GLC. CONC. (mg/dl)
125
75
25
C
1
2
3
4
TIME (hrs)
C
1
2
3

Figure 1.

the reduced forelimb blood flows, the increased vascular resistances and the degree of hypoglycemia were similar in both controlled and uncontrolled forelimb temperature groups.

Forelimb muscle glucose uptake, muscle venous PO_2 and core and isolated forelimb temperatures of both controlled and uncontrolled forelimb temperature shock groups are graphically illustrated in Figure 2. Following shock induction in the controlled forelimb temperature group, forelimb muscle glucose uptake increased at 30 minutes following shock induction and remained elevated throughout the 4 hour experimental period ($P<0.05$). In contrast, shock induction in the uncontrolled forelimb temperature group resulted in no change in forelimb glucose uptake for the entire 3.5 hour shock episode ($P>0.05$). In the controlled forelimb temperature group, forelimb muscle venous PO_2 decreased from 47 mmHg to 24 mmHg at 30 minutes of shock and remained depressed for the duration of the experiment ($P<0.01$). In the uncontrolled forelimb temperature group, muscle venous PO_2 decreased to only 39 mmHg from a control value of 47 mmHg ($P<0.05$) at 30 minutes of shock and remained below control throughout the experiment ($P<0.05$).

In the controlled forelimb temperature group, isolated forelimb temperature was not different from core temperature at any time before or during shock ($P>0.05$). However, isolated forelimb temperature did decrease slightly at 2 and 2.5 hours ($P<0.05$) and did increase slightly at 3 and 3.5

Figure 2. Muscle metabolic and temperature variables in the controlled and uncontrolled forelimb temperature groups during *E. coli* endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; c=$P<0.05$ comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

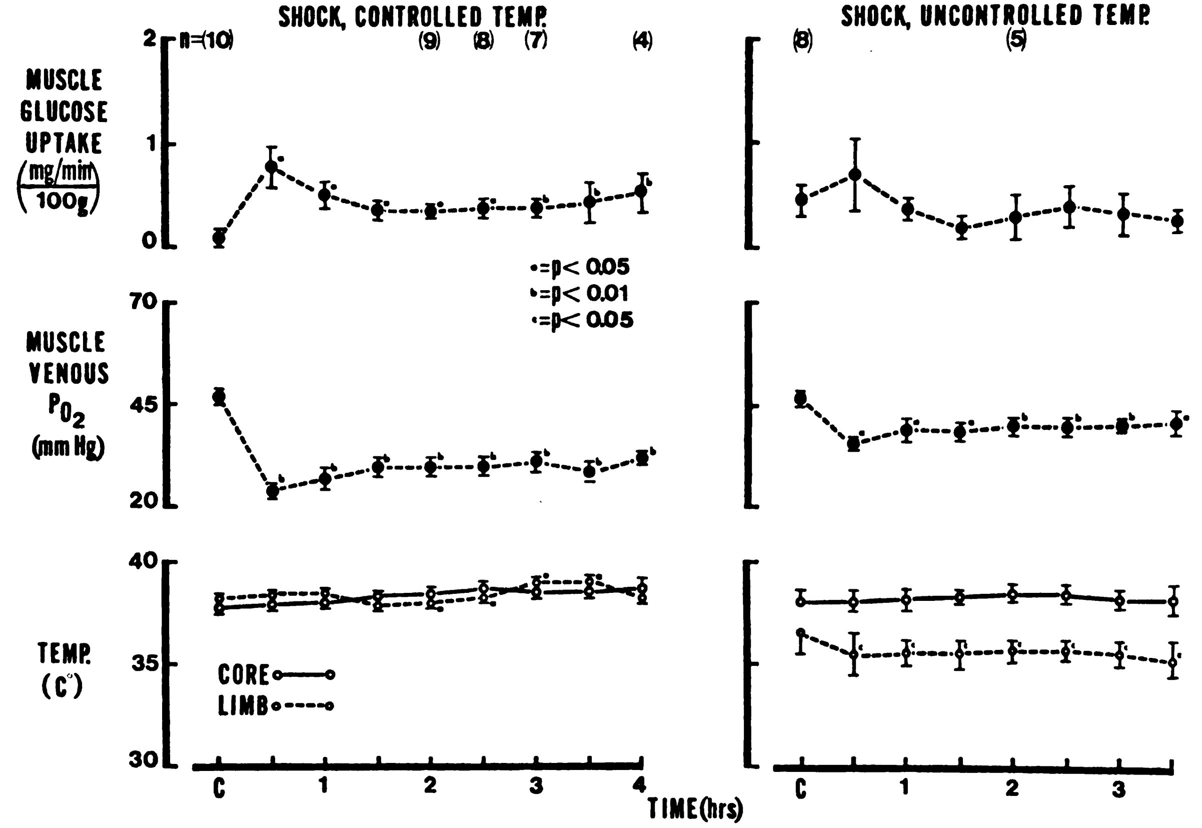
SHOCK, CONTROLLED TEMP.
SHOCK, UNCONTROLLED TEMP.
n=(10)
(9)
(8)
(7)
(4)
(8)
(5)
MUSCLE GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
^{a}=p< 0.05
^{b}=p< 0.01
^{c}=p< 0.05
MUSCLE VENOUS P_{O_2} (mm Hg)
70
45
20
TEMP. (C°)
40
35
30
CORE
LIMB
C
1
2
3
4
TIME(hrs)
C
1
2
3

Figure 2.

hours ($P<0.05$) of shock compared to the control, preshock value. These temperature changes did not exceed 0.2 degrees celcius. Core temperature did not change significantly ($P>0.05$) in either the temperature controlled or uncontrolled temperature group ($P>0.05$). However, in the uncontrolled forelimb temperature group, isolated forelimb temperature decreased 3 degrees celcius on the average ($P<0.05$) by 30 minutes of shock and remained at this lowered level for the remainder of the experiment.

Forelimb skin glucose uptake, skin venous PO_2 and core and isolated forelimb temperatures of both controlled and uncontrolled forelimb temperature shock groups are graphically illustrated in Figure 3. Temperature data shown in Figures 3 and 4 are identical to those shown in Figure 2. After administration of endotoxin in the controlled forelimb temperature group, forelimb skin glucose uptake increased at 30 minutes following shock induction ($P<0.05$) and remained elevated throughout the experimental period ($P<0.05$) except at the fourth hour ($P>0.05$). However, shock induction in the uncontrolled forelimb temperature group resulted in no change in forelimb skin glucose uptake for the entire 3.5 shock episode ($P>0.05$). In the controlled forelimb temperature group, forelimb skin venous PO_2 decreased from 47 mmHg to 30 mmHg at 30 minutes of shock and remained depressed for the duration of the experiment ($P<0.01$). In the uncontrolled forelimb temperature group, skin venous PO_2 decreased to only 39 mmHg from a control value of 54 mmHg ($P<0.05$) at 30

Figure 3. Skin metabolic and temperature variables in the controlled and uncontrolled forelimb temperature groups during *E. coli* endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; c=$P<0.05$ comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

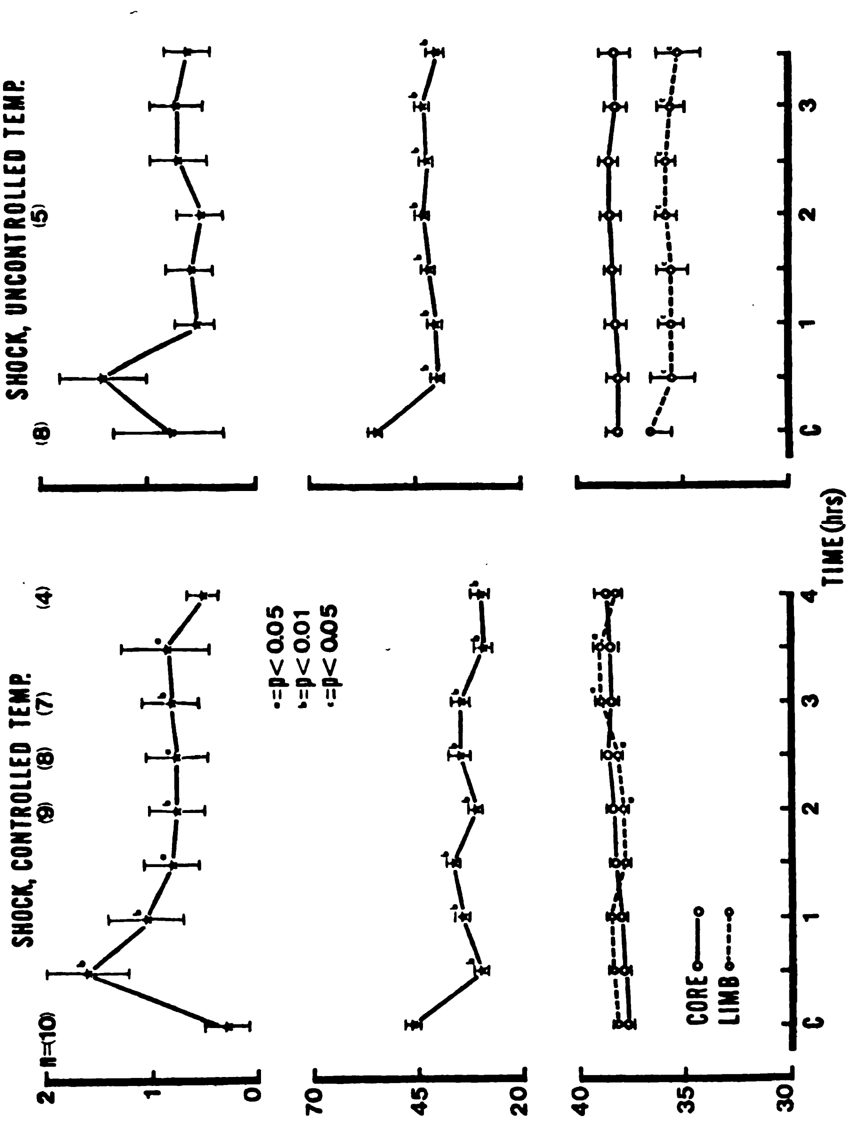
SHOCK, CONTROLLED TEMP.
SHOCK, UNCONTROLLED TEMP.
n=(10)
(9)
(8)
(7)
(4)
(8)
(5)
SKIN GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
SKIN VENOUS P_{O_2} (mm Hg)
70
45
20
TEMP. (C)
40
35
30
a=p< 0.05
b=p< 0.01
c=p< 0.05
CORE
LIMB
C
1
2
3
4
TIME (hrs)

Figure 3.

minutes of shock and remained below control throughout the experiment ($P<0.05$).

