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thesis entitled

MECHANISM OF SKELETAL MUSCLE CARBOHYDRATE METABOLISM DURING ESCHERICHIA COLI ENDOTOXIN SHOCK IN THE DOG

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

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Date 9/12/79

O-7639

MECHANISM OF SKELETAL MUSCLE CARBOHYDRATE METABOLISM DURING ESCHERICHIA COLI ENDOTOXIN SHOCK IN THE DOG

By

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A DISSERTATION

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

DOCTOR OF PHYSIOLOGY

Department of Physiology

1979

ABSTRACT

MECHANISM OF SKELETAL MUSCLE CARBOHYDRATE METABOLISM DURING ESCHERICHIA COLI ENDOTOXIN SHOCK IN THE DOG

By

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Carbohydrate metabolism by skeletal muscle was studied in spontaneously respiring dogs following intravenous administration of 2 mg/kg (LD_{100}) Escherichia coli endotoxin.

Close intra-arterial infusion of Endotoxin stimulated skeletal muscle (gracilis muscle) to increase glucose uptake, which was independent of shock alterations in hemodynamics, blood gases, pH, insulin and oxygen uptake by the gracilis muscle. These results indicate that endotoxin has an insulin-like effect by increasing glucose transport into skeletal muscle when infused locally. When shock was induced by the intravenous administration of Endotoxin (2 mg/kg, LD_{100}) glucose uptake increased throughout the six-hour experiment when gracilis muscle blood flow was allowed to flow freely. The increase in skeletal muscle glucose uptake occurred in the presence of muscle ischemia and muscle hypoxia. However, during the constantly perfused gracilis muscle, endotoxin shock failed to alter glucose uptake by the skeletal muscle. Unlike the naturally perfused gracilis muscle preparation, constant flow to the gracilis muscle prevented changes in blood flow or tissue

oxygenation. Gracilis muscle glucose uptake increased significantly during the six-hour experiment when blood flow to the muscle was reduced causing local gracilis muscle ischemia. The increase in glucose uptake was independent of systemic metabolic or hemodynamic alterations and occurred in the absence of endotoxemia. The results gave evidence that glucose uptake by skeletal muscle was mediated through local tissue ischemia and/or hypoxia. To differentiate between ischemia and hyposia in regulating skeletal muscle glucose metabolism, two sets of experiments were conducted: When gracilis muscle blood flow was held constant and made hypoxemic via an extracorporeal heart-lung preparation, glucose uptake by the gracilis muscle increased and remained elevated above control for the duration of the six-hour experiment; however, when gracilis muscle blood flow was decreased to very low levels concommitant with hyperoxia, no increase in glucose uptake was noted -- in fact, glucose uptake decreased. These data, therefore, provide evidence that endotoxin has an insulin-like action in promoting glucose uptake when given locally, but failed to change glucose uptake by the constantly perfused gracilis muscle when given intravenously. Under natural flow conditions, endotoxin shock induction produced muscle ischemia and hypoxia and caused an increase in skeletal muscle glucose uptake, which was mediated by muscle hypoxia and not ischemia.

DEDICATION

To my wife, Barbara, for her relentless patience during the past five years and also for her love and caring in giving me enough time by myself so I could finish this degree. To my children, Ricky and Amy for absorbing the hard times with smiles and open arms and love.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the research guidence from his main professor, Dr. Thomas E.Emerson, Jr., and also for his unending support when times were tough. Dr. Emerson's trust, support and help were truly beyond what was expected of him and for that, the author has gained much, not only in prefessional development but also in personal gratification and awareness. To Dr. Jerry B. Scott for his help and support. To Dr. Lester Walterink who was always there to listen and advise. To Amylou Davis who helped guide the author through many difficult times and to the skillful technical assistance of Ms. Marilynn A. DeBussey for her help and patience.

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INTRODUCTION

Endotoxin shock is a serious clinical problem with recent reports estimating between 70,000 and 130,000 fatalities a year in the United States (McCabe, et. al., 1970; McCabe, et al., 1972). Historically, early emphases were placed on metabolic alterations of shock where it was reported that endotoxins, given as crude vaccines, deplete animals of their carbohydrate reserves, resulting in early hyperglycemia and eventual hypoglycemia (Menten and Manning, 1924). The mechanism(s) responsible for endotoxin-induced hypoglycemia has received much attention. While the literature dealing with specific organ utilization of carbohydrates in shock is sparse, several organ systems have been ruled out. Hinshaw, et al. (1975) reported that glucose uptake by the heart-lung preparation is not increased and Raymond and Emerson (1978) have shown that glucose uptake by the central nervous system is decreased. Filkins and Buchanann (1977) have shown the hemidiaphragms taken form shocked rats show marked increases in glucose uptake; however, hemidiaphragms from normal, non-shocked rats did not exhibit any glucose uptake when indotoxin was added directly to the in vitro preparation. Recently, Furr, et. al. (1977) reported that the isolated, innervated,

constantly-perfused forelimb preparation of the dog did not show altered glucose metabolism following endotoxin shock induction. Their data are, however, vague and incomplete as it seemed evident that venous samples of blood, taken to calculate glucose uptake, were drawn from a common reservoir that contained both cephalic and brachial venous blood which came from skin and skeletal muscle respectively. This common pooled venous efflux from the forelimb was used to determine skeletal muscle glucose metabolism during endotoxin chock. Their experimental design and results do not seem to correctly report on glucose metabolism of skeletal muscle following endotoxin shock.

The hypothesis being tested in this study is that skeletal muscle, which represents approximetely 50-60% body mass, accounts for a marked increase in glucose uptake during E. coli endotoxin shock in the dog, and could give a better understanding of the mechanism of hypoglycemia during endotoxin shock.

SURVEY OF THE LITERATURE

Biochemical composition of gram negative microbial cell walls and the relationship of chemical structure to the pathophysiology of endotoxemia or shock.

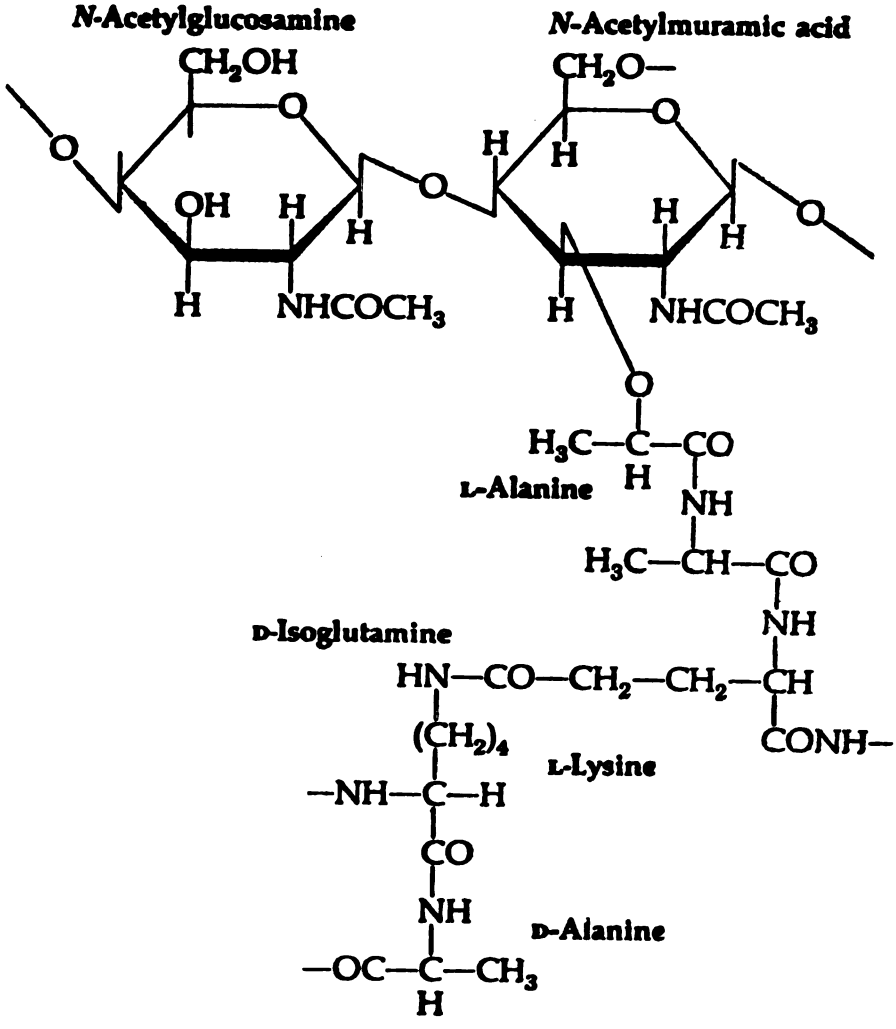
In order to provide a reliable structural defense to withstand large changes in osmotic force in the surrounding environment, many bacteria possess a cell wall component which encapsulates and protects the inner cell membrane.

Universally present among bacterial cell walls is a large single macromolecule, murein or peptidoglycan. This structure consists of two repeating units: 1) a linear polysaccharide component composed of N-acetylglucosamine and N-acetylmuramic acid which are chemically bound to each other; and 2) a small polypeptide which is attached to N-acetylmuramic acid and consists of l-alanine, d-isoglutamine, l-lysine and d-alanine. A schematic representation is seen below.

In many organisms, such as Escherichia coli, diaminopimelic acid replaces lysine in the polypeptide chain.

Attached to the cell wall outer covering of many bacteria, including E. coli, are complex structures composed of lipids, proteins and polysaccharides. These peptido-lipopolysaccharides form elaborate capsules that cover the murein macromolecule, or cell wall, and serve as the

Figure 1: Schematic representation of murein or peptidoglycan.


N-Acetylglucosamine
N-Acetylmuramic acid
L-Alanine
D-Isoglutamine
L-Lysine
D-Alanine

characteristic somatic O antigens, of which some are highly toxic to animals.

Peptido-lipopolysaccharides or endotoxins of many gram negative bacteria have received much interest in regards to their chemical structure and toxicity. Much debate has occurred over the years concerning which fraction of the endotoxin molecule possesses the toxic properties exhibited during infection.

Early studies were done in which endotoxins from a variety of gram negative bacteria (Raistric and Topley, 1934) were fractionated to characterize its structure and to identify the acitve toxic component. Endotoxins from enteric bacilli were extracted utilizing techniques from a number of investigators (Boivin and Mesrobeanu, 1935, 1936; Boivin, 1938, 1940, 1946; Haas, 1937, 1938; Wagner-Jauregg and Helmert, 1942; Boroff, 1949; Morgan, 1937; Morgan and Partridge, 1940, 1941). Results from these early studies indicate that endotoxins have a lipid component composed of palmitic acid, oleic acid, alpha-glycerophosphoric acid and many nitrogenous compounds not readily identified. Attached to the lipid moiety is a protein complex which, according to Boivin, could be dissociated from the lipid moiety by appropriate biochemical fractionating techniques. The separation was based on qualitative tests in which the dissociated lipid complex did not show any protein

characteristic; however, the lipid residue did behave as a protein. In addition to the lipid and protein components comprising 9-12% and 17-20% of the total crude endotoxin molecule respectively, there was also a polysaccharide complex. The chemical compostion of the saccharides is variable from one type bacteria to another, but the main products from hydrolysis extractions were: N-acetylglucosamine, d-galactose, l-rhamnose and a variety of small monosaccharides. The polysaccharide fraction of the endotoxin molecule was found to comprise 55-60% of the total weight.

Biochemical studies of other enteric bacilli, such as the Flexner dysentery bacilli have revealed important information concerning the nature of endotoxin toxicity (Goeble, et al., 1945; Binkley, et al., 1945). As previously mentioned, fractionating techniques gave similar lipid, protein and polysaccharide complexes. Tal and Goeble in 1950, using different analytical procedures, found that the polysaccharide complex contained a small (about 1%) organically bound phosphorous ion. Later, it was shown that the protein moiety also contained a small amount of phosphorous. Toxicity studies in mice showed that the active or toxic portion of endotoxin was associated with the presence of organic phosphorous in combination with either protein or polysaccharide portions of the endotoxin molecule. In 1954, Westphal and Luderity, using acid hydrolysis, identified a phosphorous-containing lipid from

purified endotoxin, which they named lipid A. Lipid A was shown to have approximately one-tenth the pyrogenic activity as the parent endotoxin molecule. It was thought that the explanation for the reduced activity of the isolated lipid A molecule was that it was no longer contianed in the lyophilic polysaccharide carrier which makes it water soluble (Westphal, et al,. 1958; Westphal, 1960). Westphal and associates therefore concluded that the lipid A portion of endotoxin was the active portion of the molecule; however, other investigators disagreed with the findings of Westphal, in that endotoxins with varying concentrations of lipid did not correlate with increases or decreases in biolobical acitivity (Ribi, et al., 1961, 1962). Conclusive proof could have been presented only by producing an entirely lipid-free endotoxin molecule with full biologic activity; however, such preparations still do not exist. Ribi and co-workers felt that the biological activity of endotoxin was determined by its size, degree of agregation or degradation. They found this result to be consistent with the hypothesis that a macromolecular complex of critical size is one of the major requirements for endotoxin to elicit its characteristic effects in the mammalian animal host (Haskins, et al., 1961; Ribi, et al., 1962).

Recent analyses of lipid A portion of endotoxins have shown the major constituents to be a phosphomucolipid and that the residual endotoxicity of the lipid A mixture is due to the presence of a remnant from the parent endotoxin

(Nowotny, 1965). Nowotny also performed new, unique detoxification studies of endotoxin and found that organic chemical reactions which split O-acly linkages of carbohydrates were able to diminish the lethality of endotoxin preparations. These O-acly linkages were found to exist throughout the entire lipopolysaccharide complex. Recent evidence has confirmed that the lipopolysaccharide portion of endotoxin in responsible for the pathophysiological effects seen in mammals (Benjamin, et al., 1974). It has also been shown that, during the fractionating of endotoxin, both simple proteins and lipopolysaccharides retained a portion of the lipid A moiety. Although the lipopolysaccharide portion contained only small amounts of lipid A, it exhibited high levels of toxicity.

Complete fragmentation of endotoxin, using acetic acid, produced fractions of conjugated protein, O-specific side chains and a polysaccharide core (Wolber and Alaupovic, 1971). The conjugated protein consisted of intact lipid A moieties which proved to be equal or greater in toxicity than that of lipopolysaccharide.

The lipid portions of conjugated proteins or lipopolysassharide resembled the lipid A configuration. Mortality rates were only observed with whole endotoxin or fragments that contained at least a portion of the lipid A moiety. It has recently been demonstrated that lipid A, isolated from E. coli bacteria and solubilzed by complexing

it with bovine serum albumin (BSA), is the component responsible for pyrogenicity and toxicity (Galanos, et al., 1972). In other experiments dealing with lipid A preparatoins, it was found that the active sites of the lipid A molecule were side branches of ester-bound fatty acids.

In summary, the early results which emphasized protein as the active component were correct, but for the wrong reason. The early experiments did not detect the lipid component within the proteins, which has since been identified as lipid A and is universally found throughout the peptido-lipopolysaccharide molecule. The lipid A moiety, which contains phosphorous, confirmed the results presented earlier that phosphorous is the active component responsible for the pyrogenicity of endotoxins, independent of its presence within proteins or polysaccharides. Detoxification studies of lipid A produced evidence that implicated side branches of lipid A as the active component which have been identified as ester-bound fatty acids.

Hemodynamic alterations during endotoxin shock

Shock induced by intravenous injection of Escherichia coli endotoxin is a widely used model in experimental shock work because it resembles clinical septic shock in a variety of respects and is an easy and relatively reproducible way of inducing hypotension (Gilbert, 1960).

When endotoxin is given intravenously to the dog, there is an initial rapid decline in systemic arterial blood pressure followed by a temporary recovery which is then succeeded by a progressive hypotensive period. This early fall in blood pressure must either be due to a decline in total peripheral resistance or cardiac output. Weil, et al., in 1956, found that endotoxin caused a decrease in cardiac output which was accompanied by a slight elevation in total peripheral resistance. Their results showed that a decrease in cardiac output was the cause for the initial decline in arterial blood pressure rather than peripheral dilation being the cause.

These results were further corroborated with venous return-constant flow experiments, that is, the total venous return of the dog was allowed to fill an extracorporeal reservoir from which it was returned at a constant rate to the right atrium of the heart. (Weil et al., 1956, 1956a). Under these conditions, left ventricular output was held constant. The intravenous administration of endotoxin into the dog during these venous return-constant flow preparations failed to show any decrease in systemic arterial blood pressure over the first few minutes. These results demonstrated two important findings: 1) that systemic arterial blood pressure did not decrease over the initital endotoxin period, indicating no decrease in peripheral resistance for if resistance vessels had dilated under a constant cardiac output, arterial blood pressure

would also have decreased, and, 2) the results from these observations also indicated that the heart was capable of pumping the constant amount of blood returned to it, which demonstrated that the decrease in cardiac output seen in the intact dog was clearly not due to myocardial failure or weakness. These data, therefore, could only point to a decrease in venous return, as witnessed by the rapidly falling blood level in the reservoir, as the primary means for the decrease in arterial blood pressure during endotoxin shock in the intact dog. Throughout the years, many investigators have also shown similar responses to endotoxin administration in the dog. The overwhelming evidence shows that the early decrease in arterial blood pressure is caused by a fall in cardiac output subsequent to a decrease in venous return (Chien, et al., 1966; Hinshaw and Nelson, 1962; Weil, et al., 1956). Similar results were also obtained following endotoxin administration in unanesthetized dogs (Prinao, et al., 1971). These animals demonstrated the classic decrease in mean arterial blood pressure, accompnaied by diminished cardiac output, increased total peripheral resistance, increased heart rate and decreased stroke volume.

The mechanism by which venous return is decreased initially in the dog following endotoxin administration is an interesting one. Gilbert, in 1960, preported that the decrease in venous return was the result of marked hepatic venoconstriction resulting in protal hypertension and venous

pooling of blood in the hepatosplanchnic region. Further substantiation came from Meyer and Visscher in 1962 when they reported on the hemodynamic responses of intestinal vascular segments in the dog. Their results showed that following E. coli endotoxin administration, pre-capillary arterial resistance increased by 50% whereas it increased by 500% in the venous vascular segment and decreased by 40% in the segment form small artery to venule (Pre/post capillary resistance ratio). Chien, et al., in 1966, also presented similar results following the administration of E. coli endotoxin to dogs. Their results were broken down, however, into two groups, one in which hemodynamic alterations were studied over the first twenty minutes of shock (early phase), and, second, where these alterations were studied during the late phase of the shock period (greater than 20 minutes). Their data showed marked hepatic venoconstriction which caused rises in portal and wedged hepatic venous pressures. These increases in venous resistance were, however, independent of the sympathetic nervous system for they were also observed in totally sympathectomized dogs. In the late phase of shock, there was a decrease in venous resistance in the hepatosplanchnic areas as compared to the early period of shock but was still high relative to control. As shock progressed, intestinal venous resistance increased significantly which was similar to the findings of Meyer and Visscher in 1962. Other investigators have also shown an increased hepatosplanchnic sequestration or pooling

of blood as the mechanism for decreased venous return in the dog during endotoxin shock (Moreno, et al., 1962; Hinshaw, et al., 1962; Lillehei, et al., 1964).

On the other hand, Blattberg and Levy in 1971 supplied evidence that if portal venous pressure was prevented from rising utilizing the aid of a Servo-pump, endotoxin administration to dogs still produced blood pooling. By appropriately placed aortic ligatures, it was found that 15 minutes after endotoxin administration, approximately 8 ml/kg of blood was pooled above the celiac artery and 12 mg/kg was pooled below the superior mesynteric artery. Total pooling was estimated to be 28 ml/kg. It was concluded that more than half of the early pooling in response to endotoxin took place outside of the hepatosplanchnic area. Extrahepatic pooling of blood was also demonstrated in evicerated dogs (Hinshaw, et. al., 1958, Brockman, et al., 1967).

In the early phase of endotoxin shock, the hemodynamic responses in the dog are followed by a decrease in arterial blood pressure subsequent to a fall in venous return caused by hepatosplanchnic and extrahepatosplanchnic pooling of blood. During the late phase of endotoxin shock, however, hemodynamics in the dog are different from the early phase.

The initial hypotensive period following endotoxin administration is uaually followed by a recovery of blood pressure towards control values. This could be explained by either a decrease in hepatovenous resistance which would

"free" some of the sequestered pooled blood or to increased catecholamine release or both (Gilbert, 1960). Following this recovery, blood pressure then declines with the animals dying in shock. Wiggers, in 1950, reported that the final, prolonged hypotensive period could not result from either trapping of blood in the liver or result from the early decline in arterial blood pressure for the initial fall in blood pressure would have to be more prolonged to be a factor of irreversibility. It has, however, been well established that cardiac output is decreased during late endotoxin shock (greater than 20 minutes) (Chien, et al., 1966; Dedichen and Schenk, 1967; Martin, et al., 1965; Freidberg, et al., 1944; Ebert, et al., 1955), but the mechanisms for the decline in cardiac output is still controversial. Late is shock, venous return is also decreased as it was during the early period. This probably resulted from pooling of blood in the gut and elsewhere, for it has been shown that pooling of blood does occur in the noneviscerated dog for at least thirty minutes and in the eviscerated dog for one hour (Meyer and Visscher, 1962; Chien, et al., 1966, Hinshaw, et al., 1958; Weil, 1956). Venous return-constant flow experiments are very reliable in determining the degree of blood pooling or sequestration as evident by changes in reservoir blood volume. However, these experiments are not usually maintained beyond one hour because of deterioration in the preparation (Gilbert, 1960). Other experimental evidence, such as low right and left

atrial pressures, increases in cardiac output, following an infusion of fluid and small decremental changes in blood volume producing large decreased in output have supplied good evidence for a decrease in venous return in the late phase of shock (Hagano, et al., 1974; Freedberg and Altschule, 1945; Pennington, et al., 1973; Ebert, et al., 1955; Lansing, 1963; Kuida, et al., 1958).

Decreases in venous return other than by blood pooling or sequestration are possible. Blood volume changes in the late phase of endotoxin shock could potentiate the effects of vascular pooling and cause a further decline in venous return. Early reports have stated that plasma volume reduction must eventually occur during endotoxemia or shock because of the occurrence of diarrhea and vomitting in unanesthetized animals, including man. There are also reports suggesting loss of fluid into the tissues, based on histological studies where edema was evident in the intestine along with the presence of I^{131}-labelled albumin (Pemer and Bernheim, 1942; Kraneberg and Sandritter, 1953; Aust, et al., 1957; Aust 1959).

In the hepathosplanchnic bed of the dog, fluid filtration has been shown to occur during endotoxin shock resulting in a bloody diarrhea (Chien, et al. 1964; Hinshaw, et al., 1964). Chien, et al., in 1964 and 1966, have reported evidence for fluid filtration in other vascular beds, often based upon measurements of thoracic lymph flow, increased hematocrit and decreases in plasma volume. When

hematocrit is inhibited form rising following splenectomy, plasma volume actually increased during endotoxin shock in the dog. In addition, the means by which plasma volume is measured in endotoxin shock are questionable. These techniques offer employ dye-dilution and it has been shown by Horwitz, et al., in 1972, that dye-dilution techniques are invalid because of slower, uneven mixing and sequestration of blood during shock. In the dog, these techniques for measuring blood volume may be of little significance as opposed to primate studies, where the degree of pooling or sequestration is limited.

If net fluid filtration in extra-hepatosplanchnic areas does occur, the specific location of this efflux has not yet been identified. On the contrary, net fluid reabsorption has been reported throughout the entire shock period (Hinshaw and Owens, 1971; Weidner, et al., 1971). These reports were based on actual organ weights and vascular resistances in skin and skeletal muscle. Weidner, et al., in 1971, and Hinshaw and Owens, in 1971, reported that decreases in canine forelimb weight were associated with declines in vascular resistance. This suggests that the weight loss was due to interstitial fluid reabsorption. Similar changes in other species such as the cat and monkey during endotoxin shock also supports this concept. Thus, blood volume loss subsequent to transcapillary fluid loss cannot explain the decrease in venous return in the dog during the late phase of endotoxin shock.

A possibility which has to be considered as a probable mechanism for the decrease in venous return in the late phase of endotoxin shock is the involvement of the myocardium. Most authors agree that the myocardium will fail in shock, although reports vary as to the mechanism of the dysfunction (Alican, et al., 1962; Boho, et al., 1973; Brand and Lefer, 1966; Hinshaw, et al., 1974, 1971, 1972). Hinshaw, et al., in 1971, 1972 and 1974 were unable to demonstrate any cardiotoxic effects of endotoxin during the first 4 hours of shock. Their preparation consisted of isolated working hearts. Six hours after endotoxin shock induction, consistent changes in myocardial failure were reported. Their results, however, were not consistent with the theory that enodtoxin produces direct myocardial failure but rather its effects are indirect; failure arising from prolonged hypotension and low flow. Contrary to their findings, Brand and Lefer in 1966, reported on the discovery of a myocardial depressant factor (MDF) which was obtained from blood of shocked animals and possessed cardiodepressant or negative inotropic actions (Lefer, 1970, 1973). They later reported that the pancreas was the major site of MDF production. The production was dependent on pancreatic ischemia which caused tissue hypoxia. Pancreatic hypoxia is reported to stimulate the release of lysosomal hydrolase, particularly acid proteased (Glenn and Lefer, 1971; Herlihy and Lefer, 1975). These enzymes were shown not only to catalyse the proteolysis leading to MDF formation, but also

directly exert deleterious effects on the micorcirculation and myocardium (Ferguson, et al., 1972, Glenn, et al., 1972). In contrast, Hinshaw, et al., 1972a, reported that although the heart failed after four to six hours of shock, this did not result from the direct action of MDF on myocardial cntractility. To demonstrate their hypothesis, pancreotectomized animals were studied and found that the presence of the pancreas bore no relationship to the degree of myocardial failure in endotoxin shock (Hinshaw, et al., 1974).

The role of the heart late in shock (greater than 4-6 hours) in regulating cardiac output and blood pressure is clear, however, the mechanism(s) for dysfunction remians controversial.

Carbohydrate Metabolism During Shock

Reports by Menten and Manning in 1924 and Zechmer and Goodell in 1925 presented evidence concerning rabbits injected with crude bacterial extracts from the Enteritidis-Parathyphoid B group. Blood glucose concentrations in these rabbits increased from approximately 130-160 mg/dl to 235-250 mg/dl over the first few hours following injection of the crude bacterial extracts, then progressibely decreased, with the rabbits dying with a blood glucose condentration of 30-40 mg/dl.

One characteristic hallmark of endotoxin of gram negative septic shock is the appearance of hypoglycemia

(Beck, et al., 1970). Some reports have documented hyperglycemia during endotoxin shock as well as during live E. coli septicemia (Cryer, et al., 1971; Hinshaw, et al., 1975). These differneces between hypoglycemia versus hyperglycemia may reflect only different time courses of shock.

Cryer, et al. in 1971, reported that during the administration of live E. coli bacteria (10^{10} organisms/ml given at 10 ml/kg body wt.) into unanesthetized baboons (Papio Doguera) plasma glucose concentrations increased significantly and remained elevated during the 6-hour experimental protocol. Along with the measurements of glucose, they also measured insulin, 11-hydroxycortisteroids, growth hormone and urinary catecholamines. Glucose, 11-hydroxycortisteroids, growth hormone and catecholamines were elevated, whereas insulin was decreased. Based on their results, they concluded that the initial and prolonged elevated glucose concentration was a result of impaired insulin release from the pancreatic islet cells. This is consistent with the fact that glucose appearance and disappearance from plasma is modulated by insulin which not only promotes peripheral glucose uptake but also depresses hepatic glucose release (LeCocq, et al., 1964; Mack and Egdahl, 1970; Madison, et al., 1959). Their findings emphasized a major role for depressed insulin in causing hyperglycemia. This was based on data showing a decrease in plasma insulin concentration during E. coli septicemia in

the baboon and suggested that the observed hyperglycemia was due, in part, to lack of insulin at the appropriate receptor site. Their interpretation of the results may be incomplete. They concluded that the increased catecholamine levels may have played a role in the development of hyperglycemia because it has been well-documented that catecholamines (norepinpherine, epinepherine) released from either sympathetic nerve terminals or the adrenal medulla or both in shock have profound effects in stimulating glycogenolysis from the liver (Filkins and Cornell, 1974; McCallum and Berry, 1973; Zwadzk and Snyder, 1973; Groves, et al., 1974; Blackwood, et al., 1973). Many investigators have also shown that during endotoxin or septic shock, plasma catecholamines are elevated (Sanford, et. al., 1960, LeCocq, et al., 1964; Guetner, et al., 1969; Spink et al., 1966; Nykiel and Glaviana, 1961; Rosenberg, et al., 1959, Hinshaw, et al., 1964; Zweifach, et al., 1956; Thomas, 1956). Insulin depletion, together with the presence of anti-insulin mediators, such as 11-hydroxycortisteroids, catecholamines and growth hormone, need not be the only answer to elevated plasma glucose concentrations associated with endotoxin or septic shock in the baboon. Since Cryer, et al., did not measure either the rate of appearance or disappearance of glucose form the plasma, it connot be assumed that glucose efflux from plasma is not increased during low inculin cncentrations. It has been demonstrated that glucose transport into skeletal muscle and cardiac

muscle can occur independent of insulin, provided the tissues are hypoxic (Morgan, et al., 1958; Randle and Smith, 1958). Morgan, et al., in 1958, and Randle and Smith, in the same year, demonstrated that cardiac or skeletal muscle glucose uptake increased by 100% at any given insulin concentration when the bathing fluid was made hypoxic. The increase in glucose transport was dependent on the degree of hypoxia. Cryer, et al., also showed that venous PO_2 values were greatly reduced, which would further support an anaerobic membrane transport of glucose. It is possible that the hyperglycemia could have resulted from glycogen breakdown only, its rate of production of glucose being greater than the rate of disappearance from plasma into muscle via anaerobiosis--which is independent of plasma insulin concentrations. In 1972, Cryer, et al., published another report showing similar hyperglycemic responses to E. coli septicemia in the baboon. This study was undertaken to clarify the effects of hypoinsulinemic hyperglycemia witnessed in the previous study. Their assumption, based on the previous data, was that hypoinsulinemia was the cause for hyperglycemia in the septic baboon. To eliminate catecholamine inhibition of insulin release through alpha-adrenergic stimulation of islet cells, baboons were pre-treated with an alpha-adrenergic inhibitor, phenotlanine. E. coli septicemia did not produce any increase in glucose concentration or depression in the plasma insulin. These results were interpreted to

substantiate previous data where decreases in insulin were implicated as the major cause of hyperglycemia in septic baboons. As in their previous data, these results only demonstrated that insulin, allowed to increase in response to the increased plasma glucose concentration, following E. coli septic shock induction, responded predominantly to facilitate transfer of glucose into insulin-dependent cells, reducing blood glucose concentrations to control levels. With the mass of information dealing with carbohydrate metabolism in the shock state, it was generally accepted that hyperglycemia is generally described in primates (Cryer, et al., 1971, 1972) and hypoglycemia is emphasized in canines after injection of live Escherichia coli organisms or endotoxin (Berk, et al., 1970; Griffits, et al., 1973; Groves, et al., 1974; Peyton, et al., 1974; Raymond and Emerson, 1978). Hinshaw, et al., in 1975, repeated Cryer's previous work in baboons and found that, if the animals were monitored until death or recovery, blood glucose concentrations and insulin concentrations were similar over the first 4 hours of the study. As the time course of shock progresses, 86% of the baboons died within 26 hours after E. coli septic shock induction (mean survival time of 15 hours). There was also a progressive decline in blood glucose concentration in the presence of a decreased plasma insulin concentration. Hinshaw correlated death of the animal to its blood glucose concentration, one annimal dying with a blood glucose concentration of 0 mg/dl at 14

hours into the experiment. These data provided evidence that hypoglycemia did occur in the baboon if the experiments were followed long enough and the decrease in blood glucose concentration occurred in the presence of a diminished plasma insulin concentration. Hinshaw demonstrated clearly the progressive debelopment of hypoglycemia in the baboon administered lethal infusions of live E. coli bacteria. The nature of hypoglycemia was that it was not merely a terminal event reflecting obvious deterioration of major organ systems, but rather it represented an early metabloic deficiency in the animal, lasting many hours and becoming progressibely worse with time. Further corroborative indirect evidence for an increase efflux of glucose from plasma was seen by the increased lactate concentrations in the baboon studies, indicatng a shift from aerobic to anaerobic metabolism in which glucose-derived pyruvate produces lactate (O'Donnell, et al., 1974). Blackwood, et al., in 1973, and Groves and associates, in 1974, reported elevations in lactate 1 and 2 hours after endotoxin or live E. coli organism admistration. O'Donnell, et al., in 1974, and Rackwitz, et al., in the same year, have reported significantly elevated lactate in septic shock, concimitant with severe hypoglycemis.

Intravenous injections of live E. coli organisms into dogs also resulted in hypoglycemia; glucose was reported to decrease from 90 mg/dl to 70 mg/dl within 4 hours of injection (Griffits, et al., 1973) and to decrease from 102

mg/dl to 62 mg/dl in 6 hours (Groves, et al., 1974). When an LD_{70} injection of E. coli endotoxin was administered to dogs, hyopglycemia occurred (Berk, et al., 1970). These animals showed progressively developing systemic hypotension and acidosis and, within three hours, dogs were dying in convulsions with a plasma glucose concentration of 25 mg/dl. Hinshaw, et al., in 1974, also demonstrated the occurrance of hpyoglcemia in dogs given an LD_{70} concentraion of E. coli endotoxin. In both studies by Berk, et al., and Hinshaw, et al., there were animals occasionally developing initial, transient hyperglycemia. Similar hypolycemia was also noted in unanesthetized dogs given lethal doses of live E. coli organisms (Archer, 1976). To summarize these events, it is clear that following either endotoxin or live gram-negative bacteria in a variety of animal species, the predominant effect is the occurence of hypoglycemia occasionally preseeded by a transient hyperglycemic period. The initial hyperglycemia has been attributed to depletion of liver glycogen subsequent to hypotensive-induced catecholamine release. Hyperglycemia persists for longer periods of time in the primate as compared to the dog, but beyond 6 hours, progressive hypoglycemia ensues in the baboon, with animals dying with very low plasma glucose concentrations.

In previous studies, Griffits, et al., in 1973, demonstrated that hypoglycemia, hypertriglyceridemia and hypoinsulinemia occur in dogs with E. coli bacteremia during twelve hour esperiments. The hypoglycemia and

hypoinsulinemia suggest that the septic dogs had hepatic dysfunction resulting in impaired gluconeogenesis. A key amino acid precussor for gluconeogenesis is alanine. This amino acid is deaminated to pyruvate and is subsequently converted to glucose (Felig and Wahren, 1971). Impaired hepatic utilization of alanine is thought to cause the hyperalanemia which has been observed during lactic acidosis and shock (Marliss, et al., 1972; Marchidk, et al., 1976.

Decreased oxidation of lactate via pyruvate in the tricarboxylic acid cycle is generally considered responsible for the lactic acidosis of shock (Schumer, 1968). Recent evidence from Wolfe, et al., 1977, has also shown an increased lacaite in endotoxin shock in dogs together with hypoglycemia. Their results provided indirect evidence that endotoxin hypoglycemia developed because of an enhanced peripheral uptake of glucose and a failure of the liver to maintain an increased glucose production. Their results also concluded that lactate became an important precussor for gluconeogenesis and an important metabolic substrate.

Filkins, et al., in 1975, provided direct evidence for impaired hepatic gluconeogenesis in endotoxin shock. The estimates of gluconeogenesis in vivo were accomplished utilizing alanine loading of insulin-suppressed rats. In their model, alanine is used primarily by the liver to produce glucose. However, under normal conditions, as the hepatic glucose output increases, thereby transiently increasing plasma glucose, insulin release will be augmented

while glycogen release is inhibited. These changes would effectively blunt hepatic gluconeogenesis thereby augmenting tissue use and storage. In order to estimate hepatic gluconeogenesis better, they used mannoheptulose to block insulin secretion which would allow hyperglycemia to better reflect the status of hepatic gluconeogenesis. Conversion of ^{14}C-alanine to ^{14}C-glucose was another technique used to estimate hepatic gluconeogenesis. Their results provided direct evidence that during endotoxin shock, gluconeogenesis as measured either by the hyperglycemic response to alanine loading or by ^{14}C-alanine conversion to ^{14}C-glucose, is greatly depressed. Several other reports have also noted a decrease in hepatic gluconeogenesis in a variety of animal models in shock (Filkins and Cornell, 1974; Schumer, et al., 1970; Mela, et al., 1971; Freinkel, et al., 1965; LaNoue, et al., 1968).

A malfunctioning liver, however, need not be the sole explanation of progressively developing hypoglycemia because notable hypoglycemia has been reported in evicerated dogs within two hours following endotoxin administration, whereas control, evicerated dogs demonstrated relatively constant levels of glucose (Peyton, et al., 1974). Experiments carried out in evicerated rats by Russell, et al., in 1944, revealed the occurrence of hypoglycemia within three hours in contrast to control, non-hemorrhaged evicerated rats. The liver and pancreas were removed in the studies and the

mechanism for the marked fall glucose was ascribed to anaerotic metabolism occuring in the presence of hemorrhage at rates exceeding those in control, evicerated, non-hemorrhaged rats.

Mechanism(s) of Shock Induced Hypoglycemia

Lindsgaard-Hansen, et al. in 1972, considered circulatory deterioration to be the primary determinant of the degree of shock due to enodtoxin because of its adverse effect on tissue metabolism. They ascribed a lesser influence of early toxic effects of endotoxin on liver metabolism. Glucose levels, however, were not significantly altered in their studies. Impairment of hepatic oxidative metabolism which could be accounted for on the basis of circulatory-toxic interactions has been documented following endotoxin injection in rats (Mela, et al., 1971). Circulatory factors indirectly influencing the release of insulin (via catecholamine release) from the pancreas were suggested (Spath, et al., 1974; Lefer and Spath, 1974). The low cardiac output stage of septic or endotoxin shock and the extremely low plasma concentration of insulin may be primarily due to the impoverished metabolic status of a poorly perfused pancreas (Clowes, et al., 1974; O'Donnell, et al., 1974).