Total forelimb glucose uptake, forelimb venous PO_2 and core and isolated forelimb temperature of both controlled and uncontrolled forelimb temperature shock groups are graphically illustrated in Figure 4. Following shock induction in the controlled forelimb temperature group, forelimb glucose uptake increased 30 minutes after shock induction ($P<0.01$) and remained elevated throughout the experimental period ($P<0.05$), except at 90 minutes following shock induction ($P>0.05$). In contrast, shock induction in the uncontrolled forelimb temperature group resulted in no change in total forelimb glucose uptake for the entire 3.5 hour shock episode ($P>0.05$). In the uncontrolled forelimb temperature group, forelimb venous PO_2 decreased to only 37 mmHg from a control value of 50 mmHg ($P<0.01$) at 30 minutes of shock and remained below control throughout the experiment ($P<0.05$).

Table 1 shows changes in metabolic variables in the controlled forelimb temperature-natural flow group during *E. coli* endotoxin shock. After the administration of endotoxin, arterial PO_2 and arterial PCO_2 were not different from control at any point during the shock episode ($P>0.05$). However, brachial venous PCO_2 increased from a control value of 33.9 mmHg to 43 mmHg by 60 minutes of shock and remained elevated until 150 minutes of shock ($P<0.05$), after which it was not different from control ($P>0.05$). Cephalic venous

Figure 4. Total forelimb metabolic and temperature variables in the controlled and uncontrolled forelimb temperature groups during *E. coli* endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; c=$P<0.05$ comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

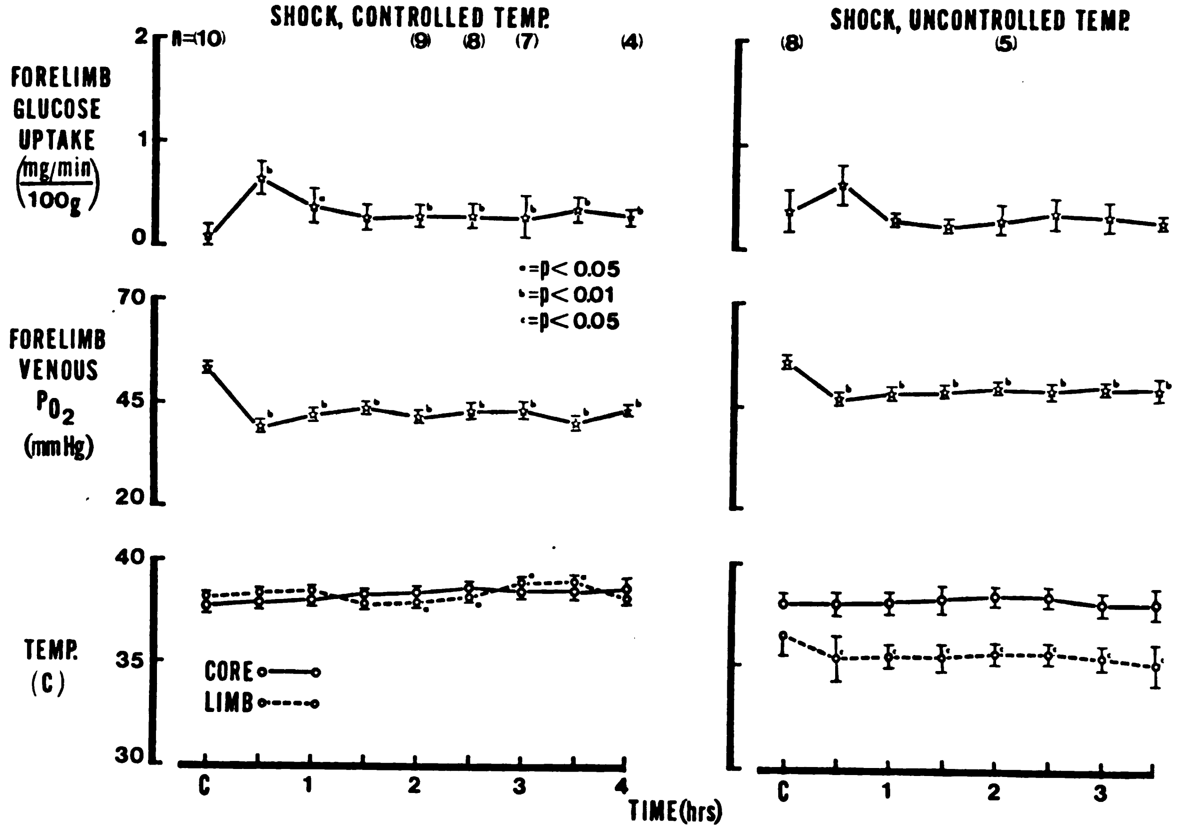
SHOCK, CONTROLLED TEMP.
SHOCK, UNCONTROLLED TEMP.
n=(10)
(9)
(8)
(7)
(4)
(8)
(5)
FORELIMB GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
FORELIMB VENOUS P_{O_2} (mmHg)
70
45
20
^{a}=p< 0.05
^{b}=p< 0.01
^{c}=p< 0.05
TEMP. (C)
40
35
30
CORE
LIMB
C
1
2
3
4
TIME(hrs)
C
1
2
3

Figure 4.

Table 1. Changes in metabolic variables in the controlled forelimb temperature-natural flow group during E. coli endotoxin shock.

Table 1. Changes in metabolic variables in the controlled forelimb temperature-natural flow group during E. coli endotoxin shock.

	Control	Endotoxin							
		Time (min)							
Parameter	0	30	60	90	120	150	180	210	240
Number (n)	10	10	10	10	9	8	7	7	4
Arterial PO_2	80.4 ±3.0	69.9 ±3.8	63.8 ±4.1	66.9 ±4.1	64.0 ±4.2	66.6 ±3.5	62.4 ±3.1	72.3 ±6.0	69.5 ±7.1
Arterial PCO_2	32.8 ±1.5	31.8 ±2.1	36.3 ±2.1	33.7 ±2.1	32.3 ±2.3	30.0 ±1.9	29.6 ±2.2	27.6 ±2.2	30.8 ±1.6
Brachial Venous PCO_2	33.9 ±1.4	40.4 ±2.2	43.0* ±2.2	42.5* ±2.2	41.9* ±2.2	43.0* ±2.9	41.0 ±3.0	38.3 ±2.9	35.5 ±2.8
Cephalic Venous PCO_2	35.0 ±1.2	42.5 ±2.7	44.2* ±2.6	44.9* ±3.5	43.2 ±3.1	46.4* ±3.8	43.7 ±2.8	39.3 ±3.5	37.3 ±4.4

(Continued on next page)

Table 1 (cont'd).

	Control	Endotoxin							
		Time (min)							
Parameter	0	30	60	90	120	150	180	210	240
Arterial pH	7.39 ±0.02	7.30* ±0.03	7.25* ±0.03	7.28* ±0.03	7.28* ±0.03	7.28* ±0.03	7.31* ±0.02	7.27* ±0.02	7.19* ±0.07
Brachial Venous pH	7.40 ±0.01	7.25* ±0.03	7.19* ±0.03	7.21* ±0.03	7.24* ±0.03	7.22* ±0.03	7.24* ±0.02	7.21* ±0.02	7.24* ±0.02
Cephalic Venous pH	7.40 ±0.02	7.23* ±0.03	7.17* ±0.03	7.22* ±0.03	7.23* ±0.03	7.21* ±0.04	7.21* ±0.02	7.19* ±0.03	7.26* ±0.02
Hematocrit	34.0 ±1.0	41.8* ±2.4	42.5* ±2.0	42.7* ±2.1	43.1* ±2.4	45.1* ±1.6	46.9* ±1.9	47.4* ±2.7	46.5* ±2.5

*denotes values which are significantly different from control ($P<0.05$).
Values are expressed as mean ± S.E.

PCO_2 was elevated at various times throughout the experimental period ($P<0.05$). Arterial pH progressively decreased from a control value of 7.39 units to 7.19 units by 240 minutes of shock ($P<0.05$). Brachial venous pH and cephalic venous pH were also below control throughout the shock episode ($P<0.05$). Hematocrit was increased by 30 minutes of shock ($P<0.05$) and remained above control for the duration of the shock episode ($P<0.05$).

Table 2 shows changes in metabolic variables in the uncontrolled forelimb temperature-natural flow group during E. coli endotoxin shock. Following shock induction arterial PO_2 and arterial PCO_2 were not different from control at any point during the experiment ($P>0.05$). Brachial venous PCO_2 was not different from control at any point during the shock episode ($P>0.05$) except at 90 minutes of shock when it was significantly elevated ($P<0.05$). Sixty minutes after the administration of endotoxin, cephalic venous PCO_2 increased ($P<0.05$) from a control value of 31.3 mmHg to 46.6 mmHg and remained elevated until 150 minutes of shock when it was not different from control ($P>0.05$). Arterial pH, brachial venous pH and cephalic venous pH all decreased immediately after shock induction ($P<0.05$) and remained below control for the duration of the shock episode ($P<0.05$). Hematocrit was elevated by 30 minutes of shock and remained elevated for the entire experimental period ($P<0.05$).

Hemodynamic variables and arterial glucose concentration of both control groups are shown graphically in Figure 5.

Table 2. Changes in metabolic variables in the uncontrolled forelimb temperature-natural flow group during *E. coli* endotoxin shock.

Table 2. Changes in metabolic variables in the uncontrolled forelimb temperature-natural flow group during E. coli endotoxin shock.

	Control	Endotoxin						
		Time (min)						
Parameter	0	30	60	90	120	150	180	210
Number (n)	8	8	8	8	5	5	5	5
Arterial PO_2	82.9 ±4.4	74.8 ±4.5	73.1 ±2.4	75.3 ±3.3	75.4 ±4.4	81.6 ±7.2	83.8 ±6.6	85.4 ±6.5
Arterial PCO_2	31.3 ±2.1	33.0 ±4.2	36.4 ±3.4	35.5 ±3.4	31.2 ±3.5	28.6 ±3.3	30.6 ±4.4	28.8 ±4.1
Brachial Venous PCO_2	33.6 ±2.1	43.8 ±3.2	46.3 ±3.3	48.3* ±3.8	45.0 ±5.3	43.0 ±2.8	41.4 ±2.5	43.0 ±2.0
Cephalic Venous PCO_2	31.3 ±1.6	44.3 ±4.4	46.6* ±3.8	50.0* ±4.6	48.2* ±6.5	42.4 ±1.9	41.4 ±3.3	41.0 ±4.5

(Continued on next page)

Table 2 (cont'd).

Table 2 (cont'd).

	Control	Endotoxin						
		Time (min)						
Parameter	0	30	60	90	120	150	180	210
Arterial pH	7.40 ±0.02	7.27* ±0.02	7.22* ±0.03	7.20* ±0.03	7.22* ±0.03	7.26* ±0.03	7.26* ±0.03	7.26* ±0.03
Brachial Venous pH	7.38 ±0.02	7.22* ±0.03	7.11* ±0.07	7.13* ±0.04	7.17* ±0.04	7.19* ±0.03	7.17* ±0.03	7.17* ±0.04
Cephalic Venous pH	7.36 ±0.03	7.21* ±0.03	7.18* ±0.04	7.08* ±0.07	7.16* ±0.06	7.18* ±0.03	7.18* ±0.04	7.18* ±0.05
Hematocrit	37.6 ±2.0	40.5* ±2.0	42.0* ±2.9	43.3* ±3.5	48.0* ±3.8	47.8* ±3.5	49.2* ±3.2	49.8* ±3.0

*denotes values which are significantly different from control ($P<0.05$).
Values are expressed as mean ± S.E.

In both groups, arterial blood pressure remained relatively constant, decreasing slightly during the last hour and the last 2 hours in the controlled and uncontrolled forelimb temperature groups, respectively ($P<0.05$). Muscle, skin and total forelimb vascular resistances did not change significantly ($P>0.05$). Muscle vascular resistance in the uncontrolled forelimb temperature group increased significantly by 1.5 hours of shock ($P<0.05$), while skin and total forelimb vascular resistance did not change significantly in either group ($P>0.05$). This figure illustrates the stability of the experimental preparation and the similarities between the two groups as evidenced by stable hemodynamic variables and constant blood glucose levels for the duration of the four hour experimental period.

Figure 6 shows muscle, skin and total forelimb glucose uptakes and venous PO_2, along with isolated forelimb and core temperature for both control, non-shocked groups. Glucose uptake did not change significantly in muscle, skin or total forelimb in either group ($P>0.05$). Muscle, skin and total forelimb venous PO_2 decreased slightly ($P<0.05$), but remained above 45 mmHg in both groups. In the controlled forelimb temperature group, isolated forelimb temperature was slightly lower than core temperature (0.2 degrees celcius) at several points during the experiment ($P<0.05$). In the uncontrolled forelimb temperature group, core temperature gradually increased throughout the control episode ($P<0.05$). Isolated forelimb temperature was significantly

Figure 5. Muscle, skin and total forelimb hemodynamics and arterial glucose concentration in the controlled and uncontrolled forelimb temperature non-shocked, control dogs. (n)=number of animals; a=P<0.05 relative to time zero; b=P<0.01 relative to time zero; c=P<0.05 comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

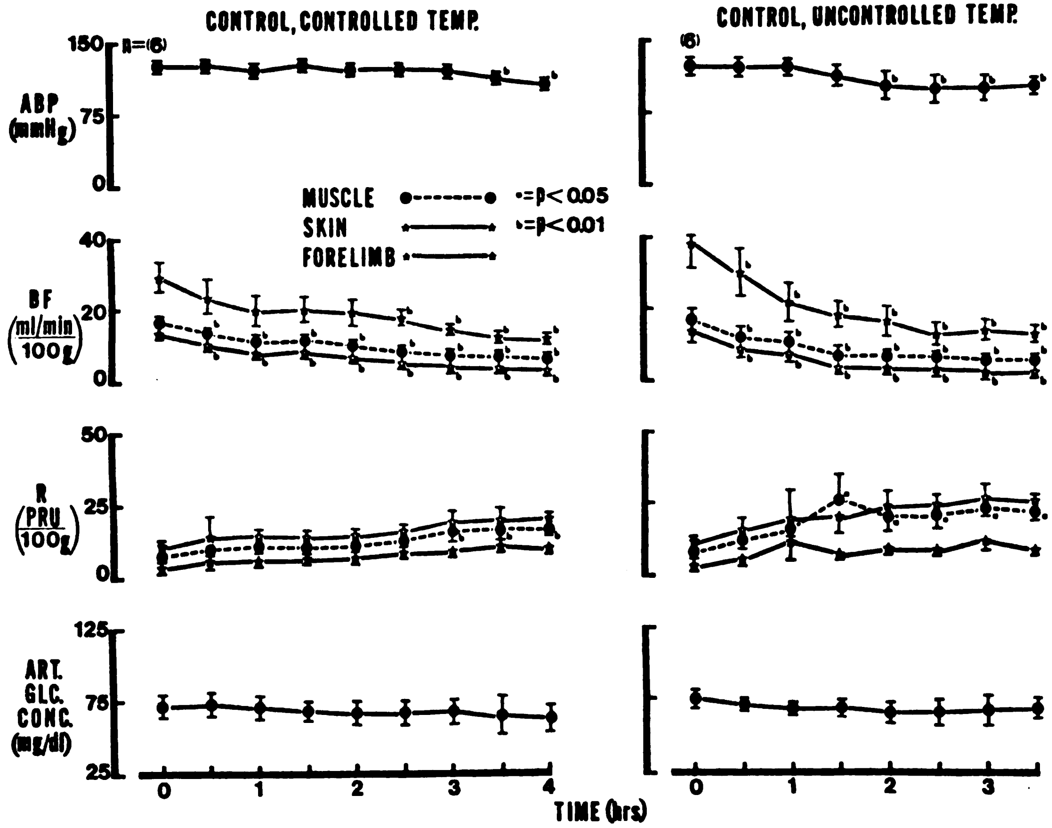
CONTROL, CONTROLLED TEMP.
CONTROL, UNCONTROLLED TEMP.
n=(6)
(6)
ABP (mmHg)
150
75
0
BF (ml/min / 100g)
40
20
0
R (PRU / 100g)
50
25
0
ART. GLC. CONC. (mg/dl)
125
75
25
MUSCLE
SKIN
FORELIMB
a=p<0.05
b=p<0.01
0
1
2
3
4
TIME (hrs)
0
1
2
3

Figure 5.

Figure 6. Control muscle, skin and total forelimb metabolic and temperature variables in the controlled and uncontrolled forelimb temperature groups in non-shocked control animals. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; c=$P<0.05$ comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

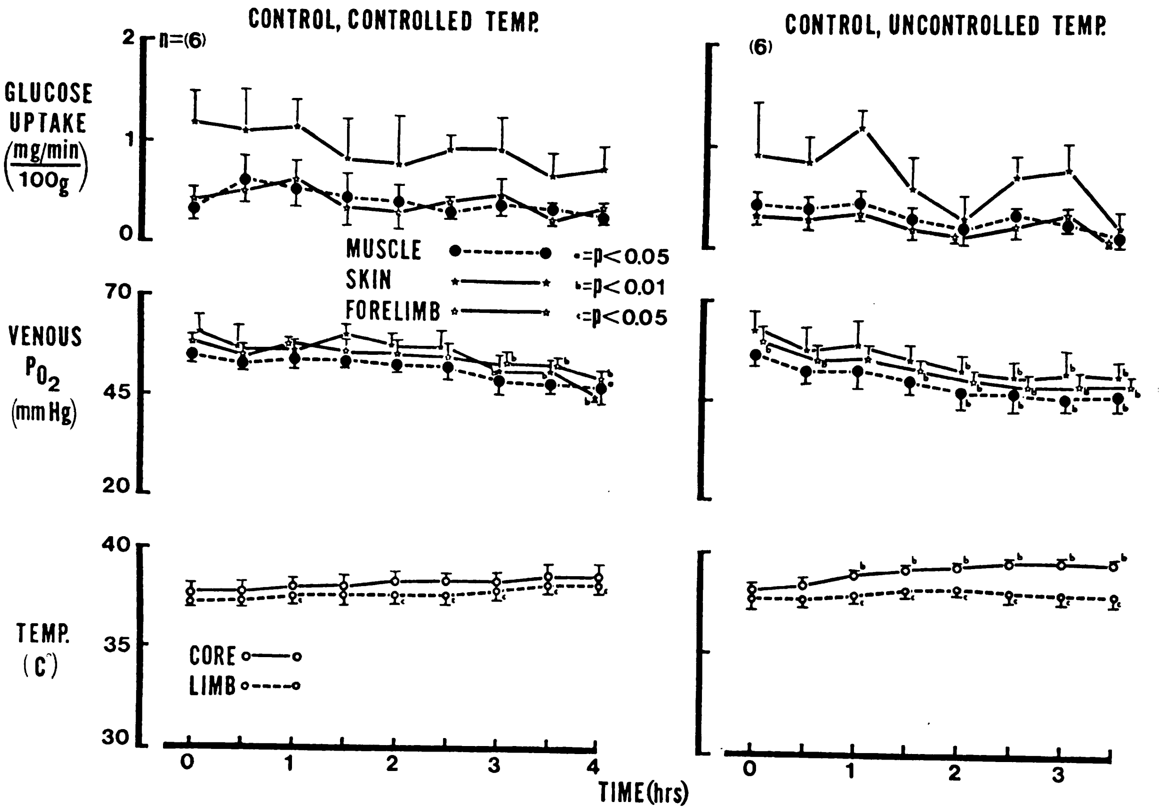
CONTROL, CONTROLLED TEMP.
CONTROL, UNCONTROLLED TEMP.
n=(6)
(6)
GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
VENOUS P_{O_2} (mmHg)
70
45
20
TEMP. (C°)
40
35
30
MUSCLE
SKIN
FORELIMB
a=p<0.05
b=p<0.01
c=p<0.05
CORE
LIMB
0
1
2
3
4
TIME(hrs)
0
1
2
3

Figure 6.

different from core temperature between 1 and 4 hours of the control period ($P>0.05$). This temperature difference never exceeded 1 degree celcius and occured because core temperature increased.

An additional statistical analysis was made to compare the glucose uptake of the endotoxin-shocked animals and the non-shocked animals of the controlled forelimb temperature-natural flow groups. In this analysis, the "Students T" test was used to compare the control group to the shock group to determine whether there were any differences in the magnitude of the calculated glucose uptake between the groups at each data point. Results of this analysis indicate that the absolute values of glucose uptake by skeletal muscle, skin and total forelimb are not different between the two groups ($P>0.05$). However, as previously indicated (Figures 2-4), glucose uptake by skeletal muscle, skin and total forelimb does increase from control values during endotoxin shock in the temperature controlled-natural flow group (Figures 2-4) while there was no change in the non-shocked animals (Figure 6).

Table 3 shows changes in metabolic variables in the controlled forelimb temperature-natural flow group in non-shocked, control animals. Arterial PO_2 remained relatively constant throughout the experimental period with no significant differences from control ($P>0.05$). Arterial PCO_2 was significantly different from control only at 180 minutes of shock ($P<0.05$), otherwise arterial PCO_2 was never different

Table 3. Changes in metabolic variables in the controlled forelimb temperature–natural

Table 3. Changes in metabolic variables in the controlled forelimb temperature-natural flow group in non-shocked, control animals.