Aside from circulatory deterioration which would cause a decrease in pancreatic release of insulin, hypoglycemia in shock may be ralated to a reduced rate of glucose production

because the tissue demand for blood glucose might then outstrip the supply mechanism, eventually resulting in lethally low concentrations of glucose in peripheral cells. Berry, in 1971, concluded that endotoxin administration depleted animals of their carbohydrate reserves by virtue of an impaired ability to carry out glycogenesis and gluconeogenesis. This statement is in agreement with other reports, showing impaired hepatic metabolism (Filkins and Cornell, 1974; Mela, et al., 1971). Williamson, et al., in 1970, provided direct evidence for a malfunctioning liver (impaired gluconeogenesis) after endotoxin induction in rats. Groves, et al., have recently documented impaired hepatic gluconeogenesis in dogs administered live E. coli organisms. Their work suggested the presence of a block in gluconeogenesis between pyruvate and glucose, and they ascribed death as primarily due to derranged liver metabolsim. Blackwood and others, in 1973, found significant depressions of both glucose and glycogen stores in liver and muscle of dogs subjected to hemorrhage or endotoxin shock.

Data from the literature on trauma studies in man suggest an increased need for substrate. Evidence was provided that during endotoxin shock, glucose efflux from plasma occurs at a greater rate than would normally occur under control conditions (Kinney, 1972). Sepsis has also been shown to cause not only a greater turnover rate of glucose but also to cause greater glucose oxidation. Energy

expenditure measurements were made in severely burned patients with sepsis and it was found that the energy expenditure exceeded the caloric equivalent of fever (Roe and Kinney, 1965). Long, et al., in 1971, provided corroborative evidence of Roe and Kinney by showing that sepsis did not interfere with the body to utilize glucose, but rather the rate of glucose oxidation was elevated. Further studies dealing with increased energy expenditure in shock (Hypermetabolic state) were described by Halmagyi, et al., in 1974. Their results were broken down into two categories: 1) high resting metabolic expenditure during shock in normothermia, and, 2) a state with increased fever with an elevated caloric requirement. Their results provided evidence for increased glucose oxidation in shock of normothermic patients and a further increase in glucose utilization with increased fever. Their results were consistent with the clinical observations that large amounts of glucose are needed intraveously to maintain blood glucose concentrations constant during endotoxin shock (Hinshaw, et al., 1974). Utilizing isolated mitochondrial preparations it was also found that endotoxin or septic shock produced an uncoupling of mitochondrial oxidative-phosphorylation. This was accompanied by increased glucose oxidation, increased heat production and decreased ATP production. The prevailing results from the literature have documented an increased disappearance of glucose from plasma in septic or endotoxin shock in the presece of low plasma insulin

concentrations. The mechanism(s) for the disappearance of glucose has been the subject of recent investigations. Berry, in 1971, reported that endotoxin stimulated cellular glycolysis in "a fashion similar to that obtained with insulin" and also pointed out that endotoxin caused an increased glucose utilization by leucocytes and macrophages. Experiments done recently to specifically document the glucose utilization and role of blood in endotoxin shock (Hinshaw, et al., 1977, 1977a) following the intravenous injection of E. coli endotoxin or live E. coli organisms into the dog, show that white blood cell (WBC) concentrations were significantly increased (from 18,000/mm^3 to 29,000/mm^3). The major fraction for the elevated WBC count was due to elevated neurtophils and immature neutrophils. Lymphocytes and monocytes were not significantly altered during endotoxin challenge. Hinshaw, et al., however, did not demonstrate the mechanism for the leukocytosis, but it is known that endotoxin administration promotes the entry of new leukocytes from the bone marrow into the circlation (Herion, et al., 1965). Hinshaw's results also demonstrated increased glucose utilization by WBC, particularly the neutrophils. These calculations were based on the total amount of glucose taken up by the WBC during leukocytosis. When glucose uptake, occurring during the first hour of endotoxin administration, was divided by the average WBC count during the same time sequence, it was found that the quantity of glucose taken up per acitvated (endotoxin) WBC

was not different from non-activated WBC in vitro (11.7 x 10 $^{-9}$ vs. 7.4 x 10 $^{-9}$ mg of glucose/ WBC/60 min. respectively). The increased glucose utilization by the WBC was related to their numbers rather than enhanced metabolic activity of individual WBC. The WBC types accounting for the total increase in numbers seen in their study was shown to be the mature and immature neutrophils, which have been reported to be perticularly active in phagocytosing endotoxin (Balis, et al., 1974) or live E. coli organisms (Postel, et al., 1975). In relation to their findings, other reports have documented beneficial effects of transfused white blood cells in animals and patients in septic or endotoxin shock (Graw, et al., 1972; Epstein, et al. 1974). Nemeth, et al., in 1972, studied the metabolism of glucose in rats during Nobel-Collip drum traumatic shock. Their interest in studying glucose metabolism as a function of shodk was stimulated by the discrepancy between hypoglycemia-starved shocked rats as opposed to the hyperglycemia-fed shocked rats. These early findings of the differnces in the metabolic response to shock may also be the underlying factor for the increased resistance of fed animals to shock (Mraz, et al., 1959). During their shock protocol, they provided results in which hypoglycemia was induced during Nobel-Collip drum traumatic shock in the rat. Because of the technique used to traumatize the animals, only indirect evidence was used to determine the means for the increased rate of glucose efflux from plasma. Urinary loss was

excluded because of the low glucose concentration in plasma. Plasma glucose values were below the renal threshold (Tm) for glucose. Increased diffusion through damaged intestines was also discarded because luminal glucose concentrations were not significantly elevated. Measurements of free glucose concentrations in the liver, skeletal muscle and epididymal fat pads were also not significantly elevated above control. Their final conclusion was that glucose was being lost through anaerobic glycolysis. This was shown by pretreating the rats with 2-deoxyglucose, which blocks glycolysis. When this was done, rats traumatized by Nobel-Collip drum tumbling were seen to prevent the disappearance of the greater part of glucose. Nemeth, et al., concluded that if the glucose disappearance had been by some other route other than glycolysis, the fall in glucose should have been similar in magnitude to that which occurred without 2-deoxyglucose. Two mechanisms for the enhanced glucose disappearance are possible: 1) insulin's effect, and, 2) tissue hypoxia. Insulin's effects were discarded because the same results occurred in alloxan diabetic rats. Therefore, their conclusion was that tissue hypoxia caused an elevated glucose transport into tissues. This was substantiated by low arterial and vena caval PO_2. Engel, in 1946, and Russell, et al., in 1944, both observed a sharp fall in blood glucose in evicerated rats subjected to hemorrhagic shock in the absence of pancreatic insulin. Their findings suggested that anaerobic metabolic pathways

were utilized in the rapid disappearance of carbohydrate in the periphery during shock. This mechanism would explain the extremely marked fall of blood glucose in evicerated dogs receiving endotoxin in contrast to control, non-evicerated dogs(Peyton et al., 1974). Drucker and Dekiewiet, in 1964, measured glucose uptake of the isolated diaphragm removed from rats subjected to hemorrhagic shock. They observed an increased glucose uptake occurring in the presence of a severe depletion of intracellural energy primarily through anaerobic pathways. O'Donnell, et al., in 1974, reported that hindlimbs of animals maintained normal glucose uptake during low flow septic shock state. Filkins also has shown that hemidiaphragm muscles taken from shocked rats exhibited elevations in glucose uptake. On the other hand, a recent study by Furr, et al., in 1978, suggested that glucose uptake is not affected in the isolated, perfused dog forelimg preparation during endotoxin shock. Their results were interpreted to mean that skeletal muscle glucose uptake was not altered during endotoxin shock. Methods for their experiment could produce significant errors in that blood flow from the brachial vein, which drains mainly muscle, and cepahlic vein flow, which drains mainly skin, were pooled into a common reservoir before glucose analysis was determined. Therefore, glucose measurement came from blood of skin and skeletal muscle combined. Because the effect of endotoxin shock on skin glucose metabolism is unknown, combined with some error in

sampling techniques, it is possible that small, but significant, changes in pure skeletal muscle glucose uptake would be missed during endotoxin shock. It is also possible that skin glucose uptake actually decreased during endotoxin shock. If this happened, and increase in forelimb muscle glucose could be offset, resulting in misleading data and and erroneous interpretation.

A final possible mechanism for glucose uptake in shock during the presence of extremely low insulin cncentrations would be the actions of kinins in facilitating glucose transport. Research by Nies, et al., 1968, documented the release of kinins in monkeys administered endotoxin, whereas, Haberland, in 1972, had demonstrated insulin-like actions of kinins in glucose transport.

In summary, five mechanisms are possible for the hypoglycemia of shock. These include the insulin-like actions of endotoxin, the increased glucose utilization by WBC, the effects of kinins on glucose transport, impaired hepatic gluconeogenesis and the increased glucose utilization by anaerobic pathways.

METHODS

Fifty two mongrel dogs of either sex, weighing 20 ± 2 kg, were anesthetized with sodium pentobarbital (30 mg/kg), intubated and ventilated spontaneously. Following the surgical preparation, animals were anticoagulated with 10,000 units of sodium heparin. During the experimental protocol, supplemental administration of sodium heparin (500 units) and sodium pentobarbital (averaging 50 mg) were given hourly.

Surgical preparation of the gracilis muscle during either natural or constant flow

A longitudinal skin incision, using a thermal cautery, was made over the right gracilis muscle. The incision was made from a point near the origin of the gracilis muscle to approximately 2-3 cm below its insertion. Skin covering the muscle and neighboring femoral artery and vein were removed by cauterization. This was done to insure accessible cannulations. The gracilis muscle was isolated from all connective tissue and surrounding muscles. The obturator nerve was carefully isolated from its conective tissue near

the gracilis muscle. Care was taken not to injure the nerve during isolation. The entire length of the gracilis artery and vein were completely isolated from conective tissue and all side branches from these vessels were ligated. This was done to insure that the gracilis muscle received no collateral perfusion from non-gracilis arterial muscle sources and also to insure that gracilis venous outflow was free from venous blood not derived from the gracilis muscle. The femoral artery and vein were isolated approximately 3-5 cm proximal and distal to the insertion of the gracilis artery and vein. All side branches of the femoral vessels were ligated to prevent collateral perfusion by non-gracilis sources. To further decrease the possibility of collateral perfusion of the gracilis muscle, heavy ligatures were placed around the tendons at the insertion and origin of the muscle. The left femoral artery was cannulated with polyethylene tubing (PE 320) and the other end of the cannula was inserted retrogradely into the right remoral artery at a point approximately 2 cm below the level of the gracilis artery. The tip of the cannula in the right femoral artery was positioned so that it was below the level of the gracilis artery. A sigmamotor pump was interposed in the perfusion circuit and was used for constant flow perfusion of the gracilis muscle. The femoral artery, above the gracilis artery, was cannulated with polyethylene tubing (PE 240), and advanced to a level of the abdominal aorta. This cannula was used to measure arterial blood pressure.

Interposed in the polyethylene tubing perfusion the right femoral artery was a piece of rubber tubing. This rubber tubing was placed close to the right frmoral artery. A needle, attached to polyethylene tubing (PE 90) was inserted into the rubber tubing to measure gracilis muscle perfusion pressure. Perfusion pressure was set at a level approximating arterial blood pressure. The right femoral vein was connulated with polyethylene tubing (PE 320) at a level 2-3 cm below the gracilis vein. Above the gracilis vein, the femoral vein was ligated to prevent any backflow from the systemic circulation. Gracilis venous outflow drained by gravity into a reservoir that was primed with approximately 200-300 ml physiological saline and/or dextran, and was returned continually to the cannulated (PE 320) left femoral vein via a variable speed Holter blood pump.

Completeness of vascular isolation was confirmed by stopping the perfusion pump and observing the perfusion pressure and gracilis venous outflow. A drop in muscle perfusion pressure to 25 mm Hg or below during the stopping of the perfusion pump was considered acceptable provided venous outflow was zero. Muscle blood flow was determined by timed collection of venous outflow with a graduated cylinder and stopwatch and was expressed per 100 grams by removing and weighing the muscle at the end of the experiment. Muscle drying was prevented by bathing the muscle with warm mineral oil and covering it with Saran

Wrap. Muscle temperature was maintained at 38° C with a heat lamp. A Jugular vein cathether (PE 240) was used for supplemental drug administration and endotoxin infusion.

All pressure recordings were made from appropriate pressure transducers (Statham Laboratories, Model P23Gb) connected to a Sanborn polygraph (Sanborn Co., Mode 160-1300).

During natural flow experiments, only the gracilis vein was prepared and the arterial inflow remained intact. The left femoral artery was used to measure systemic arterial blood pressure. All other procedures listed above were similar during the surgical preparation of the muscle.

Blood gases and chemical analyses

During the appropriate experimental protocol, blood samples were drawn anaerobically from the femoral artery and gracilis vein. Blood gases and substances were analyzed as follows: 1) PO_2, PCO_2 and pH with an Astrup Radiometer blood gas analyzer; 2) O_2 content with a Lex-O_2-con oxygen analyzer; 3) glucose concentration with a Yellow-Springs glucose analyzer; 4) insulin by radioimmunoassay; 5) hematocrit by microcentrifugation; 6) muscle surface temperatube with a celsius thermometer. Oxygen and glucose uptake by the gracilis muscle were calculated as the product of the appropriate arteriovenous difference muluiplied by muscle blood flow. Resistance was calculated by dividing perfusion pressure by muscle blood flow.

Experimental Protocol

Series I. Direct effects of E. coli endotoxin on gracilis muscle hemodynamics and metabolism during constant flow conditions.

To test the direct effects of E. coli endotoxin on gracilis muscle glucose uptake and hemodynamics, the inervated gracilis muscle, perfused under constant flow, was used. E. coli endotoxin, prepared in physiological saline, was infused at 0.382 mg/min at a volume flow rate of 0.382 ml/min via a needle inserted into the gracilis artery inflow tubing behind the blood pump so as not to alter the constant flow perfusion rate of the muscle. The saline vehicle (control) was infused at an identical volume rate. The amount of endotoxin infused approximated a gracilis artery blood concentraion of endotoxin which occurs when 2 mg/kg E. coli endotoxin is given intravenously as a bolus injection. The estimate was based upon an average blood volume of 8.6% body weight, and average body weight of 20 kg, and an average gracilis blood flow rate of 15 ml/min/100 grams. The previously described hemodynamic and metabolic parameters were determined before, during and after a five to ten minute infusion of saline or purified E. coli endotoxin.

The gracilis venous blood was diverted from the reservoir and discarded during intra-arterial endotoxin infusion in order to avoid any endotoxemia or shock in the animal; however, uncontaminated blood from the venous

reservoir was returned continually to the dog to prevent any systemic volume depletion. The initial blood volume in the venous reservoir was approximately 200-300 ml and the amount of blood returned to the animal during intra-arterial endotoxin infusion never exceeded 100 ml. The reservoir was primed with either normal physiological saline, dextran (M.W. 40,000) or cross-matched whole blood from a donor animal. No difference in any variables were noted when saline, dextran or whole blood were used.

Series II. Hemodynamic and metabolic alterations of the gracilis mescle under natural flow conditions, during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin.

Experiments were conducted to determine the effects of E. coli endotoxin shock on gracilis muscle hemodynamics and metabolism. In this series of experiments, the innervated naturally perfused gracilis muscle preparation was used. Following a ten to fifteen minute stabilization period, control blood samples and hemodynamic measurements were taken and 2 mg/kg E. coli endotoxin, suspended in 20 c.c. physiological saline, was infused intravenously. Endotoxin was infused over a 5-minute period. All hemodynamic measurements and chemical analyses, except insulin, as

described previously, were made during consecutive thirty minute intervals for a total of six hours, or until death of the animal.

Series III. Effects of constant gracilis muscle blood flow on muscle hemodynamics and metabolism durng the intravenous administration of 2/ mg/kg (LD_{100}) E. coli endotoxin.

This series of experiments utilized the constant flow-gracilis muscle preparation. Muscle blood flow was maintained constant during endotoxin shock. Excluding insulin, all chemical, hemodynamic and statistical measurements were similar to those previously described in Series II.

Series IV. The effect of partial local ischemia a muscle hemodynamics and metobolism.

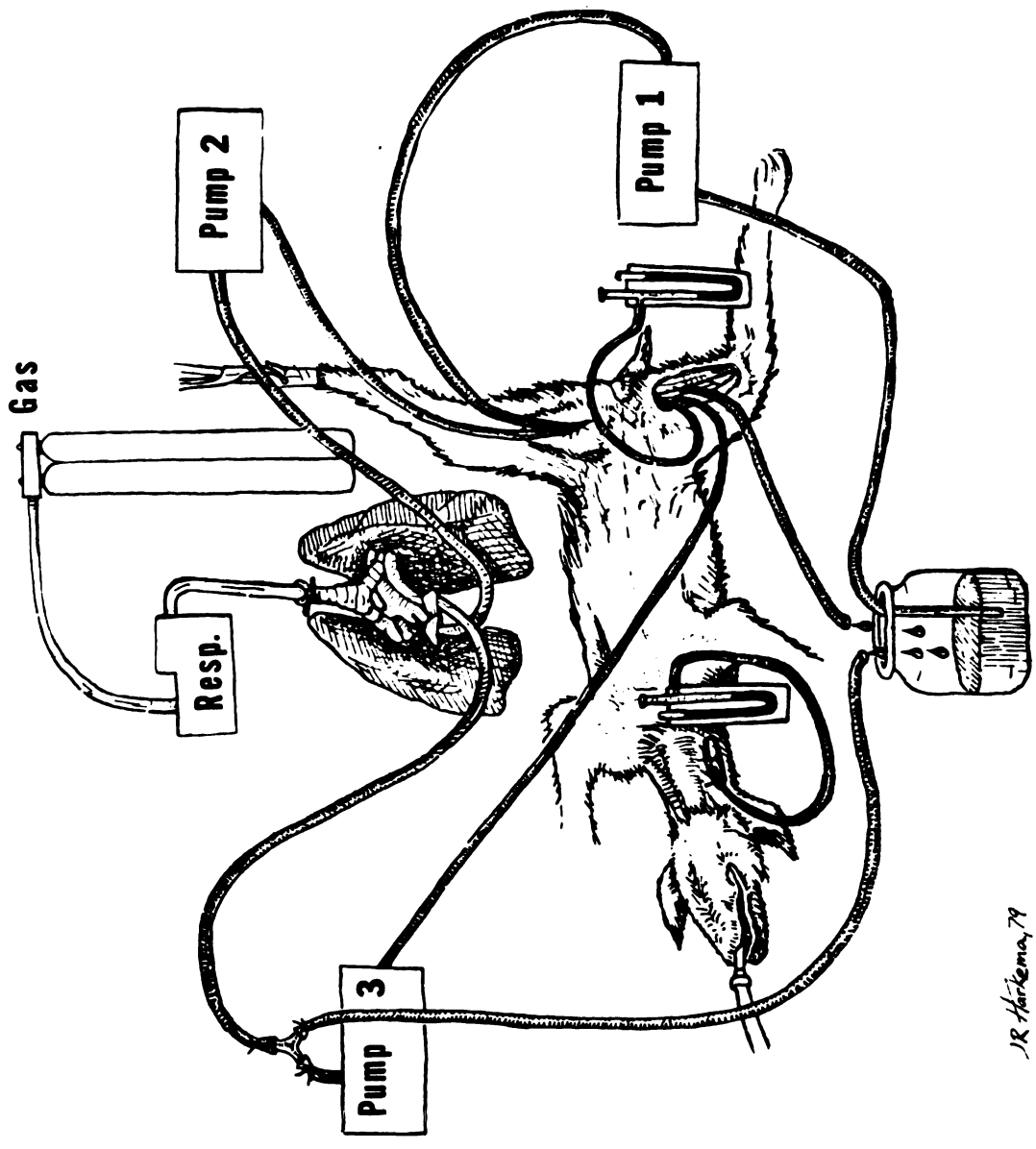
The innervated, constant flow gracilis muscle preparation was used in this series of experiments. Similar measurements were conducted as described in Series II., except endotoxin was not administered, rather, muscle blood flow was decreased to a similar level as that seen during natural flow shock (Series II). Experiments lasted 6 hours and similar hemodynamic and metabolic measurements were made as in Series II.