	Control								
	Time (min)								
Parameter	0	30	60	90	120	150	180	210	240
Number (n)	6	6	6	6	6	6	6	6	6
Arterial PO_2	84.5 ±6.4	81.0 ±6.0	83.3 ±9.2	80.7 ±5.3	79.2 ±4.6	74.8 ±5.8	70.7 ±4.1	73.3 ±5.3	71.8 ±5.8
Arterial PCO_2	35.8 ±2.0	33.7 ±1.7	33.5 ±1.4	34.0 ±1.6	35.3 ±2.3	36.3 ±1.6	38.2* ±3.1	36.0 ±1.8	35.5 ±0.9
Brachial Venous PCO_2	37.8 ±1.9	35.8 ±1.4	35.8 ±1.2	37.0 ±1.9	37.7 ±1.6	38.7 ±2.1	40.3 ±2.6	38.8 ±1.3	39.8 ±0.9
Cephalic Venous PCO_2	35.2 ±1.5	36.0 ±1.8	35.7 ±1.5	36.3 ±1.6	38.7 ±1.5	39.0 ±1.2	40.8 ±2.8	37.8 ±2.0	39.7 ±1.3

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Table 3 (cont'd).

	Control								
	Time (min)								
Parameter	0	30	60	90	120	150	180	210	240
Arterial pH	7.38 ±0.02	7.37 ±0.02	7.38 ±0.03	7.38 ±0.03	7.36 ±0.03	7.35 ±0.03	7.34 ±0.04	7.34 ±0.03	7.35 ±0.03
Brachial Venous pH	7.37 ±0.01	7.38 ±0.02	7.36 ±0.03	7.36 ±0.03	7.34 ±0.04	7.33 ±0.03	7.32 ±0.04	7.32 ±0.04	7.32 ±0.03
Cephalic Venous pH	7.38 ±0.01	7.36 ±0.02	7.35 ±0.03	7.37 ±0.03	7.34 ±0.03	7.32 ±0.03	7.32 ±0.04	7.32 ±0.04	7.32 ±0.03
Hematocrit	37.5 ±1.3	38.0 ±1.5	38.7 ±1.5	38.3 ±0.9	38.7 ±1.1	39.0 ±0.9	39.7 ±0.6	38.7 ±1.0	36.5 ±1.6

*denotes values which are significantly different from control ($P<0.05$).
Values are expressed as mean ± S.E.

from control ($P>0.05$). Brachial venous PCO_2 and cephalic venous PCO_2 were not different from control at any point during the experimental period ($P>0.05$). Arterial pH, brachial venous pH and cephalic venous pH did not change during the experimental period ($P>0.05$). Hematocrit remained at control levels throughout the experimental period ($P>0.05$).

Table 4 shows changes in metabolic variables in the uncontrolled forelimb temperature-natural flow group in non-shocked, control animals. There were no changes in arterial PO_2, arterial PCO_2 or cephalic venous PCO_2 during the entire experimental period ($P>0.05$). Arterial pH and brachial venous pH did not change during the entire experimental protocol ($P>0.05$). Cephalic venous pH was not different from control during the entire experimental period ($P>0.05$) except at 150 minutes of shock when it was decreased slightly ($P<0.05$). Hematocrit was not different from control during the entire experiment ($P>0.05$).

It should be noted that the data displayed on the left-hand side of Figures 1-4 titled "Shock, Controlled Temp." will be repeated on the left-hand side of Figures 7-10 retitled "Shock, Natural Flow". This was done so that a visual comparison between the controlled forelimb temperature-natural flow and constant flow groups could be made.

Hemodynamic changes and arterial glucose concentrations of both the natural and constant flow groups are graphically illustrated in Figure 7. In both groups, arterial blood

Table 4. Changes in metabolic variables in the uncontrolled forelimb temperature-natural flow group in non-shocked, control animals.

	Control							
	Time (min)							
Parameter	0	30	60	90	120	150	180	210
Number (n)	6	6	6	6	6	6	6	6
Arterial PO_2	80.7 ±6.0	73.0 ±6.6	75.3 ±8.8	80.3 ±8.7	79.2 ±7.8	72.7 ±8.2	76.3 ±7.4	76.7 ±7.7
Arterial PCO_2	31.3 ±1.8	30.3 ±2.0	31.8 ±1.7	30.0 ±1.4	32.7 ±2.9	33.2 ±2.6	31.8 ±2.2	32.7 ±2.7
Brachial Venous PCO_2	30.7 ±1.4	31.2 ±1.5	31.8 ±1.4	30.2 ±1.5	35.2 ±2.3	37.0 ±3.1	35.5 ±1.7	36.2 ±2.9
Cephalic Venous PCO_2	31.8 ±2.0	30.5 ±1.2	31.3 ±1.6	29.8 ±1.5	33.7 ±2.2	35.3 ±2.8	32.8 ±2.1	32.3 ±1.9

(Continued on next page)

Table 4 (cont'd).

Table 4 (cont'd).

Parameter	Control Time (min) 0	30	60	90	120	150	180	210
Arterial pH	7.40 ±0.02	7.39 ±0.02	7.41 ±0.02	7.39 ±0.02	7.36 ±0.03	7.36 ±0.03	7.37 ±0.01	7.35 ±0.02
Brachial Venous pH	7.40 ±0.02	7.40 ±0.02	7.38 ±0.02	7.38 ±0.01	7.37 ±0.02	7.34 ±0.02	7.34 ±0.02	7.34 ±0.03
Cephalic Venous pH	7.40 ±0.02	7.40 ±0.02	7.38 ±0.02	7.38 ±0.02	7.36 ±0.02	7.33* ±0.02	7.36 ±0.02	7.35 ±0.02
Hematocrit	41.0 ±6.9	41.7 ±2.3	42.5 ±2.0	43.5 ±2.0	43.5 ±2.7	44.0 ±2.8	44.8 ±2.8	43.3 ±2.2

*denotes values which are significantly different from control ($P<0.05$)
Values are expressed as mean ± S.E.

pressure decreased to approximately 55 mmHg by 30 minutes of shock and remained below control for the duration of the experiment ($P<0.05$). Muscle, skin and total forelimb blood flows in the natural flow group decreased at 30 minutes of shock ($P<0.05$) and remained below control for the duration of the experiment ($P<0.05$). In the constant flow group, blood flows were maintained at control levels throughout the experimental protocol. Muscle, skin and total forelimb vascular resistances were significantly increased throughout the shock period (P0.05) in the natural flow group. In the constant flow group, muscle and forelimb vascular resistances did not increase until the third hour of shock ($P<0.05$) and remained elevated for the remainder of the experiment ($P<0.05$). In both groups of animals, arterial glucose concentration increased ($P<0.05$) at 30 minutes following shock induction ($P<0.05$); then progressively decreased for the duration of the experiment ($P<0.05$). This figure illustrates that the degree of shock was similiar in both groups of animals.

Forelimb muscle glucose uptake, muscle venous PO_2 and core and isolated forelimb temperatures of both natural and constant flow experiments are graphically illustrated in Figure 8. Following shock induction in the natural flow group, forelimb muscle glucose uptake increased at 30 minutes of shock ($P<0.05$) and remained elevated throughout the experimental period ($P<0.05$). In contrast, shock induction in the constant flow group resulted in an increased forelimb

Figure 7. Muscle, skin and total forelimb hemodynamics and arterial glucose concentration in the natural (free flow) and constant blood flow perfused isolated forelimb during *E. coli* endotoxin shock. (n)=number of animals; ABP=mean systemic arterial blood pressure; BF=blood flow; R=vascular resistances; PRU=peripheral resistance units expressed in mmHg/ml/min/100gm; Art. Glc. Conc.=arterial blood glucose concentration; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

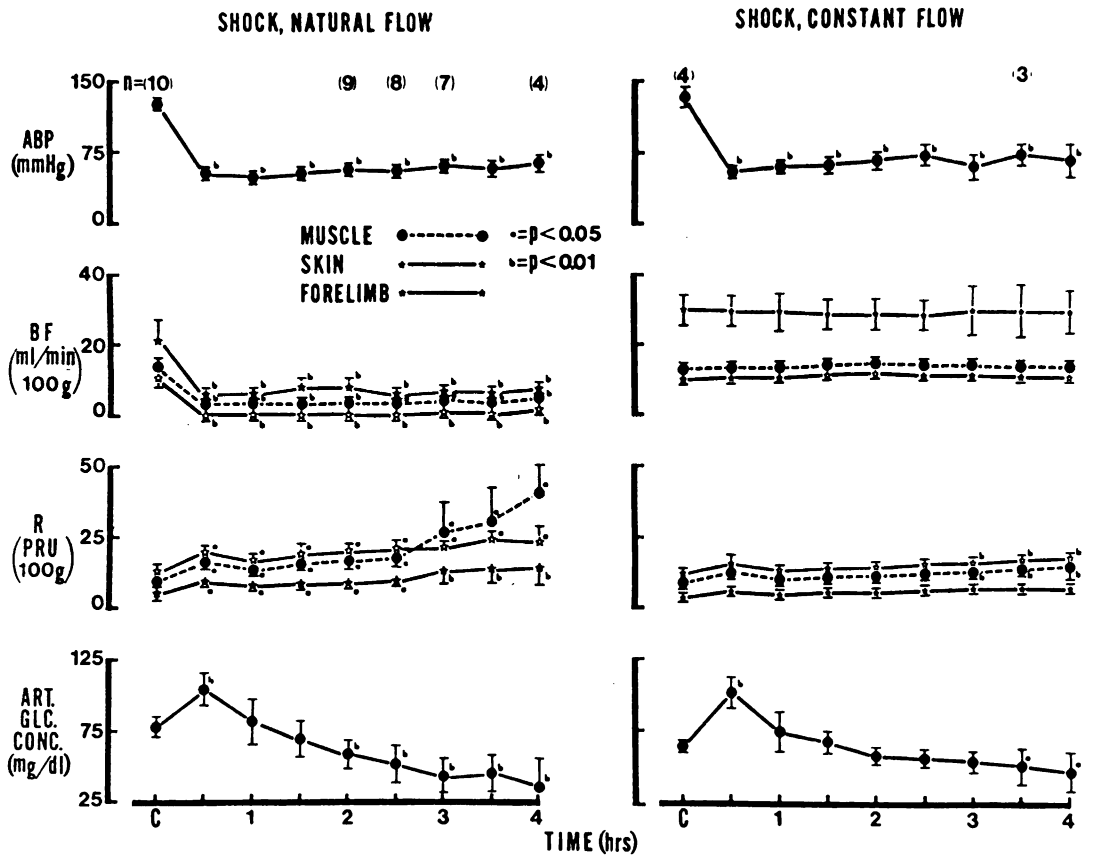
SHOCK, NATURAL FLOW
SHOCK, CONSTANT FLOW
n=(10)
(9)
(8)
(7)
(4)
(4)
(3)
ABP (mmHg)
150
75
0
MUSCLE
SKIN
FORELIMB
a=p<0.05
b=p<0.01
BF (ml/min / 100 g)
40
20
0
R (PRU / 100g)
50
25
0
ART. GLC. CONC. (mg/dl)
125
75
25
C
1
2
3
4
TIME (hrs)

Figure 7.

muscle glucose uptake only at 30 minutes of shock ($P<0.01$), after which it returned to values not different from control for the duration of the experiment ($P>0.05$). Forelimb muscle venous PO_2 decreased ($P<0.01$) following shock induction and remained below control throughout the experiment ($P<0.01$) in the natural flow group. However, forelimb muscle venous PO_2 was unaltered in the constant flow group ($P>0.05$) throughout the 4 hour shock period.

In both groups of animals, isolated forelimb temperature was never different from core temperature ($P>0.05$). In the natural flow group, isolated forelimb temperature was slightly below control at 2 and 2.5 hours ($P<0.05$) and slightly increased at 3 and 3.5 hours ($P<0.05$) when compared to their own control values. These temperature changes did not exceed 0.2 degrees celcius. Core temperature of the natural flow group was not significantly different from control ($P>0.05$). Subsequent temperature illustrations of Figures 9 and 10 are identical to that in Figure 8.

Forelimb skin glucose uptake, skin venous PO_2 and core and isolated forelimb temperatures of both natural and constant flow experiments are graphically illustrated in Figure 9. Following shock induction in the natural flow group, forelimb skin glucose uptake increased at 30 minutes of shock ($P<0.01$) and remained elevated throughout the experimental period ($P<0.05$) except at the 4 hour reading ($P>0.05$). In contrast, shock induction in the constant flow group resulted in an increased forelimb skin glucose uptake

Figure 8. Muscle metabolic and temperature variables in the natural (free flow) and constant blood flow perfused isolated forelimb during *E. coli* endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

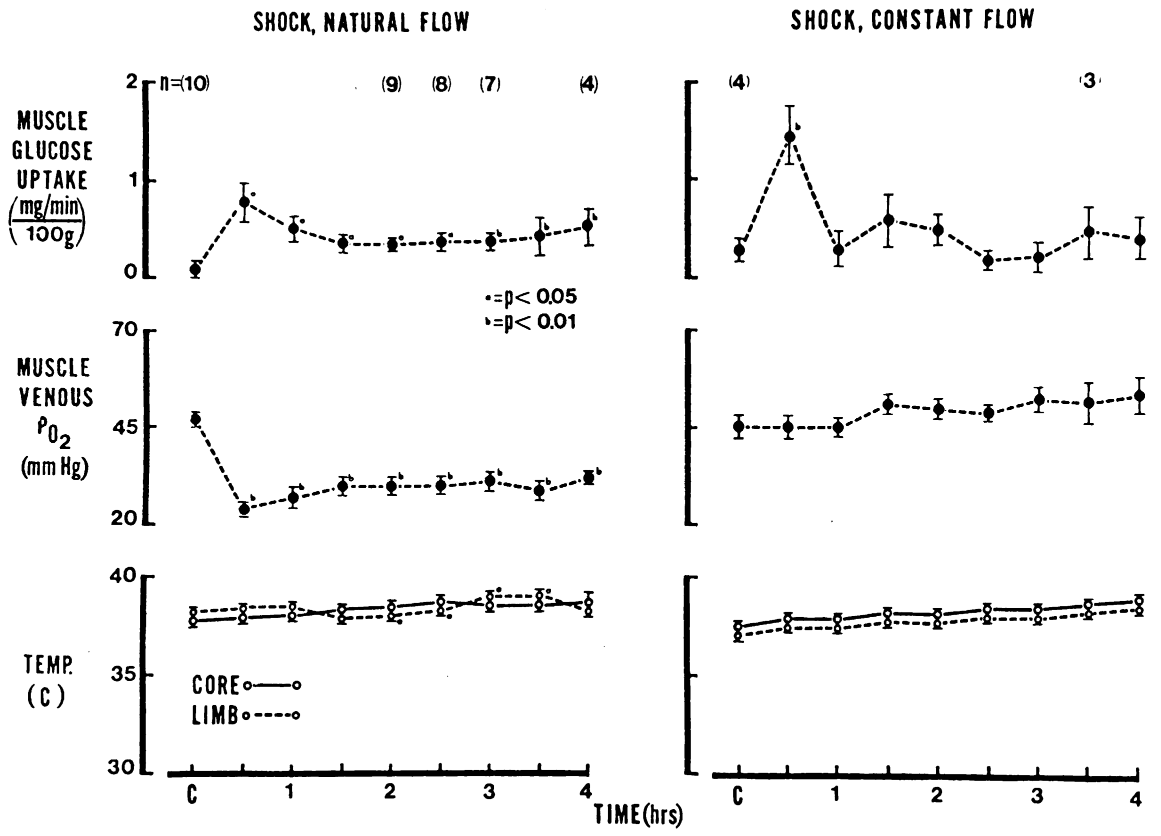
SHOCK, NATURAL FLOW
SHOCK, CONSTANT FLOW
MUSCLE GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
n=(10)
(9)
(8)
(7)
(4)
(4)
(3)
•=p< 0.05
ᵇ=p< 0.01
MUSCLE VENOUS P_{O_2} (mm Hg)
70
45
20
TEMP. (C)
40
35
30
CORE o——o
LIMB o----o
C
1
2
3
4
TIME(hrs)
C
1
2
3
4

Figure 8.

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at only 30 minutes of shock ($P<0.05$), after which it returned to values not different from control for the duration of the experiment ($P<0.05$). Forelimb skin venous PO_2 decreased ($P<0.01$) following shock induction in the natural flow group. However, forelimb skin venous PO_2 was unaltered in the constant flow group ($P>0.05$) throughout the 4 hour shock period.

Total forelimb glucose uptake, total forelimb venous PO_2 and core and isolated forelimb temperatures of both the natural and constant flow experiments are graphically illustrated in Figure 10. Following shock induction in the natural flow group, total forelimb glucose uptake increased at 30 minutes of shock ($P<0.01$) and remained elevated throughout the experimental period ($P<0.05$) except at 1 hour following shock ($P>0.05$). In contrast, shock induction in the constant flow group resulted in an increased forelimb glucose uptake only at 30 minutes of shock ($P<0.05$), after which it returned to values not different from control for the duration of the experiment ($P>0.05$).

Forelimb venous PO_2 decreased following shock induction ($P<0.01$) and remained below control throughout the experiment in the natural flow group. However, forelimb venous PO_2 was not altered in the constant flow group ($P>0.05$) throughout the 4 hour shock period.

As previously mentioned the numbers in parentheses at the top of Figures 1-12 and the numbers in the first line of Tables 1-6 represent the number of animals at each data

Figure 9. Skin metabolic and temperature variables in the natural (free flow) and constant blood flow perfused isolated forelimb during *E. coli* endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

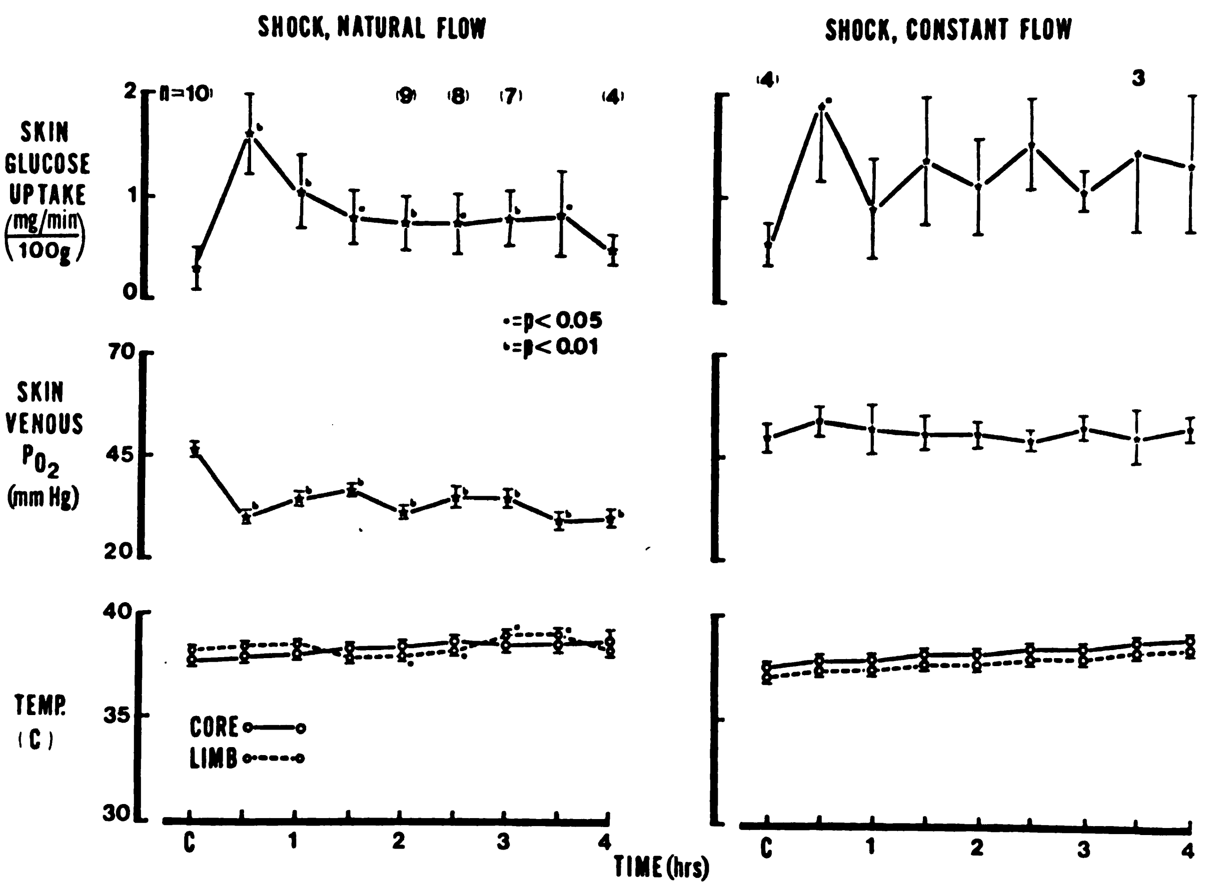
SHOCK, NATURAL FLOW
SHOCK, CONSTANT FLOW
SKIN GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
n=10
(9)
(8)
(7)
(4)
(4)
3
• = p< 0.05
ᵇ = p< 0.01
SKIN VENOUS P_{O_2} (mm Hg)
70
45
20
TEMP. (C)
40
35
30
CORE
LIMB
C
1
2
3
4
TIME (hrs)

Figure 9.