Series V. Effects of local muscle hypoxia, independent of ischemia, on muscle metabolism and hemodynamics.

The isolated, innervated, constantly perfused gracilis muscle preparation was used as previously described except that an extracorporeal heart-lung preparation was interposed between the left femoral artery and right gracilis artery (Figure 2).

A small donor dog (8-10 kg) was used for the extracorporeal heart-lung preparation. The heart and lung were removed from the donor dog by a left thoracotomy made in the fourth intercostal space. Two lobes from the left side of the lung were isolated and the remaining lobes on the left side and all the lobes on the right side were ligated and sectioned free from the main pulmonary tree. The trachea was isolated and cut approximately 5-7 cm above its insertion into the lung. The vena cava, aorta, esophagus and all connective tissue and vessels were sectioned and the heart and remaining lung were removed. The heart was transversely sectioned at a position approximately half-way between the base and apex. The left atrium was cannulated with large bore tygon tubing having a plastic fitting over the end of the tygon tubing. The plastic fitting was flared so that its placement into the left atrium could be secured by surrounding the atrium with a tight ligature.

Figure 2: Schematic representation of the gracilis muscle perfusion system utilizing an extra corporeal heart-lung preparation.


Gas
Pump 2
Pump 1
Resp.
Pump 3
JR Harkema, 79

The cannula from the left femoral artery (PE 320), attached to a sigmamotor blood pump, was inserted into the main pulmonary artery. Flow was adjusted so that approximately 25-30 ml/min passed through the lung. As flow from the lung filled the left atrial cannula, it traversed along polyethylene tubing (PE 320) to a plastic "Y" connection. One section of the "Y" connector was attached, by polyethylene (PE 320) and rubber tubing to another sigmamotor pump which was used to perfuse the right gracilis artery via the right femoral artery as described earlier. The other portion of the "Y" connector drained excess blood not perfusing the gracilis artery via the sigmamotor pump. This blood drained into the reservoir which was also used for the gracilis venous outflow. Arterial blood perfusing the gracilis artery was sampled via the cannula draining excess pulmonary blood. Pulmonary venous pressure was measured via a cathether (PE 50) inserted into the large bore tygon tubing. The cathether tip was advanced to a level inside the left atrium, and pulmonary venous pressure was set at approximately 0 - 5 mm Hg. This was done by adding a variable resistance clamp which drained the excess blood from the lung. During control measurements, the extracorporeal heart-lung preparation was ventilated with 20% O_2, 5% CO_2 and 75% N_2. Induction of hypoxia was accomplished by vetilating the extracorporeal heart-lung with 95% N_2 abd 5% CO_2. A typical schematic, representing the surgical and perfusion set-up, is seen in Figure 2.

Following the induction of hypoxemia, hemodynamic and chemical analyses, except insulin, were made as previously described. All measurements were taken durng subsequent thirty minute intervals for a total of six hours.

Series VI. Effects of local gracilis muscle ischemia independent of hypoxia on muscle metabolism and hemodynamics.

Previously described gracilis muscle and extracorporeal heart-lung preparations (Series V) were used in this series of experiments. Following a control period, the gracilis muscle perfusing pump speed was decreased to induce muscle ischemia. Concommitant with muscle ischemia, the extracorporeal heart-lung was ventilated with a gas mixture of 95% O_2 and 5% CO_2 (Hyperoxia). This allowed the arterial blood perfusing the gracilis muscle, along with the gracilis venous effluent, to remain at control, or higher PO_2, thereby preventing ischemic-induced tissue hypoxia. Similar chemical and hemodynamic measurements, as previously described in Series V, were made during thirty minute intervals for a total of six hours.

Statistical Analyses

Data were analyzed using one and two-way Analysis of Variance. Means were compared with the Student-Newman-Keuls test. A "P" value less than or equal to 0.05 was considered significant.

RESULTS

Series I. Direct Effect of E. coli Endotoxin on Skeletal Muscle Glusoce Uptake in the Dog.

The important findings of this study are illustrated graphically in Figure 3. The upper portion of this figure shows gracilis muscle glucose uptake, while the lower portion indicates the arterio-venous glucose difference under different conditions. Each value shown represents steady state five-minute values, i.e., saline or endotoxin was infused for a five-minute period and the values depicted in Figure 4 represent the steady state value at the end of the five-minute infusion period in each case. Glucose uptake by the isolated gracilis muscle was 0.17 $\pm$ 0.06 mg/min/100 grams tissue during the control period. Infusion of saline was associated with no signicicant change in glucose uptake. The post-control value represents post-control value saline infusion and control for the endotoxin infusion. This value was 0.26 $\pm$ 0.04 mg/min 100 grams tissue. During close intra-arterial infusion of E. coli endotoxin, glucose uptake by the gracilis muscle increased significantly ($P < 0.01$) to 0.71 $\pm$ 0.09 mg/min 100 grams tissue, which represents an increase of 173% above control. Glucose uptake returned to a level not different from control immediately after stopping the endotoxin infusion ($P > 0.05$). The bottom panel of this figure shows that the

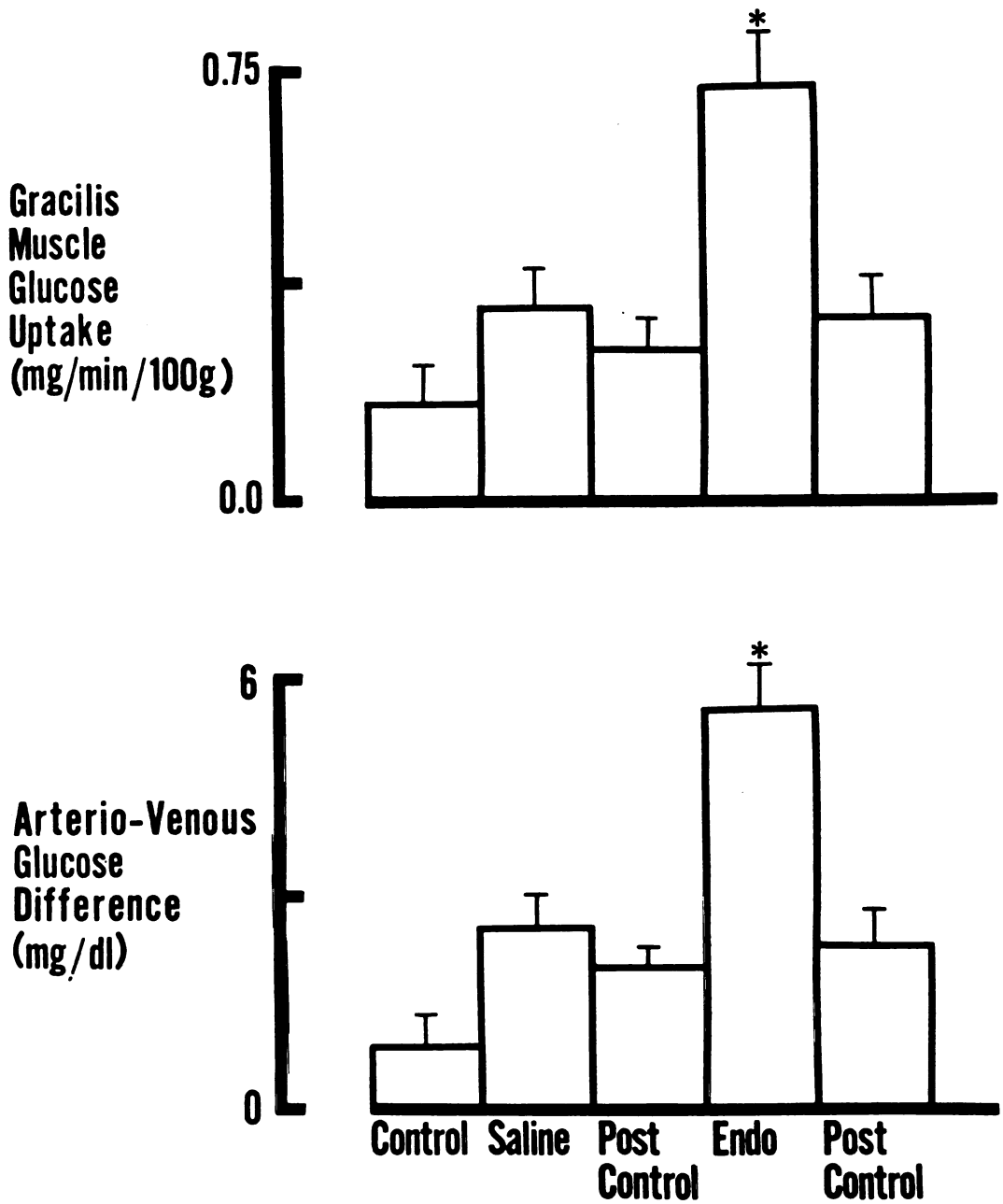
Figure 3: Direct effects of E. coli endotoxin on gracilis muscle glucose uptake and arterio-venous flucose difference.

Endo = endotoxin infusion at .382 mg/min at a volume flow rate of .382 ml/min.

Note: Saline post control is control for endotoxin infusion.

n = 11

* = p 0.01 compared to control and saline.


0.75
0.0
Gracilis
Muscle
Glucose
Uptake
(mg/min/100g)
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6
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Arterio-Venous
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Difference
(mg/dl)
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Control
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Control
Endo
Post
Control

arterio-venous glucose difference of the gracilis muscle did not change significantly during saline infusion ($P > 0.05$). There was a tendency for the arterio-venous difference to increase; however, this was probably due to dilution of the plasma glucose by the 0.382 ml/min infusion of saline. On the other hand, endotoxin infusion was associated with an increase in the arterio-venous glucose difference from 1.9 mg/dl to 5.2 mg/dl ($P < 0.01$). The arterio-venous difference returned to a level not significantly different from control immediately after stopping the endotoxin infusion ($P > 0.05$). The increase in gracilis muscle glucose uptake and arterio-venous difference during local endotoxin infusion occurred with no significant change in systemic arterial blood pressure, gracilis muscle perfusion pressure, gracilis muscle vascular resistance, hematocrit, muscle surface temperature, arterial glucose concentration, arterial or venous insulin concentration, gracilis muscle arterial or venous blood gases, muscle oxygen uptake or pH ($P > 0.05$). These values are summarized in Tables 1, 2 and 3.

Series II. Hemodynamic and Metabolic Alterations of the Gracilis Muscle Under Natural Flow Conditions During Intravenous Administration of 2 mg/kg (LD_{100}) E. Coli Endotoxin.

The intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin produced both hemodynamic and metabolic changes of the gracilis muscle under natural flow

conditions. The important findings are shown in Figures 5 and 6. Endotoxin shock caused a marked increase in gracilis muscle glucose uptake ($P < 0.01$), that was maintained above control values, except at the fourth hour, where the mean value was not different from control ($P > 0.05$.

Endotoxin administration caused the mean gluxose uptake of the grecilis muscle to increase from a control value of 0.087 mg/min/100 grams to a value of 0.511 mg/min/100 grams at sixty minutes post-endotoxin administration ($P < 0.01$). This represented a 487% increase as compared to control. Systemic arterial glucose concentration decreased significantly 120 minutes following shock induction and remained below control levels throughout the remainder of the 6-hour experiment ($P < 0.01$). During the initial ninety minute period of shock, however, arterial glucose concentration did not differ from control ($P > 0.05$) although there was a tendency for the mean values to increase. Gracilis venous glucose concentration, as seen in Table I4 also decreased following endotoxin administration; however, the decrease was seen during the first thirty minute determination and remained below control values throughout the remiander of the experiment (< 0.01).

Gracilis muscle oxygen uptake decreased from a control value of 0.448 ml/min/100 grams to a level of 0.226 ml/min/100 grams 6 hours following endotoxin administration ($P < 0.01$). At the sixth hour of shock, oxygen uptake represented a 45% reduction as compared to control. The

Tables 1. Hemodynamic Measurements of Gracilis Muscle Under Constant Flow Conditions During Close Intra-Arterial Infusion of E. Coli Endotoxin[a].

	n[b]	Control	Saline[c]	Post Control	Endotoxin[d]	Post Control
Arterial Blood Pressure (mmHg)	11	120.5 ± 3.2	117.3 ± 3.6	113.6 ± 3.7	114.1 ± 4.0	112.7 ± 3.5
Muscle Perfusion Pressure (mmHg)	11	120.0 ± 3.1	119.1 ± 4.6	121.6 ± 4.1	115.0 ± 6.7	119.1 ± 5.6
Gracilis Blood Flow (ml/min/100g)	11	13.39 ± 1.1	13.39 ± 1.1	13.39 ± 1.1	13.39 ± 1.1	13.39 ± 1.1
Gracilis Vascular Resistance (mmHg/ml/min100g)	11	9.70 ± .96	9.58 ± .97	10.00 ± 1.2	9.00 ± .67	9.64 ± 1.08
Hematocrit	11	40.82 ± 1.3	40.82 ± 1.3	40.82 ± 1.3	40.82 ± 1.3	40.82 ± 1.3
Muscle Surface Temperature[e]	6	37.73 ± .17	37.75 ± .17	37.75 ± .17	37.73 ± .17	37.75 ± .17

a = E. coli endotoxin was prepared daily before each experiment.

b = n represents number of experiments.

c = Saline infusion rate at .382 ml/min.

d = Endotoxin infusion rate at .382 mg/min at a flow rate of .382 ml/min.

e = Muscle surface temperature measured in degrees Celsius.

Table 2. Metabolic Alterations of Gracilis Muscel Under Constant Flow Conditions During Close Intra-Arterial Infusion of E. Coli Endotoxin.

	n	Control	Saline[a]	Post Control	Endotoxin[b]	Post Control
Glucose (mg/dl)						
Arterial	11	72.67 ± 2.43	73.56 ± 3.04	74.98 ± 3.06	74.36 ±3.39	74.56 ± 3.19
Gracilis Venous	11	71.46 ± 2.63	71.04 ± 2.98	73.06 ± 3.18	69.20 ± 3.93*	72.24 ± 3.19
Glucose Uptake	11	0.17 ± 0.06	0.34 ± 0.06	0.26 ± 0.04	0.71 ± 0.09*	0.31 ± 0.07
Insulin (uU/ml)						
Arterial	6	14.35 ± 2.08	15.38 ± 2.4	14.86 ± 1.73	15.31 ± 1.78	15.85 ± 1.71
Gracilis Venous	6	13.07 ± 1.37	14.36 ± 1.77	14.13 ± 1.67	14.50 ± 1.57	14.72 ± 1.52

a = saline infusion rate at .382 ml/min

b = endotoxin infusion rate at .382 mg/min at .382 ml/min

* = $P < 0.01$ compared to saline and control

Note: Saline post control was used as control for endotoxin infusion.

Table 3. Blood Gases, pH and O_2 Uptake Measurements of Gracilis Muscle Under Constant Flow Conditions During Close Intra-Arterial Infusion of E. Coli Endotoxin.

	n	Control	Saline[a]	Post Control	Endotoxin[b]	Post Control
Systemic Arterial						
PO_2 (mmHg)	11	84.86 ± 4.17	79.73 ±4.08	80.32 ± 4.45	78.78 ± 3.13	76.92 ± 3.78
PCO_2 (mmHg)	11	34.39 ± 1.68	34.16 ± 1.76	34.11 ± 1.60	33.80 ± .67	33.22 ± 1.85
pH (units)	11	7.43 ± 0.1	7.42 ± 0.1	7.41 ± .01	7.41 ± .01	7.42 ± .01
O_2 content(ml/dl)	11	16.35 ± 0.81	15.93 ± 0.76	15.35 ± 0.95	15.70 ± .83	16.21 ± 0.87
Gracilis Venous						
PO_2 (mmHg)	11	55.38 ± 1.33	54 96 ± 1.41	53.84 ± 1.60	55.74 ± 2.03	54.12 ± 1.88
PCO_2 (mmHg)	11	36.11 ± 2.09	34.89 ± 1.99	35.05 ± 2.14	35.32 ± 2.42	35.65 ±2.37
pH (units)	11	7.42 ± .01	7.41 ± .01	7.41 ± .01	7.39 ± .01	7.40 ± .01
O_2 content (ml/dl)	11	13.78 ± 0.71	13.16 ± 0.65	12.94 ± 0.68	13.22 ± 0.77	13.45 ± 0.78
V O_2 (ml/min/100g)	11	0.26 ± 0.76	0.28 ± 0.05	0.24 ± 0.04	0.24 ± 0.04	0.26 ± 0.04

a = Saline infusion rate at .382 ml/min

b = Endotoxin infusion rate at .382 mg/min at .382 ml/min

Note: Saline post control was used as control for endotoxin

Figure 4: Metabolic alterations of the gracilis muscle under natural flow conditions during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin.

P_vO_2 = Gracilis venous PO_2 in mmHg

n = Number of animals surviving at the indicated time intervals

*$p < 0.01$

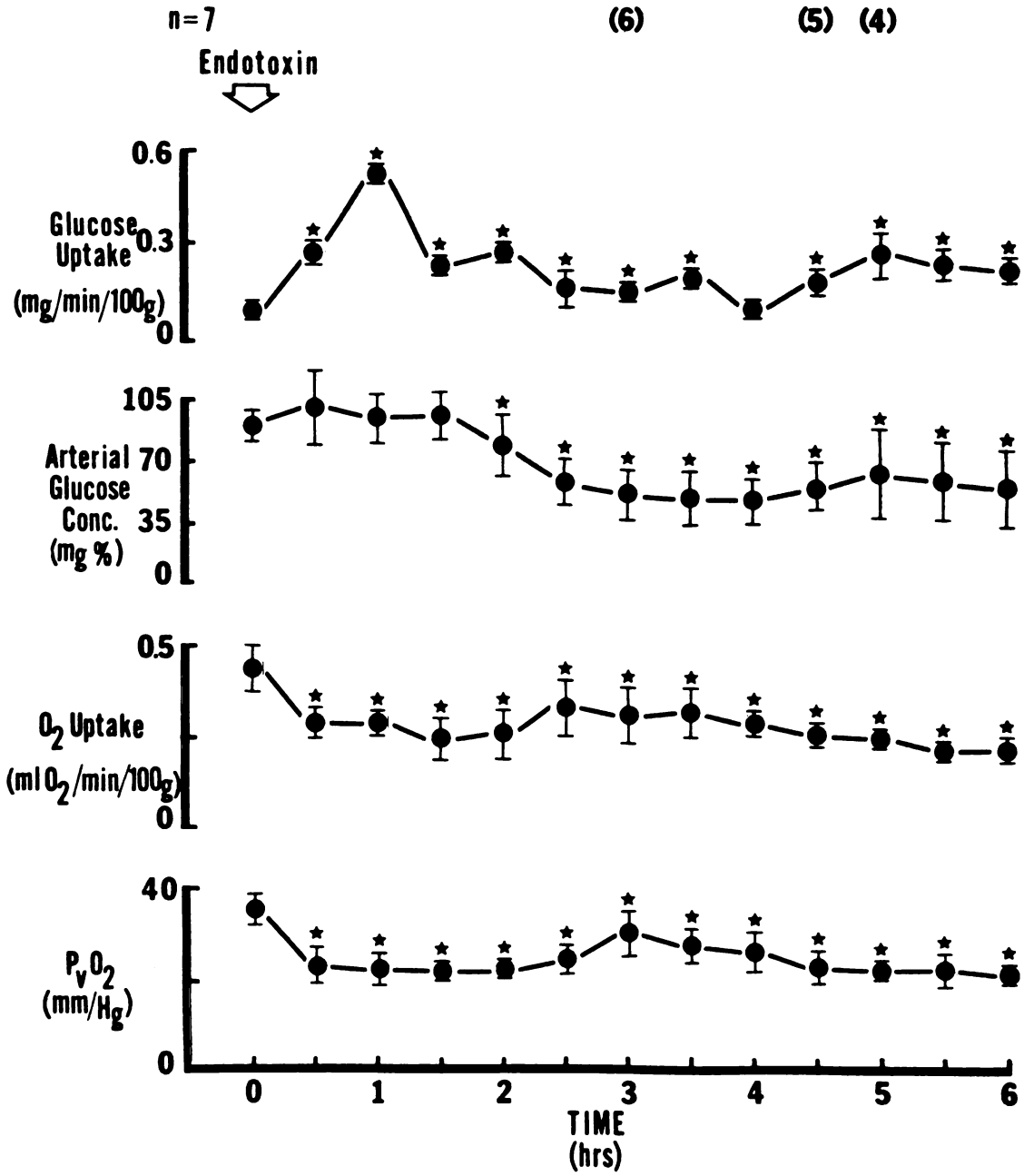
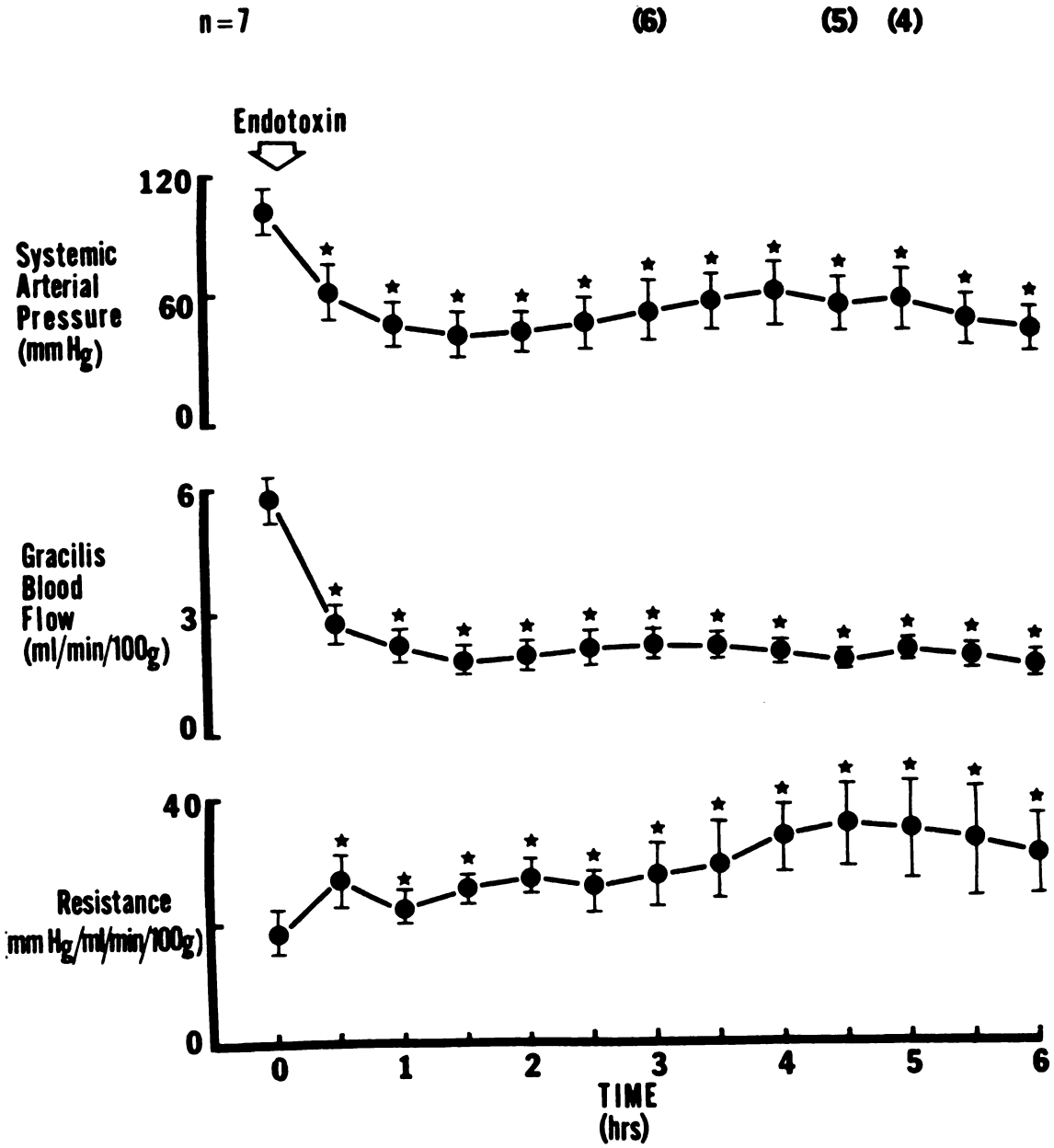

n=7
(6)
(5)
(4)
Endotoxin
0.6
Glucose Uptake
0.3
(mg/min/100g)
0
105
Arterial Glucose Conc.
70
35
(mg %)
0
0.5
O2 Uptake
(ml O2/min/100g)
0
40
PvO2
(mm/Hg)
0
0
1
2
3
4
5
6
TIME
(hrs)

Figure 5: Hemodynamic alteration of the gracilis muscle under natural flow conditions during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin.

n = Number of animals surviving at the indicated time intervals.

*$p < 0.01$


n=7
(6)
(5)
(4)
Endotoxin
120
60
0
Systemic Arterial Pressure (mm Hg)
6
3
0
Gracilis Blood Flow (ml/min/100g)
40
0
Resistance mm Hg/ml/min/100g)
0
1
2
3
4
5
6
TIME (hrs)

decrease in oxygen uptake was noted during the first thirty minute calculation and remained below control levels throughout the experriment ($P < 0.01$). Concommitant with the decrease in muscle oxygen uptake was a decrease in gracilis venous PO_2 ($P < 0.01$) throughout the 6-hour protocol.

Systemic arterial blood pressure and gracilis muscle blood flow were decreased initially thirty minutes following endotoxin administration ($P < 0.01$) and remained at these low levels throughout the 6-hour shock experiment ($P < 0.01$). Gracilis muscle vascular resistance increased during the first thirty minute calculation and remained elevated for the duration of the experiment ($P < 0.01$).

Systemic arterial and gracilis venous blood gases, O_2 content, pH, hematocrit, muscle surface temperature and gracilis venous glucose concentration are summarized in Tables 4 and 5. Systemic arterial oxygen content increased during the sixty minute time period and remained elevated throughout the remainder of the experimetn ($P < 0.01$). Systemic arterial PO_2 increased only during the later portion of the experiment, where it became significant at the 300 minute time interval and remained elevated during the last two time periods (330 and 360 minutes) ($P < 0.01$). Gracilis venous oxygen content decreased initially thirty minutes post-endotoxin infusion and remained depressed throughout the remainder of the experiment ($P < 0.01$). Together with the reduction in gracilis venous oxygen

Table 4. Systemic Arterial and Gracilis Venous Blood Gases, pH and Hematocrit Under Natural Flow Conditions During the I.V. Administration of 2 mg/kg (LD_{100}) E. Coli Endotoxin.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
PO_2 (mmHg)	66	51	63	62	61	68	65	68	63	63	67	74*	79*
	(5)	(6)	(4)	(3)	(7)	(4)	(7)	(7)	(8)	(8)	(13)	(9)	(12)
PCO_2 (mmHg)	43	42	38	36	38	35	38	41	41	31*	29*	37*	31*
	(4)	(6)	(7)	(7)	(8)	(7)	(7)	(11)	(11)	(3)	(4)	(6)	(3)
pH (units)	7.24	7.16*	7.17*	7.17*	7.14*	7.15*	7.14*	7.16*	7.15*	7.17*	7.15*	7.18*	7.13*
	(.05)	(.04)	(.05)	(.05)	(.06)	(.06)	(.07)	(.07)	(.08)	(.1)	(.12)	(.09)	(.08)
Gracilis Venous													
PCO_2 (mmHg)	43	48	52	52	52	51	55	55	56	56	64	61	63
	(4)	(5)	(6)	(7)	(8)	(9)	(14)	(12)	(14)	(13)	(15)	(10)	(12)
pH (units)	7.22	7.1*	7.1*	7.08*	7.05*	7.05*	7.05*	7.03*	7.05*	7.07*	7.03*	6.94*	6.95*
	(.05)	(.05)	(.05)	(.06)	(.06)	(.07)	(.07)	(.07)	(.08)	(.08)	(.09)	(.11)	(.12)
Hct (%)	43	50	51*	52*	53*	54*	55*	55*	53*	54*	55*	54*	54*
	(2)	(3)	(2)	(2)	(2)	(2)	(3)	(3)	(3)	(3)	(4)	(4)	(4)
n =	7						6			5	4		

1 = C equal to control
* = $p < 0.01$
Note: Numbers in parentheses are SEM. Muscle surface temperature held constant at approximately 38° C.
n = number of animals

Table 5. Alterations in Systemic Arterial and Gracilis Venous Oxygen Content and Gracilis Venous Glucose Concentration During the I.V. Administration of 2 mg/kg (LD_{100}) E. Coli Endotoxin.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
O_2 content	18.3	18.5	19.7*	20.4*	19.7*	20.0*	20.2*	20.5*	20.5*	20.0*	19.6*	20.8*	20.2*
(ml/dl)	(.2)	(.4)	(.7)	(.8)	(.6)	(.6)	(.6)	(.6)	(.6)	(.4)	(1.1)	(.8)	(.7)
Gracilis venous													
O_2 content	10.6	6.8*	6.1*	6.0*	5.3*	5.0*	6.2*	5.8*	4.9*	4.9*	5.9*	6.2*	5.8*
(ml/dl)	(.9)	(1.1)	(1.5)	(1.3)	(1.3)	(1.3)	(1.6)	(1.7)	(1.7)	(2.1)	(2.1)	(2.2)	(1.7)
Glucose concentration	88	78*	69*	68*	67*	60*	51*	53*	44*	51*	50*	56*	45*
(mg/dl)	(11)	(13)	(3)	(14)	(18)	(16)	(15)	(18)	(17)	(21)	(21)	(23)	(27)
n =	7						6			5	4		

* = $p < 0.01$
1 = C equal to control

content was an elevated venous PCO_2 ($P < 0.01$). Systemic arterial PCO_2 and pH, together with gracilis venous pH were all significantly decreased following endotoxin administration ($P < 0.01$). Gracilis venous hematocrit was increased throughout the 6-hour experimental period. Muscle surface temperature was maintained approximately at 38° C.

Series III. Hemodynamic and Metabolic Alterations of the Gracilis Muscle Under Constant Flow Conditions During Intravenous Administration of 2 mg/kg (LD_{100}) E. Coli Endotoxin.

The important results concerning changes in the hemodynamics and metabolism of the gracilis muscle, following shock induction, are shown graphically in Firuges 7 and 8. Glucose uptake by the graiclis muscle was not significantly different from control following endotoxin administration ($P > 0.05$). Glucose uptake during control was 0.01 ± 0.03 mg/min/100 grams and at 30 minutes post endotoxin administration was 0.28 ± 0.11 mg/min/100 grams. This represented the largest mean difference in glucose uptake from control. The arterio-venous (A-V) difference in glucose across the gracilis muscle is seen in Figure 9. This figure graphically illustrates the non-significant changes in A-V difference of glucose ($P > 0.05$) across the muscle. Control A-V glucose difference was $1.58 \pm .48$ mg/dl and at 30 minutes post endotoxin was $3.58 \pm .94$ mg/dl. Because muscle blood flow was held constant, any change in

Figure 6: Metabolic changes of the gracilis muscle under constant flow conditions during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endtooxin.

n = Number of animals surviving at the indicated time intervals.

*p).01

P_vO_2 = gracilis venous PO_2 in mmHg

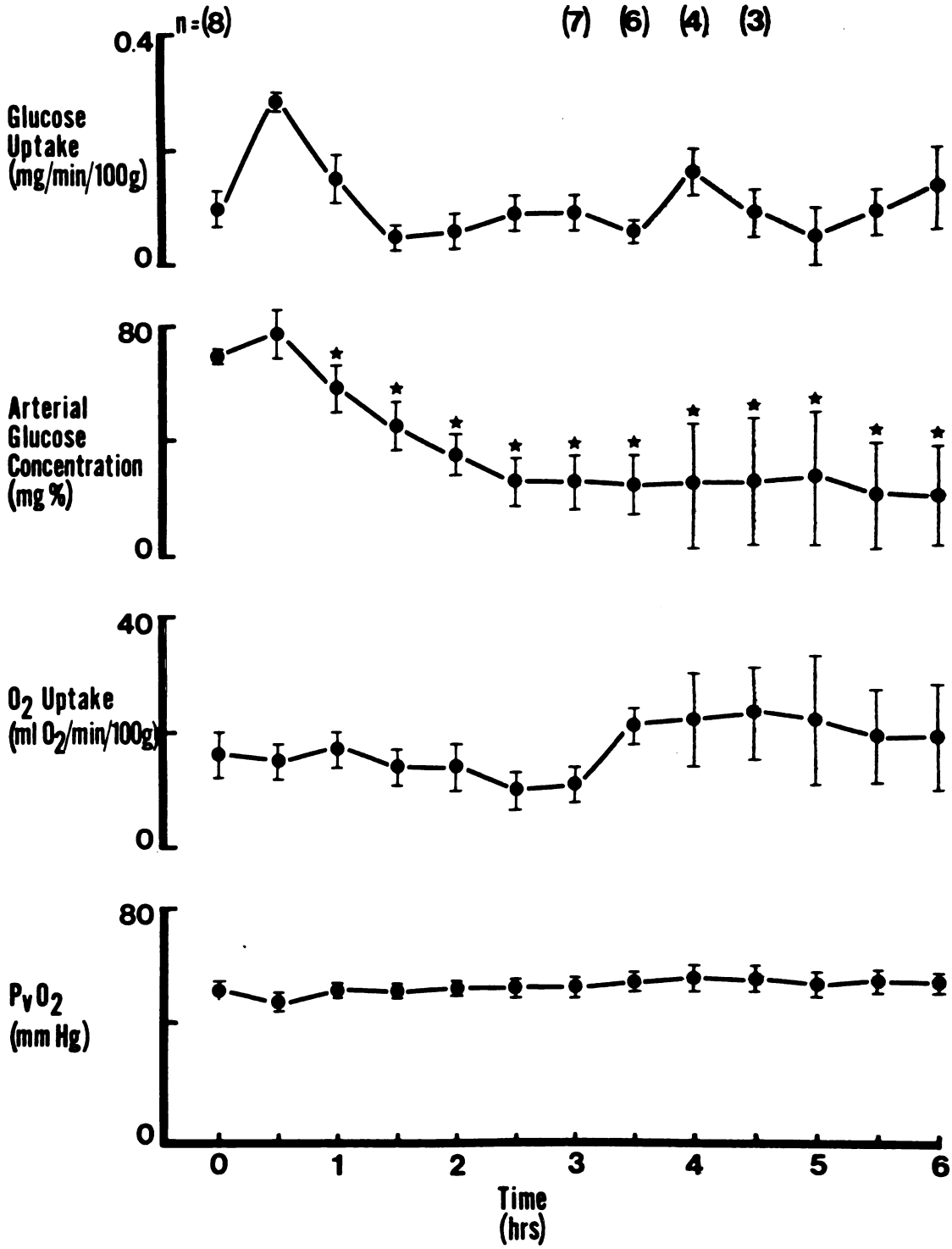
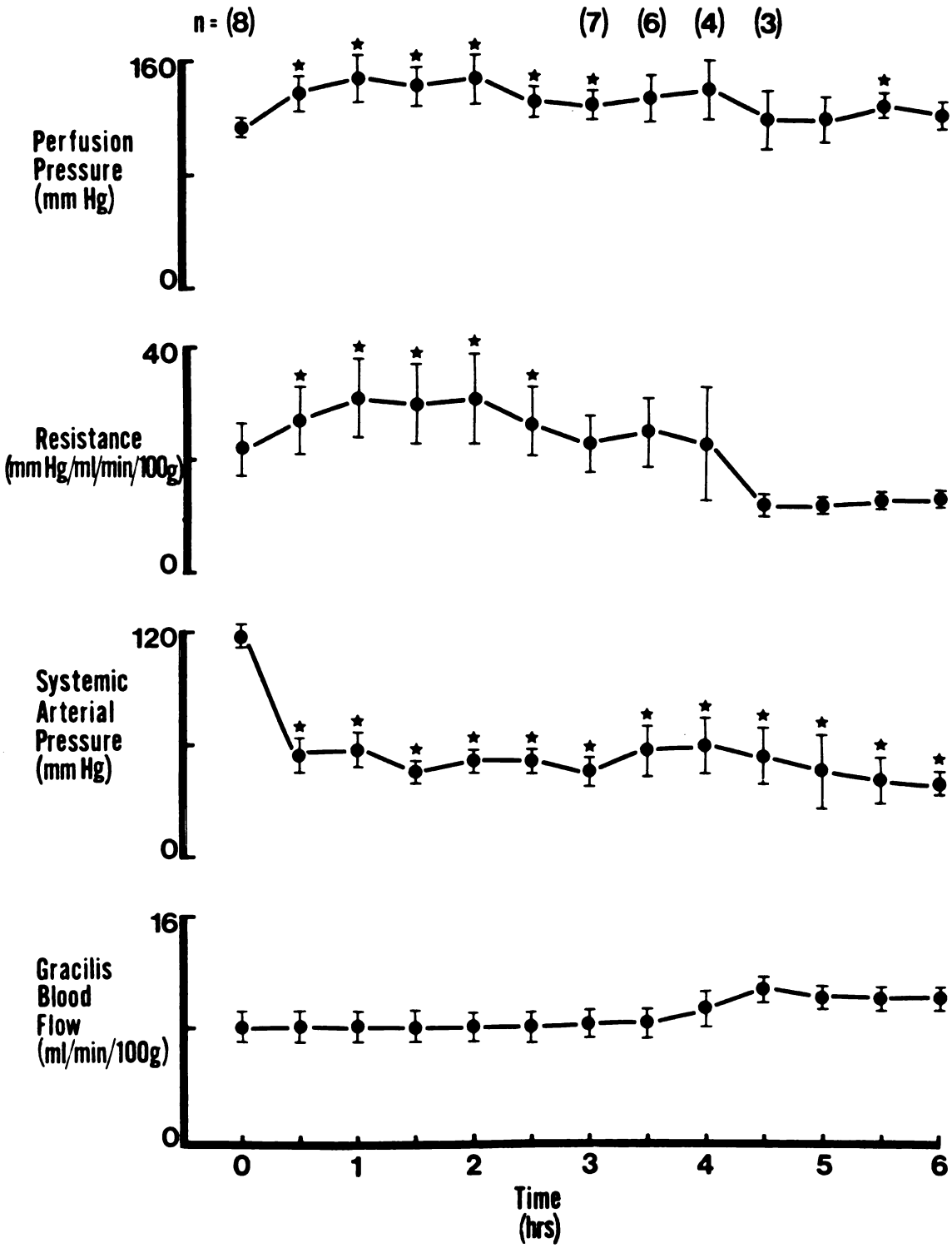

n=(8)
(7)
(6)
(4)
(3)
0.4
Glucose Uptake (mg/min/100g)
0
80
Arterial Glucose Concentration (mg %)
0
40
O_2 Uptake (ml O_2/min/100g)
0
80
P_vO_2 (mm Hg)
0
0
1
2
3
4
5
6
Time (hrs)

Figure 7: Hemodynamic changes of the gracilis muscle under constant flow conditions during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin.

n = Number of animals surviving at the indacted time intervals.