Figure 10. Metabolic and temperature variables in the natural (free flow) and constant blood flow perfused isolated forelimb during E. coli endotoxin shock. (n)=number of animals; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

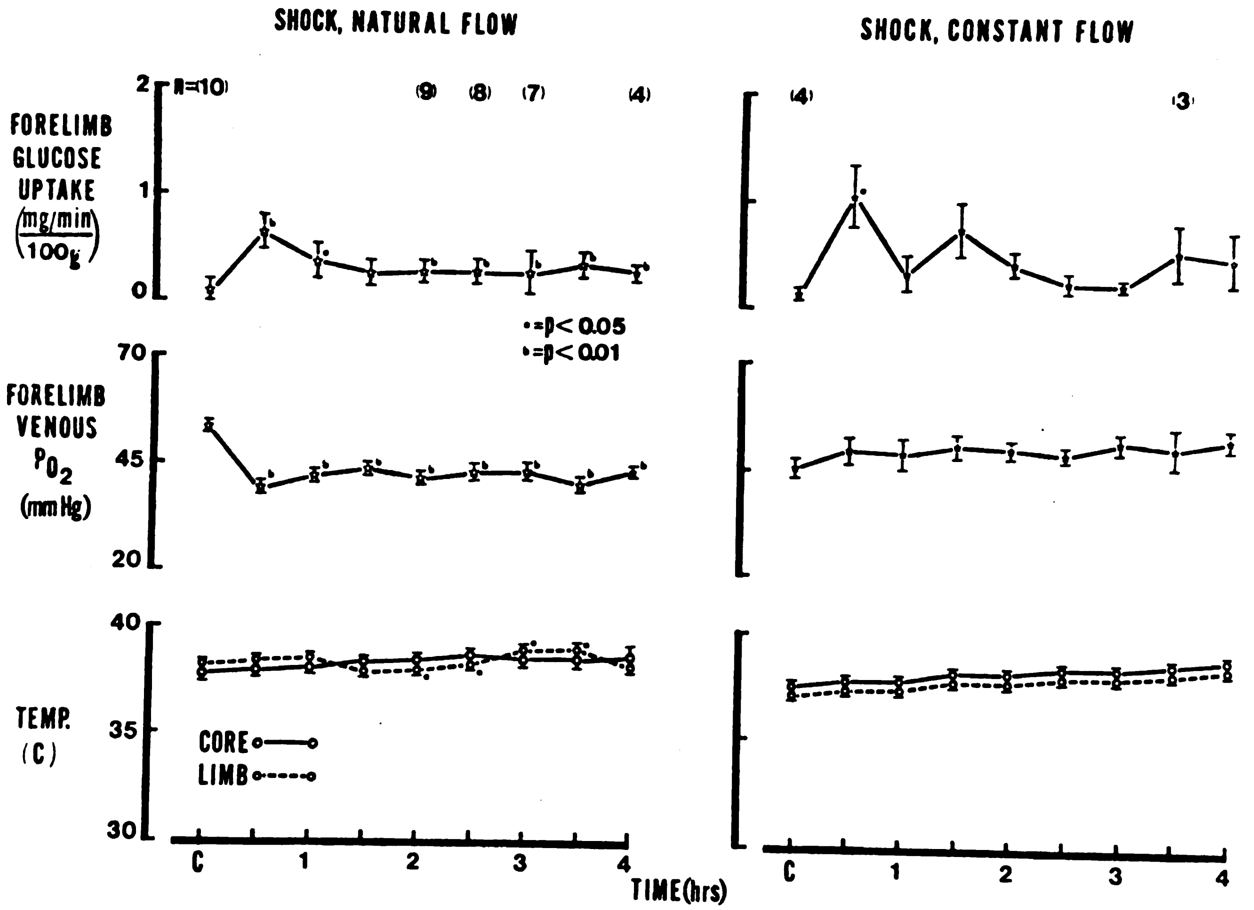
SHOCK, NATURAL FLOW
SHOCK, CONSTANT FLOW
FORELIMB GLUCOSE UPTAKE $\left(\frac{mg/min}{100g}\right)$
2
1
0
n=(10)
(9)
(8)
(7)
(4)
(4)
(3)
• = p < 0.05
• = p < 0.01
FORELIMB VENOUS P_{O_2} (mmHg)
70
45
20
TEMP. (C)
40
35
30
CORE ○——○
LIMB ○- - - -○
C
1
2
3
4
TIME(hrs)

Figure 10.

point; the smaller numbers represent animals dying during the course of the experiment.

Table 5 shows changes in metabolic variables in the controlled forelimb temperature-constant flow group during E. coli endotoxin shock. Control arterial PO_2 was 80 mmHg and did not change significantly throughout the shocked period ($P>0.05$). Arterial PCO_2, brachial venous PCO_2 and cephalic venous PCO_2 were not significantly different from control after the administration of endotoxin ($P>0.05$). Arterial pH, brachial venous pH and cephalic venous pH immediately decreased after the administration of endotoxin ($P<0.05$) and remained at this lowered level for the duration of the experiment ($P<0.05$). Hematocrit increased 30 minutes after shock induction ($P<0.05$) and remained elevated for the duration of the shock episode ($P<0.05$).

Hemodynamic variables and arterial glucose concentration of two control non-shocked groups are shown graphically in Figure 11. In both groups of animals, arterial blood pressure remained relatively constant decreasing slightly during the last hour in the natural flow group ($P<0.05$). Muscle, skin and total forelimb blood flows decreased in the natural flow group at different times throughout the experiment ($P<0.05$) whereas, in the constant flow group, blood flows were unchanged ($P>0.05$). Vascular resistances gradually increased in both groups of animals ($P<0.05$).

Muscle, skin and total forelimb glucose uptakes and venous PO_2, along with isolated forelimb and core

Table 5. Changes in metabolic variables in the controlled forelimb temperature-constant flow group during E. coli endotoxin shock.

	Control	Endotoxin							
		Time (min)							
Parameter	0	30	60	90	120	150	180	210	240
Number (n)	4	4	4	4	4	4	4	3	3
Arterial PO_2	80.0 ±4.7	72.0 ±3.5	67.5 ±4.6	70.0 ±3.9	70.5 ±3.5	70.5 ±2.7	67.8 ±4.5	67.3 ±2.9	70.3 ±2.6
Arterial PCO_2	32.3 ±2.7	31.8 ±4.0	35.8 ±3.8	34.3 ±5.7	34.8 ±5.8	32.8 ±4.4	31.0 ±5.2	29.7 ±1.3	28.7 ±0.9
Brachial Venous PCO_2	34.3 ±3.6	36.5 ±5.0	39.8 ±4.3	43.5 ±5.3	39.8 ±3.2	38.8 ±3.8	41.5 ±1.9	32.0 ±2.3	29.7 ±1.7
Cephalic Venous PCO_2	33.8 ±4.3	34.8 ±5.9	36.5 ±3.9	33.0 ±5.2	33.5 ±4.0	31.5 ±3.7	35.3 ±5.3	31.7 ±2.9	29.0 ±1.7

(Continued on next page)

Table 5 (cont'd).

Parameter	Control	Endotoxin							
		Time (min)							
	0	30	60	90	120	150	180	210	240
Arterial pH	7.41 ±0.04	7.30* ±0.04	7.27* ±0.05	7.31* ±0.06	7.29* ±0.05	7.34* ±0.05	7.30* ±0.07	7.35* ±0.09	7.35* ±0.08
Brachial Venous pH	7.41 ±0.05	7.28* ±0.04	7.26* ±0.05	7.29* ±0.05	7.27* ±0.04	7.26* ±0.05	7.27* ±0.07	7.29* ±0.08	7.31* ±0.08
Cephalic Venous pH	7.42 ±0.05	7.29* ±0.04	7.26* ±0.05	7.29* ±0.06	7.24* ±0.06	7.28* ±0.04	7.27* ±0.06	7.31* ±0.07	7.33* ±0.08
Hematocrit	34.3 ±1.7	41.0* ±3.1	43.5* ±4.1	43.8* ±3.6	44.3* ±3.4	44.3* ±3.7	44.8* ±3.8	43.3* ±5.2	44.7* ±4.4

*denotes values which are significantly different from control ($P < 0.05$).
Values are expressed as mean ± S.E.

Figure 11. Muscle, skin and total forelimb hemodynamics and arterial glucose concentration in the natural (free flow) and constant blood flow perfused isolated forelimb in non-shocked, control dogs. (n)=number of animals; ABP=mean systemic arterial blood pressure; BF=blood flow; R=vascular resistances; PRU=peripheral resistance units expressed in mmHg/ml/min/100gm; Art. Glc. Conc.=arterial blood glucose concentration; a=$P<0.05$ relative to time zero; b=$P<0.01$ relative to time zero; Bars=SEM.

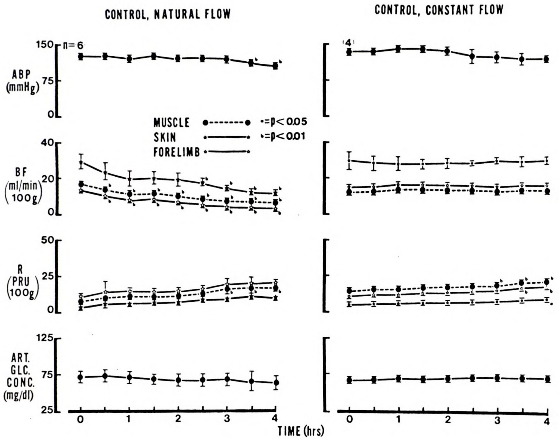
CONTROL, NATURAL FLOW
CONTROL, CONSTANT FLOW
n=6
(4)
ABP (mmHg)
150
75
0
MUSCLE ●-------● •=p< 0.05
SKIN •———• ▸=p< 0.01
FORELIMB •———•
BF (ml/min / 100g)
40
20
0
R (PRU / 100g)
50
25
0
ART. GLC. CONC. (mg/dl)
125
75
25
0
1
2
3
4
TIME (hrs)

Figure 11.

temperatures, are shown in Figure 12 for both control groups. Glucose uptake did not increase in muscle, skin or total forelimb during the experiment ($P<0.05$). Muscle, skin and total forelimb venous PO_2 decreased slightly ($P<0.05$) but remained above 45 mmHg in both groups. In the natural flow group, isolated forelimb temperature was slightly lower than core temperature (T=0.2 degrees celcius) at several points during the experiment ($P<0.05$). In the constant flow group, isolated forelimb temperature was not different from core temperature ($P>0.05$) but both increased during the experiment ($P<0.01$).

An additional analysis of the data using the "Students T" test was employed to determine whether there were any differences in the magnitude of the calculated glucose uptake by skeletal muscle, skin and total forelimb between the control animals and the shock animals of the controlled forelimb temperature-constant flow groups. Results of this analysis revealed no statistical differences ($P>0.05$) in the level of glucose uptake for skeletal muscle, skin and total forelimb between the groups at each time point.

None of the metabolic variables listed in Table 6 changed during the entire experimental protocol in the controlled forelimb temperature-constant flow non-shocked, control group ($P>0.05$).