*p 0.01

Note: Numbers in parenthesis represent the number of animals surviving at the given time interval.


n = (8)
(7)
(6)
(4)
(3)
160
Perfusion Pressure (mm Hg)
0
40
Resistance (mm Hg/ml/min/100g)
0
120
Systemic Arterial Pressure (mm Hg)
0
16
Gracilis Blood Flow (ml/min/100g)
0
0
1
2
3
4
5
6
Time (hrs)

A-V difference of glucose across the muscle would reflect changes in glucose uptake. Although this A-V glucose difference was large at 30 minutes, it was not significantly different from control ($P > 0.05$). Arterial glucose concentration decreased and remained below control values throughout the experimental period ($P < 0.01$). Oxygen uptake and gracilis venous PO_2 were not different from control during the entire six hour experiment ($P > 0.05$).

Systemic arterial blood pressure decreased during the first thirty minute measurement ($P < 0.01$) and remained below control during the experiment ($P < 0.01$). Gracilis blood flow, being held constant, was not significantly different from control ($P > 0.05$). Because this experiment caused the dogs to die at different time periods during the 6-hour observation, any mean values that were significantly altered as a consequence of small numbers of experimental animals, due to the lethality of endotoxin shock, were compared back to their original control values using paired statistics. The time periods in question in this study are form 240 minutes through 360 minutes inclusive. Gracilis muscle perfusion pressure increased iitially following endotoxin administration ($P < 0.01$) then returned to a level not different from control at 270 minutes ($P > 0.05$) and remained at control values through the 360 minute protocol ($P > 0.05$). Muscle vascular resistance increased from 30 minutes to 240 minutes (PC 0.01) then decreased at 270 minutes to control levels where it was not significantly

Figure 8: Arterio-venous glucose difference of the gracilis muscle under constant flow conditions during the intravenous administration of 2 mg/kg (LD_{100}) E. coli endotoxin.

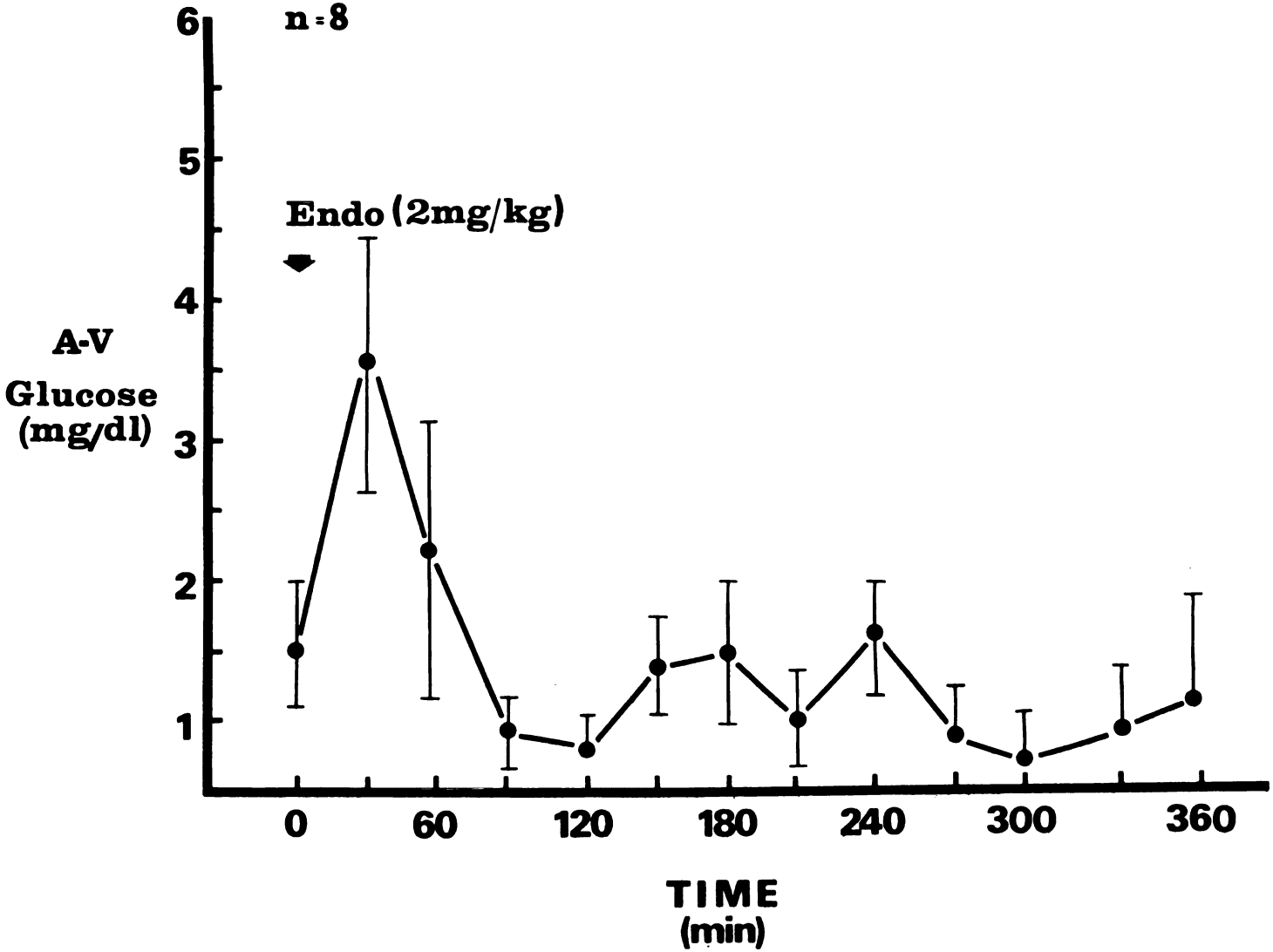

n=8
Endo (2mg/kg)
A-V
Glucose
(mg/dl)
6
5
4
3
2
1
0
60
120
180
240
300
360
TIME
(min)

Table 6. Blood Gases, pH and hematocrit Changes of the Gracilis Muscle Under Constant Flow Conditions During the I.V. Administration of 2 mg/kg (LD_{100}) E. Coli Endotoxin.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
PO_2 (mmHg)	77	69	71	68	65	67	64	79	94*	89*	83*	85*	81*
	(6)	(5)	(5)	(4)	(4)	(4)	(4)	(4)	(10)	(14)	(17)	(16)	(20)
PCO_2 (mmHg)	53	44	42*	44	44	43	43*	47	36*	31*	37*	36*	34*
	(6)	(3)	(4)	(4)	(4)	(4)	(4)	(5)	(7)	(4)	(8)	(8)	(7)
pH (units)	7.33	7.27	7.27	7.23*	7.21*	7.19*	7.17*	7.16*	7.25*	7.24*	7.17*	7.16*	7.17*
	(.04)	(.03)	(.03)	(.03)	(.03)	(.04)	(.05)	(.07)	(.12)	(.12)	(.1)	(.1)	(.09)
Gracilis venous													
PCO_2 (mmHg)	59	50*	46*	42*	40*	40*	46*	41*	35*	32*	34*	34*	36*
	(5)	(3)	(2)	(4)	(3)	(3)	(4)	(7)	(3)	(7)	(6)	(6)	(6)
pH (units)	7.31	7.26	7.23	7.15	7.15	7.14	7.13	7.10	7.21	7.22	7.14	7.14	7.11
	(.02)	(.03)	(.02)	(.04)	(.03)	(.05)	(.07)	(.09)	(.12)	(.12)	(.10)	(.08)	(.08)
Hct (%)	38	46*	50*	51*	52*	53*	54*	54*	54*	54*	53*	52*	52*
	(2)	(2)	(2)	(2)	(2)	(2)	(3)	(3)	(7)	(7)	(6)	(6)	(6)
n =	8							7	6	4	3		

1 = C equal to control
* = $p < 0.01$
Muscle surface temperature held constant at approximately 38° C.
Note: Arterio-venous difference of PCO_2 and pH were not significantly different form control ($p > 0.05$).

Table 7. Oxygen Content and Venous Glucose Measurements of the Gracilis Muscle Under Constant Flow Conditions During I.V> Admoinistration of 2 mg/kg (LD_{100}) E. coli Endotoxin.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial O_2 content (ml/dl)	13 (.9)	13 (1)	14* (.8)	14 (1)	14 (1)	14 (1)	15* (1)	15* (2)	15* (2)	15* (3)	14* (3)	14* (3)	14* (3)
Gracilis venous O_2 content (ml/dl)	10 (1)	11* (1)	11* (1)	12* (2)	12* (1)	12* (1)	13* (1)	13* (2)	13* (2)	13* (2)	13* (2)	13* (2)	13* (2)
Glucose concentration (mg/dl)	67 (2)	74 (9)	56 (8)	44* (8)	34* (7)	25* (7)	24* (9)	24* (11)	24* (20)	25* (22)	26* (23)	18* (18)	17* (17)
n =	8							7	6	4	3		

1 = C equal to control
* = $p < 0.01$
Muscle surface temperature held constant at approximately 38_o C.

different and remained at control values during the remaining experimental period ($P > 0.05$). Arterial and gracilis venous blood gases, oxygen content, pH, hematocrit, venous glucose and muscle surface temperature are listed in Tables 6 and 7. Gracilis venous glucose concentration decreased at ninetly minutes and remained below control values throughout the remainder of the experiment ($P < 0.01$). Systemic arterial and gracilis venous pH and PCO_2 both decreased following the intravenous administration of endotoxin ($P < 0.01$); however, the A-V difference of muscle pH and PCO_2 were not different from control throughout the experiment ($P > 0.05$). Systemic arterial PO_2, oxygen content, gracilis venous oxygen content and hematocrit all increased significantly from control values ($P < 0.01$). Muscle surface temperature was held constant.

Series IV. Hemodynamic and Metabolic Differences of the Gracilis Muscle During Local Muscle Ischemia, Independent of Shock.

Unlike Series II and III, which dealt with systemic shock induction, this series of experiments was undertaken to look at the response of local ischemia on gracilis muscle glucose uptake.

Local ischemia produced alterations in both gfracilis muscle metabolism and hemodynamics. These important changes are shown in Figures 10 and 11. Following the local induction of gracilis muscle ischemia, muscle glucose uptake increased during the sixty minute time period and remained

elevated throughout the six hour observation period ($P < 0.01$). The maximal increase in glucose uptake during local ischemia was seen during the 240 minute time period. Glucose uptake had increased from a control value of 0.095 mg/min/100 grams to 0.504 mg/min/100 grams which represents a 431% increase. Oxygen uptake and gracilis venous PO_2 decreased at the first thirty minute time period ($P < 0.01$) and remained below control throughout the remainder of the experimental protocol ($P < 0.01$). Arterial glucose concentration did not differ from conrol during the six hour observation period ($P > 0.05$).

Systemic arterial blood pressure did not significantly change from control values during the experiment ($P > 0.05$). Gracilis muscle blood flow decreased and remained below control throughout the experimental protocol ($P < 0.01$). Gracilis muscle perfusion pressure decreased initially at the thirty minute time period and remained below control levels throughout the six hour observation period ($P < 0.01$). Muscle vascular resistance, on the other hand, increased at the first thirty minute calculation ($P < 0.01$) and remained elevated throughout the experiment ($P < 0.01$). Remaining systemic arterial and gracilis venous blood gases, pH, oxygen content, hematocrit, gracilis venous glucose concentraiton and muscle surface temperature are summarized in Tables 8 and 9. Systemic arterial PO_2, PCO_2, pH and oxygen content did not significantly differ from control during the six hour

Figure 9: Metabolic alterations of the gracilis muscle during local muscle ischemia.

P_vO_2 = Gracilis venous PO_2 in mmHg

*p 0.01

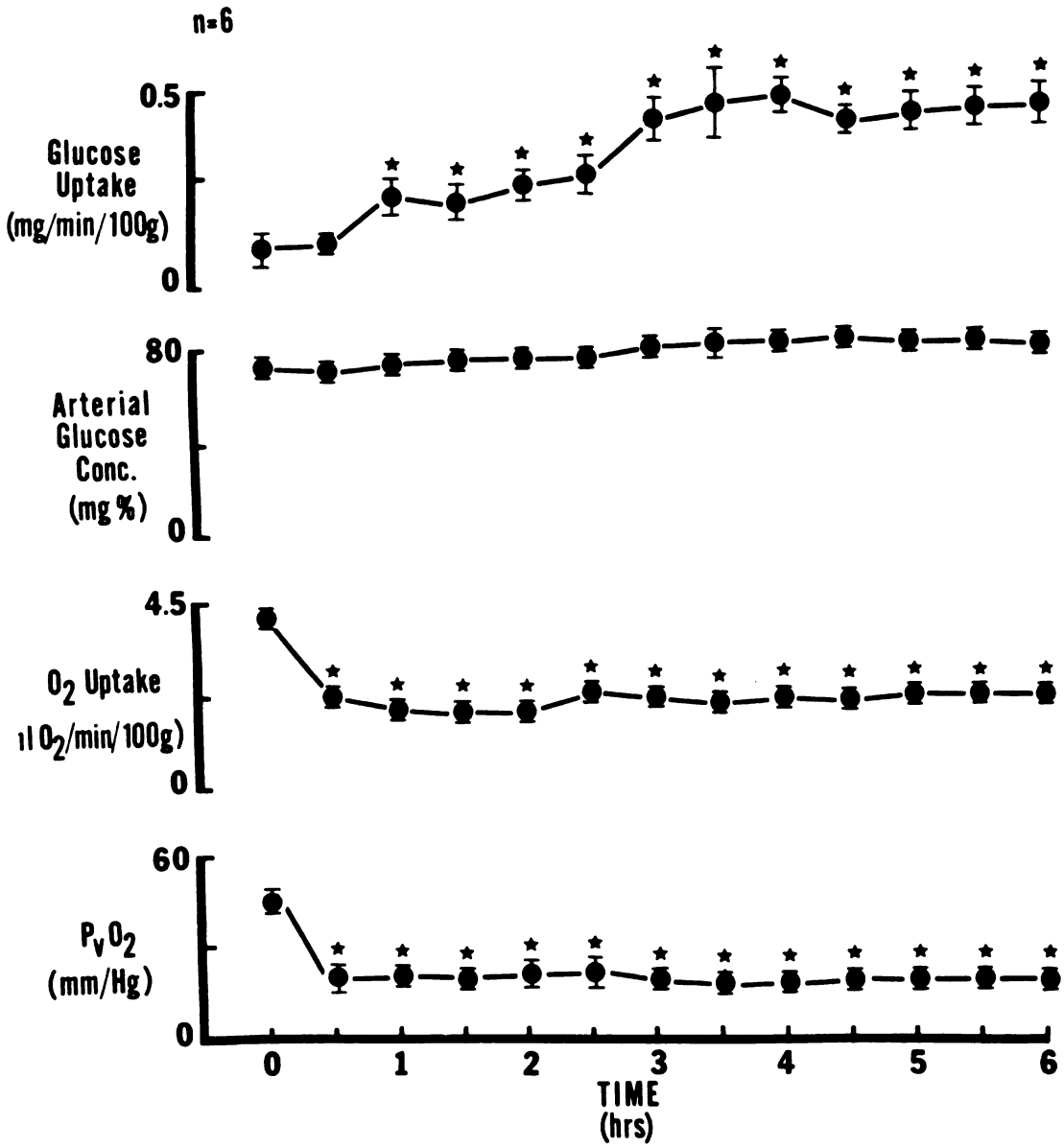

n=6
0.5
Glucose Uptake (mg/min/100g)
0
80
Arterial Glucose Conc. (mg %)
0
4.5
O_2 Uptake
$\mu l\ O_2$/min/100g)
0
60
P_vO_2 (mm/Hg)
0
0
1
2
3
4
5
6
TIME (hrs)

Figure 10: Hemodynamic alterations of the gracilis muscle during local muscle ischemia.