It should be noted that while glucose concentration of muscle and skin venous blood was usually lower than arterial blood, occasionally forelimb venous glucose concentration

Figure 12. Control muscle, skin and total forelimb metabolic and temperature variables in the natural (free flow) and constant blood flow perfused isolated forelimb in non-shocked, control dogs. (n)=number of animals; a=P<0.05 relative to time zero; b=P<0.01 relative to time zero; c=P<0.05 comparing isolated forelimb temperature to the animal's core temperature at each time point; Bars=SEM.

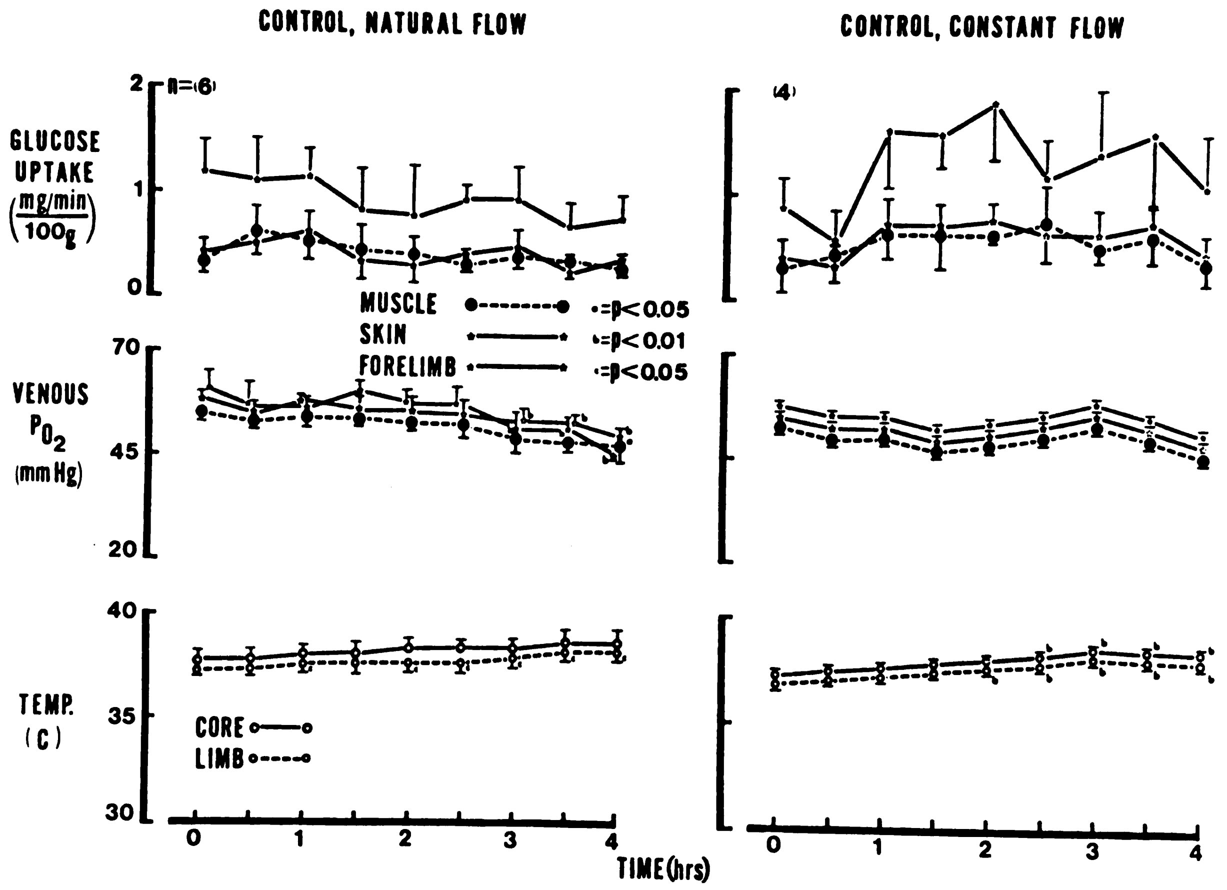
CONTROL, NATURAL FLOW
CONTROL, CONSTANT FLOW
n=(6)
(4)
GLUCOSE UPTAKE (mg/min / 100g)
2
1
0
VENOUS P_{O_2} (mm Hg)
70
45
20
TEMP. (C)
40
35
30
MUSCLE
SKIN
FORELIMB
•=p<0.05
•=p<0.01
•=p<0.05
CORE
LIMB
0
1
2
3
4
TIME(hrs)

Figure 12

was higher than arterial blood concentration at one or two points during the shock period.

Also, it should be noted that expressing blood flow on a 100 gram basis resulted in blood flow per 100 grams in muscle and skin appearing higher than total forelimb blood flow. This apparent paradox is due to the fact that bone weight was included in total forelimb weight, used to calculate total forelimb blood flow, but was not included with muscle or skin weight determinations or blood flow per 100 gram calculations. The same situation holds for calculated vascular resistance, which was obtained using blood flows per 100 grams of tissue. Therefore, resistance in the total forelimb appears to be higher than in forelimb muscle or skin.

Table 6. Changes in metabolic variables in the controlled forelimb temperature-constant flow group in non-shocked, control animals.

Parameter	Control Time (min) 0	30	60	90	120	150	180	210	240
Number (n)	4	4	4	4	4	4	4	4	4
Arterial PO_2	93.8 ±2.7	98.0 ±6.8	93.8 ±5.9	87.0 ±4.1	85.0 ±5.5	82.3 ±8.3	84.0 ±5.2	82.5 ±4.4	83.3 ±4.7
Arterial PCO_2	30.8 ±3.5	30.3 ±3.4	29.3 ±1.7	30.8 ±2.5	32.3 ±2.6	31.0 ±2.8	31.0 ±3.3	30.3 ±3.6	30.3 ±3.7
Brachial Venous PCO_2	31.0 ±3.6	31.5 ±4.0	31.0 ±3.1	32.0 ±2.6	32.8 ±2.7	33.3 ±3.0	31.8 ±3.2	31.8 ±3.0	30.5 ±2.6
Cephalic Venous PCO_2	31.5 ±3.6	31.5 ±4.6	30.8 ±3.2	31.3 ±3.0	31.8 ±3.1	32.5 ±3.5	32.0 ±4.1	31.5 ±3.2	30.0 ±2.7

(continued on next page)

Table 6 (cont'd).

Parameter	Control Time (min) 0	30	60	90	120	150	180	210	240
Arterial pH	7.44 ±0.02	7.42 ±0.04	7.42 ±0.02	7.42 ±0.02	7.42 ±0.02	7.42 ±0.03	7.43 ±0.03	7.43 ±0.03	7.41 ±0.02
Brachial Venous pH	7.39 ±0.05	7.41 ±0.01	7.41 ±0.02	7.40 ±0.01	7.41 ±0.02	7.39 ±0.03	7.41 ±0.03	7.40 ±0.03	7.40 ±0.01
Cephalic Venous pH	7.43 ±0.02	7.41 ±0.01	7.42 ±0.02	7.41 ±0.03	7.42 ±0.02	7.39 ±0.03	7.42 ±0.03	7.41 ±0.02	7.41 ±0.02
Hematocrit	33.3 ±4.3	34.5 ±4.3	33.5 ±4.4	33.8 ±4.4	33.3 ±4.0	33.5 ±3.9	35.3 ±3.4	35.0 ±3.8	35.0 ±3.3

*denotes values which are significantly different from control ($P<0.05$).
Values are expressed as mean ± S.E.

CHAPTER V

DISCUSSION

The hypotheses tested by this research were that 1) glucose uptake by skeletal muscle and skin increases during *E. coli* endotoxin shock in the dog and 2) that the mechanism for this proposed increase is related to local tissue hypoxia and/or tissue ischemia. The thesis postulated is that the hypoglycemia of endotoxin shock is, in part, due to an increased utilization of glucose by peripheral tissues, viz., skeletal muscle and skin.

In this research the surgically isolated forelimb was used to study glucose uptake by skeletal muscle and skin during *E. coli* endotoxin shock. Three groups of animals, including appropriate controls and shocked animals, were studied. In groups 1 and 2 the isolated forelimb was perfused under natural flow (free flow) conditions in order to study the hemodynamic and metabolic response of the isolated forelimb during endotoxin shock. In the first group of animals isolated forelimb temperature was maintained at the temperature of the contralateral intact forelimb which was not different from core temperature. In group 2, the temperature of the isolated forelimb was uncontrolled and allowed to change independant of core temperature. In the course of this research it was found that isolated forelimb temperature decreased approximately 3 degrees celcius within the first hour of the experiment if not maintained at core temperature. Furthermore, it was felt that this temperature drop may explain the contradictory data reported by Furr et al (101) where it was shown that glucose uptake by the isolated forelimb did not change during endotoxin shock. In their preparation the temperature of the isolated forelimb was not maintained at core temperature and presumably decreased since it was neither reported nor equipped for temperature maintenance.

In group 3 the isolated forelimb was perfused at a constant blood flow to alleviate the effects of low blood flow and the ensueing local tissue ischemia and tissue hypoxia that occurs during shock. Also, isolated forelimb temperature was maintained at core temperature in this group.

The use of the isolated forelimb as a viable isolated organ has been well established by numerous investigators (104,106). As previously stated, ligation of the median cubital vein, which represents the major anastomotic channel between the brachial and cephalic veins, ensured that brachial venous outflow was predominately from skeletal muscle and that cephalic venous outflow was predominately from skin (106). Therefore this technique allows one to study metabolic and hemodynamic variables in muscle and skin because venous drainage from either tissue can be

measured and sampled with a sufficient degree of accuracy. It was assumed that the contribution of bone blood flow was minimal and contributed little to the overall hemodynamic and metabolic response of the isolated forelimb, although we have no data to support this assumption.

Results from this study clearly show that glucose uptake by forelimb skeletal muscle and skin increases during endotoxin shock in the dog provided forelimb temperature is maintained at core temperature, and that the mechanism for this proposed increase may be related to local tissue hypoxia and/or tissue ischemia (83). While this increase in forelimb skeletal muscle glucose uptake is similar to what occurs in gracilis muscle as previously reported by Raymond et al (83) during endotoxin shock and by others (102) during mild endotoxemia in the intact hindlimb, the increase in glucose uptake by skin during endotoxin shock has to our knowledge not been previously reported. It was also noted that glucose uptake by skin is greater than muscle during resting conditions and that the increase in glucose uptake is greater by skin than muscle during shock.

It should be noted that in the analysis of the glucose uptake data using the "Students T" test modified for unpaired replicates it was found that comparison of shocked animals to control animals of the controlled forelimb temperature-natural flow group (group 1) revealed no differences at each time point between the control and the shocked animals. However when the data was analyzed with the analysis of variance test, which takes into account the variability among individual animals and the variability occuring over time, it was found that glucose uptake does increase in skin and skeletal muscle when compared back to their own control values. Because of the variability among the animals in this study it was felt that the analysis of variance was a more sensitive test than the "Students T" test to ascertain changes in glucose uptake during shock.

In this study it is not known whether the sex of the animal affected its response to shock and in particular its response to glucose metabolism. Previous studies from Dr. Emerson's laboratory have reported no differences in glucose uptake by the isolated gracilis muscle during control or shock states according to the sex of the animal (personal communication). In these studies metabolic and hemodynamic data were combined (83,84,105). In the present study the sex of the animal was not routinely recorded thereby preventing a detailed analysis of the data according to sex.

The control experiments in natural flow preparations indicated that glucose uptake by forelimb skeletal muscle and skin does not increase over time. Evidence for the viability of the isolated forelimb preparation used in this study is noted from its metabolic and vascular integrity. The appearance of a positive arteriovenous PO_2 difference, a negative arteriovenous PCO_2 difference, and an increase in the arteriovenous pH difference are qualitatively similiar to results noted by others (106). To what extent the increase in arteriovenous pH difference in the forelimb is due to carbon dioxide production or to lactate production is not known. Forelimb vascular resistance rose slightly with time, resulting in a gradual fall in limb blood flow. This results from a small fall in blood volume due to fluid loss (insensible, urine, and oozing) and perhaps from a lightening of the anesthesia. By all usual measures the isolated forelimb preparation appeared to act like an intact limb.

Data from the present study offers no explaination as to why glucose uptake by skin is greater during control and shock states, as compared to muscle. However, an earlier study from Dr. Emerson's laboratory (109) demonstrated an increase in glucose uptake by suprapubic subcutaneous adipose tissue during the first 2 and 2.5 hours of a 4 hour endotoxin shock protocol. It is possible that an increase in forelimb subcutaneous adipose tissue glucose uptake may have contributed to the "skin" glucose uptake during shock, but this study offers no data to support this possibility. It is of interest that the increase in glucose uptake in both forelimb skin and skeletal muscle reached a peak at 30 minutes of shock and then decreased, plateauing at a level still above control by approximately 1 and 1.5 hours, which roughly parallels the time course of glucose uptake observed in adipose tissue (109). Also, a partial explanation could be that even in the presence of oxygen, skin metabolizes most of the glucose only as far as to lactate (110-113). This phenomenon is unique to skin and has been attributed to the limited ability of skin to convert pyruvate to acetyl-CoA and the very high activity of lactate dehydrogenase compared to other tissue (114). Also, the majority of metabolic requirements of skin are met through anaerobic glycolysis and a small percentage by the oxidative pentose pathway, while only negligible amounts of energy requirements are provided by the Krebs cycle (115).

It has been postulated by Fusaro and Johnson (115) that skin may play a multiple, time-dependent role in glucose homeostasis. In this scheme, glucose diffuses into the dermis during periods of hyperglycemia and drains into the lymphatic system and epidermis. While glucose in the lymphatics is carried back to the vascular system eventually, that which enters the epidermal component of skin is converted to lactate which diffuses back into the blood and is transported to the liver for conversion to glycogen. As blood glucose concentration returns toward normal, the remaining excess dermal glucose diffuses back into the blood and hence helps regulate plasma glucose concentration. Again, it is not known whether this glucose regulating mechanism is involved in the greater increase in glucose uptake by skin than skeletal muscle during shock. Also the so-called back diffusion of glucose may explain the higher concentration of glucose in forelimb venous than arterial blood which was sometimes observed by us, and also by Furr et al (101).

With reference to the Furr et al (101) paper, data from their study and from our uncontrolled forelimb temperature group (Group 2) are in agreement in that we also observed no increase in glucose uptake by the isolated forelimb during endotoxin shock. However, as observed in our controlled forelimb temperature study (Group 1), glucose uptake by both skeletal muscle and skin increases substantially during shock if temperature of the isolated forelimb is maintained at the temperature of the contralateral, non-isolated forelimb, which turned out to be the same as core temperature of the animal.

An explanation for no change in glucose uptake by the isolated forelimb during shock in the uncontrolled forelimb temperature group (Group 2) of this study and in the Furr et al (101) uncontrolled forelimb temperature study may be related to a decrease in isolated forelimb temperature below contralateral forelimb temperature. The lower temperature may have resulted in a decrease in the metabolic rate of this organ and hence a decrease in substrate and oxygen need. The decrease in metabolic requirements of the isolated forelimb is further substantiated by the fact that even though oxygen delivery to the forelimb in this study was severely decreased, the limbs only developed mild hypoxia (20% decrease in forelimb venous PO_2).

The difference in forelimb skeletal muscle and skin glucose uptake between the temperature controlled and temperature uncontrolled experiments is likely due to the

3 degree celcius difference in contralateral and isolated forelimb temperature. To evaluate this, information is needed on the temperature coefficient (Q10) for the glucose uptake process under the experimental conditions of this study. Since such Q10 values are not known, it is not possible to ascertain whether or not the observed difference in glucose uptake between temperature controlled and temperature uncontrolled experiments is quantitatively equal to that due to a 3 degree celcius change.

Also of importance in this study is that glucose uptake by forelimb muscle and skin is elevated only at about 30 minutes after shock induction when blood flow was maintained constant, similar to the gracilis muscle (83). This transient increase in forelimb skeletal muscle and skin glucose uptake during the early phase of endotoxin shock can probably be explained on the basis of the endotoxin molecule acting directly on the tissue to stimulate glucose uptake (84). However, by the time the 60 minute determination was made, all or most of the circulating endotoxin should have been cleared from the blood by the reticuloendothelial system and other tissue (105). Plasma levels of endotoxin have been shown to decrease rapidly within 1 hour of shock with only negliglible amounts of endotoxin detectable 2 hours after the administration of large doses of endotoxin (5 mg/kg) to dogs (108).

This study did not specifically differentiate between the effects of local tissue hypoxia and local tissue

ischemia on glucose uptake during shock. However, Dr. Emerson's laboratory previously demonstrated the effects of local tissue hypoxia independent of local tissue ischemia by interposing an extracorporeal lung between the femoral artery and the gracilis artery for local control of blood gases (83). Under non-ischemic, hypoxic conditions, gracilis muscle glucose uptake increased by 60 minutes and remained elevated throughout the experimental period. The effects of local tissue ischemia independent of tissue hypoxia were also investigated in the same study. This was accomplished by decreasing the gracilis muscle perfusion pump speed to induce local tissue ischemia while an extracorporeal lung was ventilated with a gas mixture of 95% O_2 and 5% CO_2 to maintain arterial blood gases at hyperoxic levels supplying the gracilis muscle. Following the effects of local tissue ischemia and hyperoxia, gracilis muscle glucose uptake decreased and remained depressed for the duration of the experimental protocol. It was thus concluded that local tissue hypoxia and not local tissue ischemia was responsible for the increased glucose uptake in gracilis muscle. It is reasonable to assume that the same conditions prevail in the present study and that local tissue hypoxia per se is primarily responsible for the increase in glucose uptake in forelimb skeletal muscle and skin although the definitive experiments have not been done.

To reiterate, the present study demonstrates that glucose uptake increases substantially by both skeletal muscle

and skin in the isolated dog forelimb when temperature is maintained the same as the contralateral, non-isolated forelimb. While these data provide no information relative to the role of this phenomenon in the hypoglycemia of endotoxin shock in the dog, the skeletal muscle data supports previous work in the gracilis muscle (83), and since skeletal muscle makes up a large percentage of total body mass, an increase in glucose uptake by skeletal muscle which presumably occurs generally throughout the body must contribute substantially to the development of hypoglycemia. Also, while the mass of skin is considerably less than that of muscle, the sustained increase in glucose uptake by skin during endotoxin shock may also contribute substantially to the progressive and pathologic depletion of blood glucose.

Additionally, this study emphasizes the importance of maintaining isolated organ temperature at near body temperature, particulary during studies involving metabolism, since relatively moderate alterations in organ temperature can result in substantial alterations in the metabolic rate of that organ via the well known Q10 effect. Also, although the observations reported by Furr et al (101) were no doubt correct and were verified by the present study, these data as applied to the pathophysiology of shock should be considered artifactual, and hence not necessarily applicable because of changes in isolated organ temperature independent of temperature changes in the intact animal.

In conclusion, data from the present study support the hypotheses that glucose uptake by forelimb skeletal muscle and skin increases during *E. coli* endotoxin shock in the dog and that the mechanism for this increase in glucose uptake is related to local tissue hypoxia and/or tissue ischemia.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study was designed to determine whether glucose uptake by forelimb skeletal muscle and skin increases during *E. coli* endotoxin shock in the dog and if the mechanism for this proposed increase in glucose uptake is related to local tissue hypoxia and/or tissue ischemia. The isolated, innervated, canine forelimb perfused at either natural or constant blood flow was used as the test organ in this study. In two groups of animals (Groups 1 and 3) the temperature of the isolated forelimb was maintained at the temperature of the contralateral (intact) forelimb, which was equal to the core temperature of the animal, by wrapping the isolated forelimb in a temperature regulated water jacket. In another group of animals (Group 2) isolated forelimb temperature was allowed to change independant of core temperature. Shock was induced by a 5 minute intravenous infusion of 2 mg/kg *E. coli* endotoxin.

The results of this study indicate that if the temperature of the forelimb was maintained at the temperature of the contralateral forelimb, glucose uptake by the naturally perfused forelimb increases in skeletal muscle and skin during *E. coli* endotoxin shock in the dog and that the mechanism for this increase is related to local tissue hypoxia and/or tissue ischemia. It was also noted that constant blood flow perfusion during endotoxin shock resulted in no change in isolated forelimb glucose uptake except at 30 minutes of shock induction. This transient increase in glucose uptake by forelimb skeletal muscle and skin was attributed to the "insulin-like" effect of the endotoxin molecule acting directly on the tissue to stimulate glucose uptake.

It was also noted that when isolated forelimb temperature was allowed to change independant of core temperature (Group 2) glucose uptake by forelimb skeletal muscle and skin did not change during endotoxin shock. A decrease in the metabolic rate of the isolated forelimb during endotoxin shock was considered to be responsible for the reduction in substrate and oxygen need of the forelimb. Therefore these data emphasize the importance of maintaining temperature in isolated organ preparations during low blood flow states when metabolic variables are of importance.

In conclusion, this study demonstrates that glucose uptake by forelimb skeletal muscle and skin increases during E. coli endotoxin shock in the dog and that the mechanism for this increase in glucose uptake is related to local tissue hypoxia and/or tissue ischemia. Furthermore, since skeletal muscle and skin makes up a large percentage of total body mass, an increase in glucose uptake by these tissues, which presumably occurs throughout the body, must contribute substantially to the hypoglycemia of endotoxin shock.

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