*p 0.01

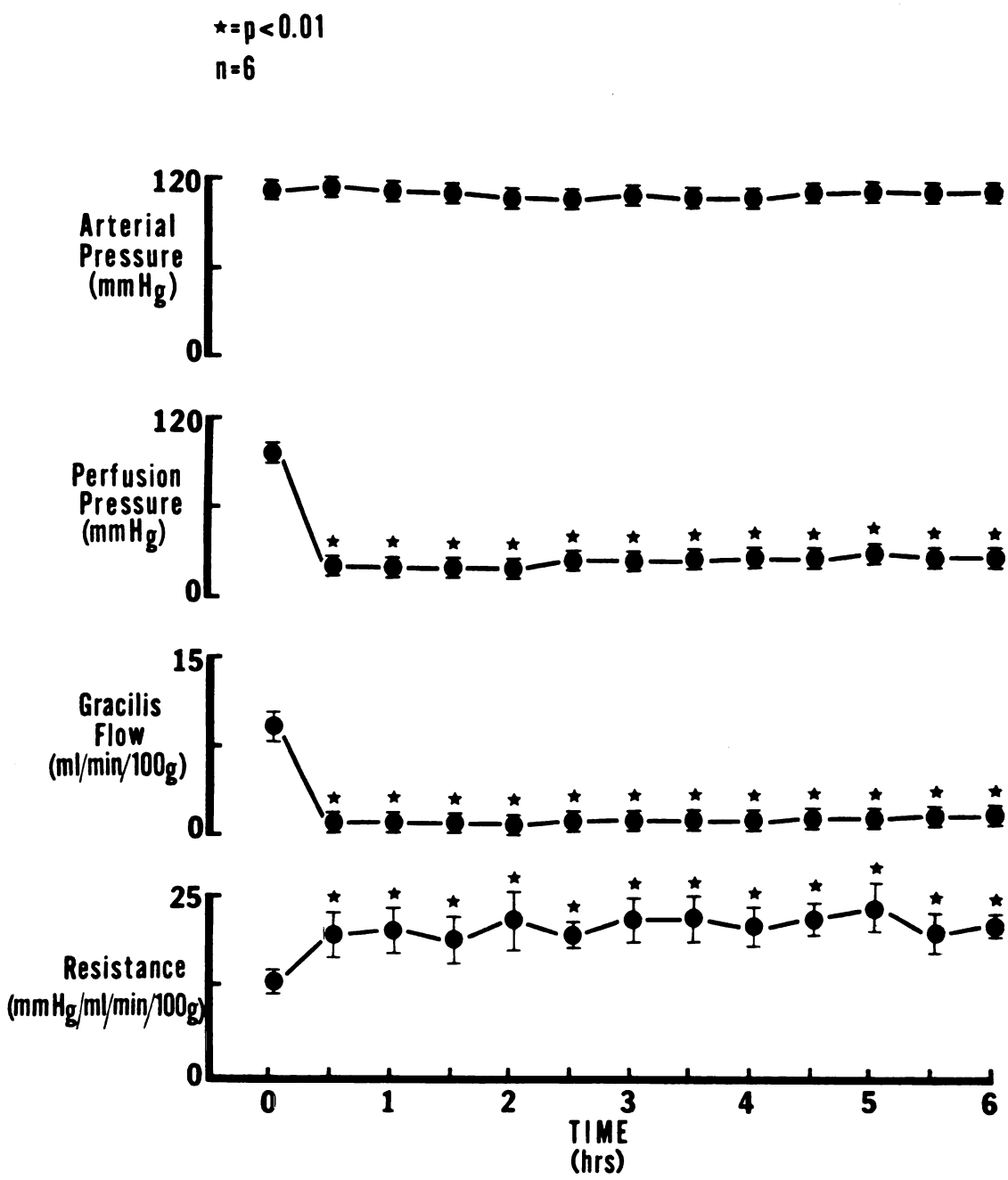

*=p<0.01
n=6
120
Arterial Pressure (mmHg)
0
120
Perfusion Pressure (mmHg)
0
15
Gracilis Flow (ml/min/100g)
0
25
Resistance (mmHg/ml/min/100g)
0
0
1
2
3
4
5
6
TIME (hrs)

Table 8. Systemic Arterial and Gracilis Venous Blood Gas, pH and Hematocrit Changes During Local Gracilis Muscle Ischemia Independent of Shock.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
PO_2 (mmHg)	66	66	70	65	68	72	74	71	69	69	69	71	72
	(6)	(5)	(3)	(5)	(4)	(3)	(3)	(3)	(3)	(3)	(4)	(4)	(4)
PCO_2 (mmHg)	43	44	39	35	37	36	37	37	40	40	39	40	40
	(4)	(3)	(3)	(2)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(4)
pH (units)	7.31	7.34	7.30	7.38	7.37	7.38	7.36	7.36	7.36	7.35	7.35	7.32	7.32
	(.02)	(.03)	(.04)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.03)	(.04)
Gracilis venous													
PCO_2 (mmHg)	47	50	49	49	50	56*	55*	54*	55*	56*	58*	59*	59*
	(3)	(3)	(3)	(2)	(2)	(7)	(6)	(6)	(6)	(6)	(5)	(6)	(6)
pH (units)	7.29	7.29	7.30	7.29	7.26	7.19*	7.16*	7.19*	7.18*	7.18*	7.20*	7.20*	7.17*
	(.01)	(.02)	(.02)	(.03)	(.03)	(.04)	(.04)	(.03)	(.03)	(.04)	(.04)	(.02)	(.05)
Hct (%)	46	45	46	46	45	45	45	46	45	45	44	45	46

1 = C equal to control
* = $p < 0.01$
Number of experimental animals was constant at 6.

Table 9. Systemic Arterial and Gracilis Venous Oxygen and Gracilis Venous Glucose Concentration During Local Gracilis Muscle Ischemia, Independent of Shock.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
O_2 content (ml/dl)	18	18	18	18	19	19	19	18	18	18	18	19	19
	(1)	(1)	(.5)	(1)	(1)	(1)	(1)	(1)	(1)	(.5)	(1)	(1)	(1)
Gracilis venous													
O_2 content (ml/dl)	13	5*	4*	4*	3*	3*	3*	3*	3*	3*	3*	3*	3*
	(1)	(1)	(1)	(1)	(.5)	(1)	(.5)	(1)	(.5)	(1)	(1)	(1)	(1)
Glucose concentration	72	61*	56*	56*	48*	44*	44*	44*	39*	43*	44*	43*	40*
(mg/dl)	(2)	(3)	(4)	(5)	(6)	(4)	(5)	(3)	(4)	(5)	(4)	(4)	(5)

1 = C equal to control
* = $p < 0.01$
Number of experimental animals was constant at 6.

experimental period ($P > 0.05$). Gracilis venous PCO_2 increased during the first thirty minute time period ($P < 0.01$) and remained elevated throughout the six hour experiment ($P < 0.01$). Gracilis venous exygen content, together with pH, were both decreased following induction of local muscle ischemia ($P < 0.01$) and remained below control values during the six hour experimental protocol ($P < 0.01$). Gracilis venous glucose concentration was decreased throughout the six hour experiment ($P < 0.01$), whereas venous hematocrit and muscle surface temperature were not significantly different form control ($P > 0.05$).

Series V. Effects of Local Gracilis Muscle Hypoxia, Independent of Ischemia, on Muscle Metabolism and Hemodynamics.

Metabolic and hemodynamic changes seen during this experimental series is graphically illustrated in Figures 12 and 13. Under constant flow, hypoxic coonditions, gracilis muscle glucose uptake increased at the sixty minute time interval and remained elevated throuthout the experimental protocol ($P < 0.01$). At the termination of the experiment (6 hours) glucose uptake by the gracilis muscle had increased from the control value of 0.14 ± 0.07 mg/min/100 grams to a level of 1.63 ± 0.44 mg/min/100 grams. This represents an increase of 1064% above control. On the contrary, muscle oxygen uptake decreased during the first thirty minute time calculation and stayed below control levels during the remainder of the experiment ($P < 0.01$).

Gracilis arterial and venous PO_2 both decreased significantly throughout the six hour observation period ($P < 0.01$). Gracilis arterial glucose concentration, systemic arterial blood pressure and gracilis muscle blood flow were not significantly different from control ($P > 0.05$). Gracilis muscle perfusion pressure decreased initially at the first thirty minute time period ($P < 0.01$) and remained below control until at the sixth hour, where it increased to a level not significantly different from control ($P > 0.05$). Gracilis muscle vascular resistance initially decreased ($P < 0.01$) until the 210 through the 360 minute calculations where it was not significantly different form control ($P > 0.05$).

In two dogs, acetylcholine was infused locally at a concentraiton of 1 ug/min. During the first hour only acetylcholine was infused and hemodynamics and metabolic (glucose) variables were measured. At the first hour, along with acetylcholine infusion, the gracilis muscle was made hypoxic and monitored for an additional five hours. A typical tracing and results are shown in Figure 14. It can be seen that acetylcholine infusion did not cause an appreciable increase in muscle glucose uptake; however, muscle hpyoxia plus acetylcholine during the first through the sixth hour observation period. caused a marked increase in muscle glucose uptake. It should also be noted that when the muscle was made hypoxic concommitant with acetylcholine infusion, (60 minutes) muscle perfusion pressure decreased

Figure 11: Changes in gracilis muscle glucose uptake, blood gases and oxygen uptake during local muscle hypoxemia under constant flow conditions.

*$p < 0.01$

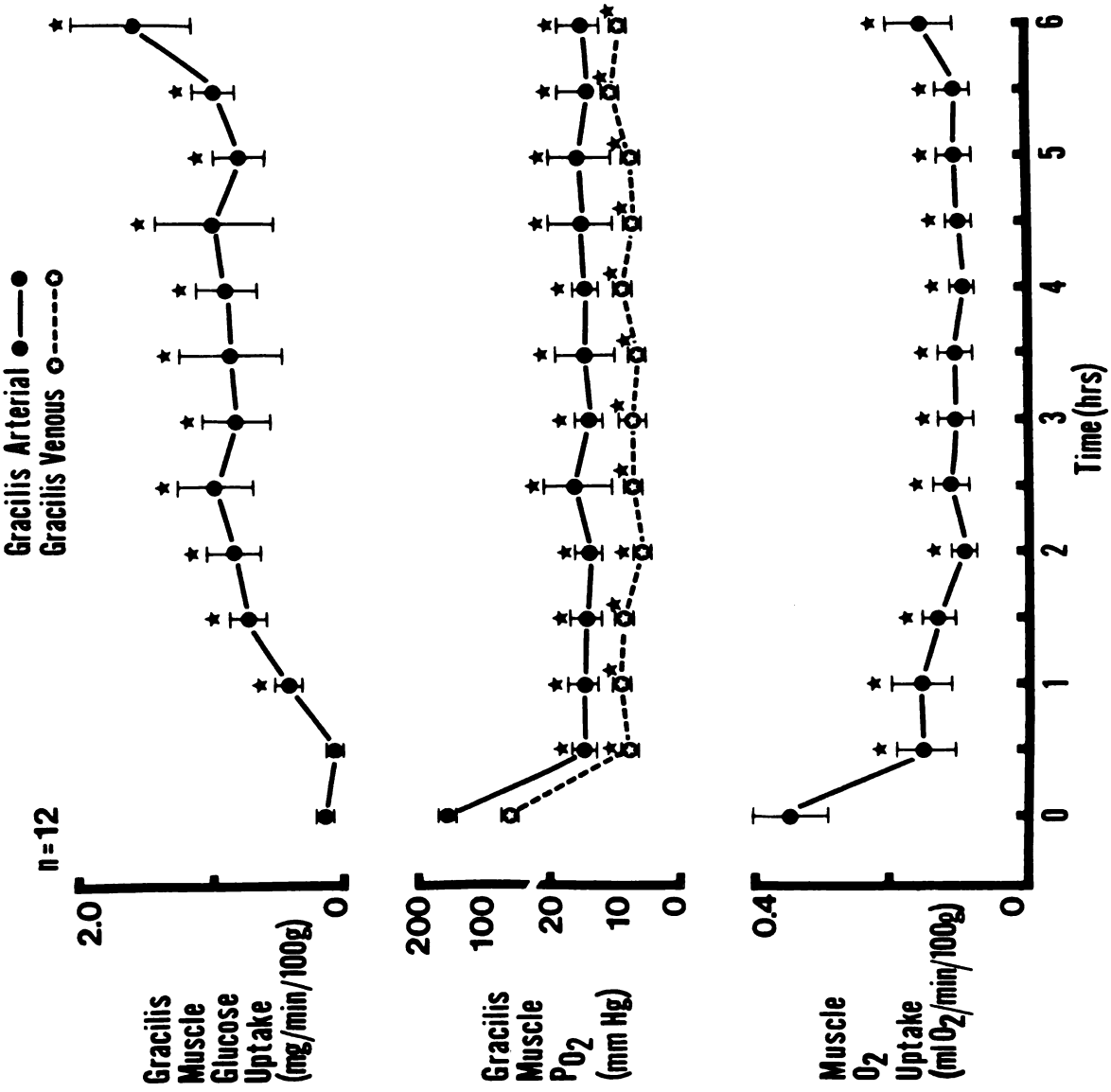

Gracilis Arterial ●——●
Gracilis Venous o- - - -o
n=12
Gracilis Muscle Glucose Uptake (mg/min/100g)
2.0
0
Gracilis Muscle P_{O_2} (mm Hg)
200
100
20
10
0
Muscle O_2 Uptake (ml O_2/min/100g)
0.4
0
0
1
2
3
4
5
6
Time (hrs)

Figure 12: Hemodynamics of the gracilis muscle and systemic arterial blood pressure during local hypoxemia under constant flow conditions.

*$P < 0.01$

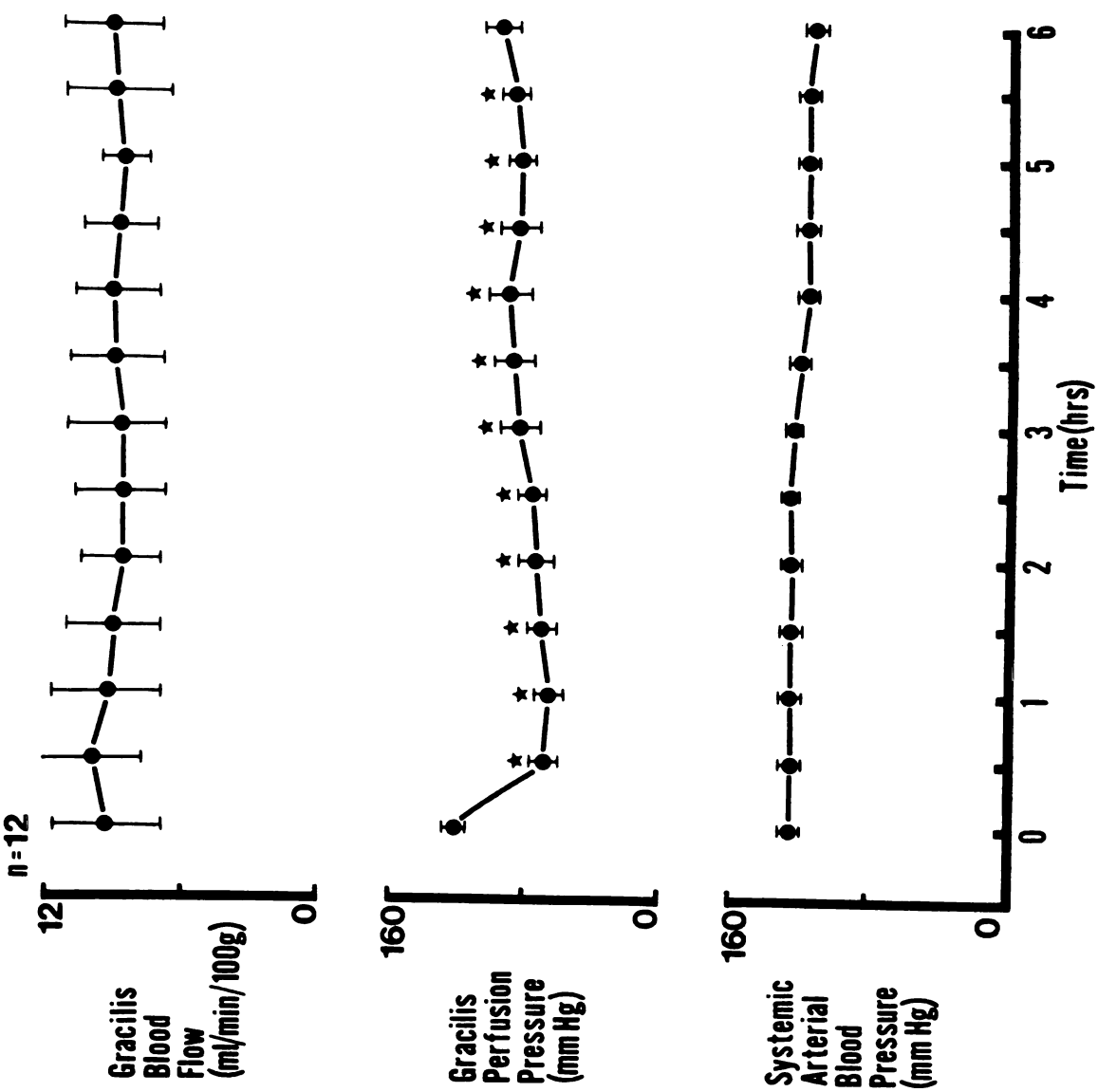

n=12
12
0
Gracilis Blood Flow (ml/min/100g)
160
0
Gracilis Perfusion Pressure (mm Hg)
160
0
Systemic Arterial Blood Pressure (mm Hg)
0
1
2
3
4
5
6
Time(hrs)

Table 10. Gracilis Arterial and Venous Blood Gas, pH and Hematocrit During Local Gracilis Muscle Hypoxia, Independent of Ischemia.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
PCO_2 (mmHg)	47	48	51	51	52	52	52	51	50	51	51	52	52
	(2)	(5)	(2)	(2)	(5)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
pH (units)	7.33	7.34	7.32	7.31	7.31	7.31	7.29	7.29	7.30	7.30	7.29	7.31	7.32
Gracilis venous													
PCO_2 (mmHg)	50	54*	54*	55*	54*	54*	54*	56*	56*	56*	58*	58*	60*
	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
pH (units)	7.30	7.25*	7.19*	7.16*	7.13*	7.13*	7.14*	7.10*	7.12*	7.13*	7.11*	7.09*	7.08*
	(.03)	(.03)	(.03)	(.04)	(.03)	(.03)	(.03)	(.03)	(.03)	(.02)	(.03)	(.04)	(.03)
Hct (%)	41	42	42	42	40	40	40	40	40	41	40	41	41
	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)

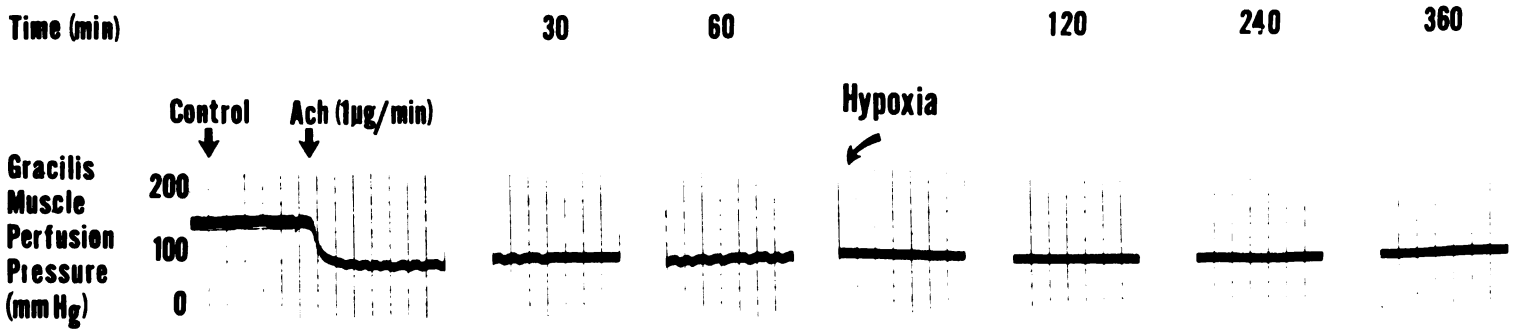
1 = C equal to control
* = $p < 0.01$
Note: Number of experimental animals throughout the the experiment were constant at 12 and muscle surface temperature was maintained at approximately 38° C.

Table 11. Gracilis Arterial and Venous and Gracilis Venous Glucose Concentration During Local Gracilis Muscle Hypoxia, Independent of Ischemia.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial O_2 content (ml/dl)	18 (.5)	1.9* (.2)	1.8* (.3)	1.8* (.2)	1.3* (.1)	1.5* (.2)	1.5* (.2)	1.4* (.2)	1.4* (.1)	1.5* (.2)	1.5* (.2)	1.7* (.2)	1.8* (.2)
Gracilis venous O_2 content (ml/dl)	14 (1)	.49 (.08)	.34* (.06)	.38* (.06)	.55* (.07)	.42* (.04)	.49* (.06)	.35* (.05)	.50* (.07)	.48* (.07)	.39* (.07)	.45* (.06)	.38* (.04)
Glucose concentration (mg/dl)	78 (2)	80 (4)	76 (4)	73 (5)	69* (5)	69* (4)	71 (4)	69 (5)	64 (5)	63 (4)	61 (4)	61 (4)	57 (5)

1 = C equal to control
* = $p < 0.01$
Number of animals held constant at 12 throughout the experiment, and muscle surface maintained at approximately 38° C.

Figure 13: Representative tracing of gracilis muscle hemodynamics and metabolism during the infusion of 1 ug/min acetylcholine and during hyposemia coupled with acetylcholine infusion under constant flow conditions.



Gracilis Muscle Glucose Uptake (mg/min/100g)	.146	.144	.142	.560	.450	.420
Gracilis Venous PO_2 (mm Hg)	50	65	52	11	10	9

Gracilis Blood Flow (ml/min/100g)

8.0

Constant

only slightly (approximately 10 mmHg), indicating that the muscle bed was nearly maximally dilated during acetylcholine infusion.

Remaining gracilis arterial and venous blood gases, oxygen content, pH, hematocrit, gracilis venous glucose concentration and muscle surface temperature are listed in Tables 10 and 11. Gracilis areterial PO_2, pH and oxygen content, together with venous hematocrit, were not different from control ($P > 0.05$). Gracilis venous PCO_2 increased while pH decreased from control ($P < 0.01$). Gracilis venous oxygen content and glucose concentration were decreased and remained depressed throughout the six hour experimental period ($P < 0.01$). Muscle surface temperature and pulmonary venous pressure in the extracorporeal heart-lung preparation were not significantly altered ($P > 0.05$).

Series VI. Effects of Local Gracilis Muscle Ischemia, Independent of Hypoxia, on Muscle Metabolism and Hemodynamics.

Metabolic and hemodynamic changes associated with local gracilis muscel ischemia, independent of hypoxia are graphically presented in Figures 15 and 16. Following local ischemia together with hyperoxia, gracilis muscel glucose uptake decreased ($P < 0.01$) and remained depressed for the duration of the experiment. Gracilis muscle blood flow was decreased by the slowing of the blood infusion pump ($P < 0.01$) and was held at a constant level of approximately 2.5 ml/min/100 grams during the experimental protocol. Muscle

Figure 14: Gracilis muscle glucose uptake, oxygen uptake and muscle arterial and venous PO_2 during local gracilis muscle ischemia, independent of hypoxia.

* = $p < 0.01$

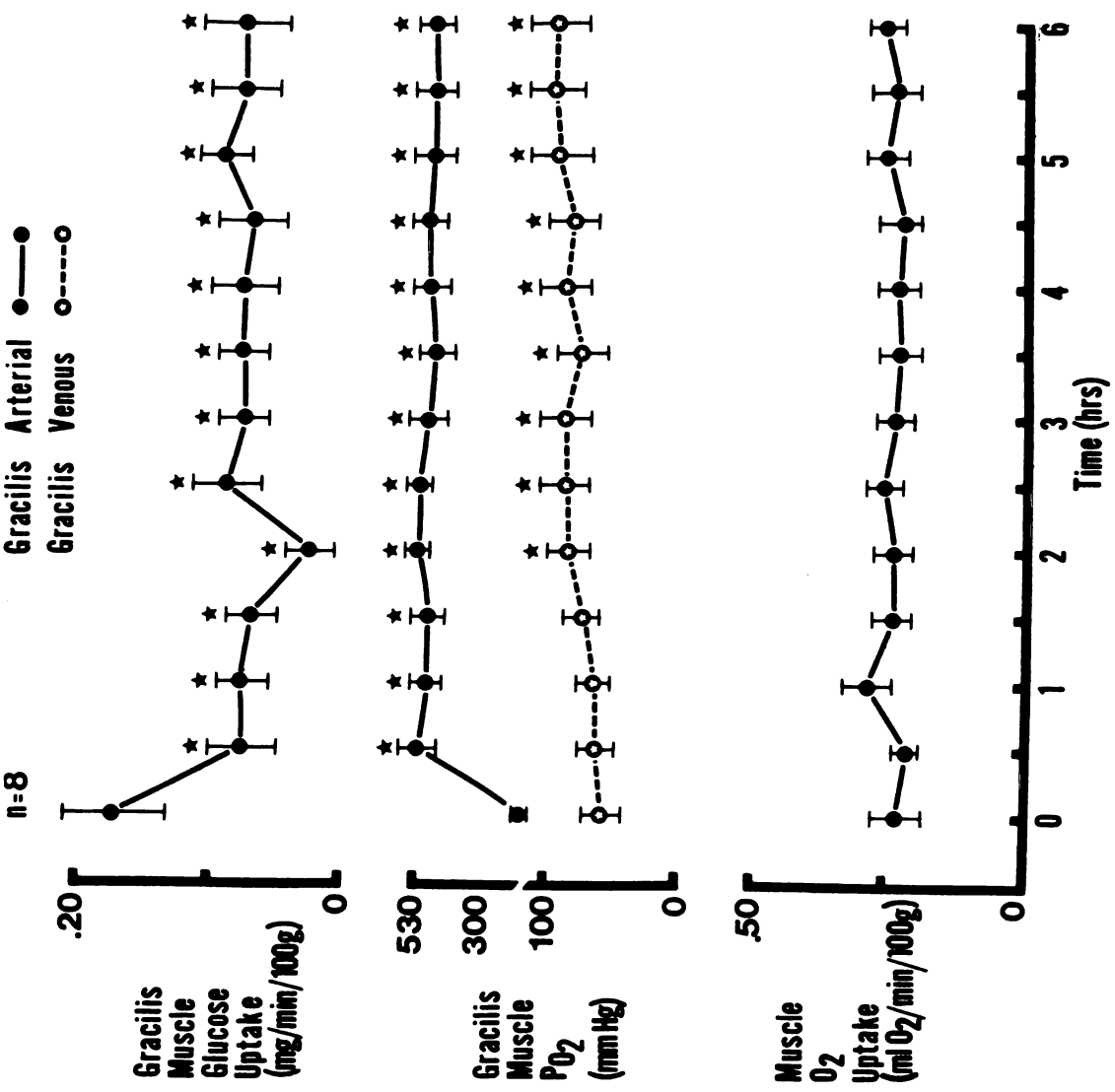

n=8
Gracilis Arterial
Gracilis Venous
Gracilis Muscle Glucose Uptake (mg/min/100g)
.20
0
Gracilis Muscle P_{O_2} (mm Hg)
530
300
100
0
Muscle O_2 Uptake (ml O_2/min/100g)
.50
0
0
1
2
3
4
5
6
Time (hrs)

Figure 15: Systemic arterial blood pressure and gracilis muscle hemodynamics during local gracilis muscle ischemia, independent of hypoxia.

* = $p < 0.01$

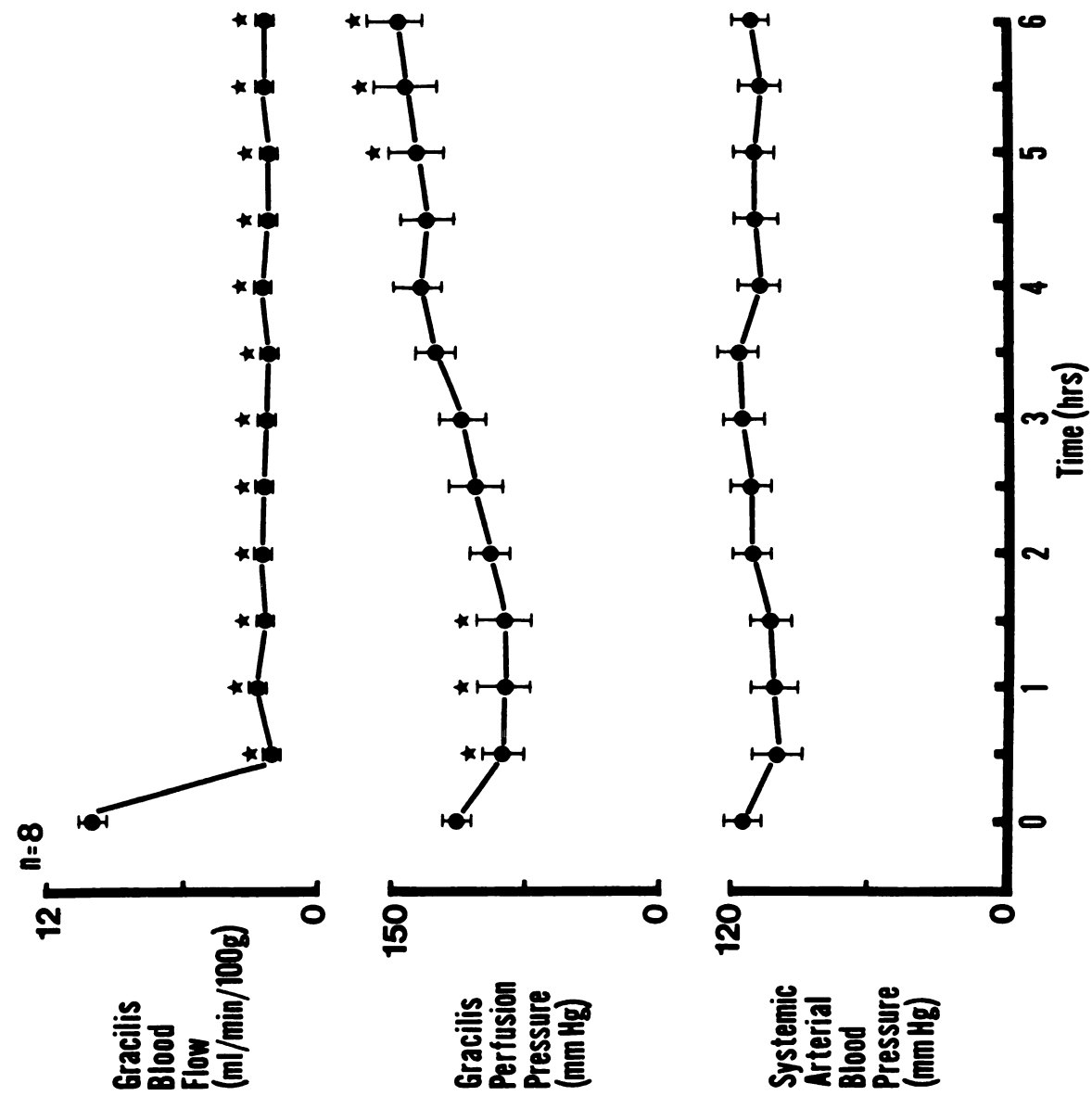

n=8
12
0
Gracilis Blood Flow (ml/min/100g)
150
0
Gracilis Perfusion Pressure (mm Hg)
120
0
Systemic Arterial Blood Pressure (mm Hg)
0
1
2
3
4
5
6
Time (hrs)

Table 12. Gracilis Arterial and Venous Blood GAses, pH and Hematocrit During Local Gracilis Muscle Ischemia Independent of Hypoxia.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial													
PCO_2 (mmHg)	48	48	50	51	50	52	50	52	51	50	50	49	50
	(2)	(2)	(3)	(3)	(3)	(3)	(2)	(3)	(3)	(3)	(3)	(2)	(3)
pH (units)	7.32	7.31	7.31	7.31	7.32	7.32	7.32	7.32	7.32	7.31	7.32	7.32	7.32
Gracilis Venous													
PCO_2 (mmHg)	49	53*	54*	55*	54*	55*	54*	55*	54*	54*	54*	55*	56*
	(1)	(2)	(2)	(1)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
pH (units)	7.29	7.20*	7.17*	7.17*	7.16*	7.17*	7.17*	7.18*	7.17*	7.17*	7.17*	7.16*	7.17*
	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)
Hct (%)	44	42	41	43	42	44	44	42	40	40	41	39	39
	(4)	(2)	(2)	(3)	(2)	(2)	(3)	(1)	(1)	(1)	(2)	(3)	(6)

1 = C equal to control
* = $p < 0.01$
Number of animals was constant at 8, throughout the 360 minute experimental protocol. Muscle surface temperature was held constant at approximately 38° C. Pulmonary venous pressurewas maintained between 0 and 5 mmHg.

Table 13. Gracilis Arterial and Venous Oxygen Content, Gracilis Venous Glucose Concentration and Muscle Vascular Resistance During Local Gracilis Muscle Ischemia, Independent of Hypoxia.

Time (min.)	C[1]	30	60	90	120	150	180	210	240	270	300	330	360
Systemic arterial O_2 content (ml/dl)	17.4 (.4)	19.5* (.5)	20.6* (.6)	20.1* (.4)	19.6* (.2)	20.8* (.5)	20.9* (.6)	20.2* (.2)	20.1* (.3)	19.8* (.2)	21.0* (.3)	20.1* (.3)	20.8* (.4)
Gracilis venous O_2 content (ml/dl)	15.1 (.3)	10.9* (.7)	10.4* (.8)	10.5* (.8)	10.1* (.8)	10.6* (.7)	10.8* (.8)	10.5* (.8)	10.6* (.8)	10.4* (.7)	10.6* (.7)	10.4* (.7)	10.6* (.6)
Glucose concentraion (mg/dl)	76 (5)	76 (5)	74 (5)	73 (5)	73 (6)	72 (6)	73 (7)	75 (6)	75 (6)	73 (5)	76 (3)	73 (5)	72 (6)
Muscle vascular Resistance (mmHg/ml/min/100g)													

1 = C equal to control
* = $p < 0.01$
Number of animals were constant at 8. Muscle surface temperature was held constant at approximately 38° C. Pulmonary venous pressure was maintained between 0 and 5 mmmHg.

perfusion pressure initially decreased during the first 90 minutes post ischemia ($P > 0.01$) then increased back to control levels ($P > 0.05$) until 300 minutes when it became significantly elevated above control values ($P < 0.01$) and remained high for the duration of the six hour experimet. Systemic arterial blood pressure, muscle oxygen uptake and arterial glucose concentraiton were not different from control ($P > 0.05$). Gracilis arterial and venous PO_2 both increased following ischemia plus hyperoxia ($P < 0.01$). Remaining gracilis arterial and venous blood gases, oxygen content, pH, venous glucose concentration, hematocrit, muscle surface temperature and pulmonary venous pressure are summarized in Tables 12 and 13. Gracilis arterial PCO_2 and pH were not significantly changed during the six hour experiment ($P > 0.05$). Gracilis arterial oxygen content was increased ($P < 0.01$) wheareas gracilis venous oxygen content and pH were decreased throughout the entire experiment ($P < 0.01$). Gracilis venous PCO_2 increased initially and remained elevated throughout the experimental protocol ($P < 0.01$). Gracilis venous glucose concentration, muscle surface temperature and pulmonary venous pressure in the extracorporeal heart-lung preparation were not statistically different from control ($P > 0.05$).

DISCUSSION

Four important findings resulted from this study: 1) E. coli endotoxin infused locally into the constant flow perfused gracilis muscle promoted glucose uptake by the muscle; this was not associated with alterations in muscle metabolism or insulin concentration across the muscle; 2) when 2 mg/kg (LD_{100}) E. coli endotoxin was infused itravenously to induce shock, gracilis muscle glucose uptake was not significantly different from control provided the gracilis muscle blood flow was held constant; 3) under natural flow conditions to the gracilis muscle, glucose uptake increased markedly during endotoxin shock; 4) the mechanism by which glucose uptake is increased under natural flow conditions during endotoxin shock was shown to be related to local tissue hypoxia and not to any metabotite that might be released during shock.

Hypoglycemia is a characteristic feature of gram-negative endotoxin or septic shock in a number of animal species (Hinshaw, 1976). While it is well known that glucose production by the liver is depressed during endotoxin or septic shock, the study of Peyton, et al., 1974, suggested that this alone cannot account for the severity of hypoglycemia. Therefore, and increased uptake of glucose by peripheral organ system or systems is likely involved. Hinshaw and colleagues, in 1975, showed that the

heart-lung system is not involved in this response. Raymond and Emerson, in 1978, demonstrated that glucose uptake by the centrol nervous system decreased during endotoxin shock in the dog. On the other hand, Hinshaw, et al., in 1977, observed an increase in glucose uptake by white blood cells during E. coli endotoxin shock, which may account for part of the hypoglycemia. More recently, spitzer, et al., in 1978, demonstrated that enodtoxin directly stimulated glucose uptake in isolated epididymal fat pad cells cultered in vitro. Although it has been suggested that endotoxin may exert an insulinomimetic acion which could increase skeletal muscle glucose uptake, this possibility has been relatively unexplored to date. Furr, et al., in 1977, recently reported that glucose uptake in the dog forelimb does not increase during endotoxin shock, which was interpreted to mean no increased glucose uptake by skeletal muscle. However, the degree of shock produced in their study was moderate and many of the hemodynamic and metabolic alterations normally associated with endotoxin shock were very mild. Also, results obtained from the perfused forelimb preparation are not necessarily representative of pure skeletal muscle since contamination of muscle flow by skin and bone cannot be excluded.

Results from Series I demonstrated that E. coli endotoxin, given locally to the gracilis muscle under constant flow conditions, increased glucose uptake by the muscle independent of muscle metabolism. This study,

therefore, supports the hypothesis that endotoxin has insulinomimetic properties, as speculated by other investigators (Wolfe, et al., 1977; Hinshae, 1976). Endotoxin infusion caused an icrease in gracilis muscle glucose uptake of 173% on the average and occurred with no change in muscle arterio-venous difference of oxygen, carbon dioxide, insulin, pH, oxygen uptake, vneous hematocrit or muscle surface temperature. Hence, the observed phenomenon does not appear to be dependent on insulin or alterations in gracilis muscle metabolism. These data, therefore, support the hypothesis that endotoxin acts like insulin to directly increase uptake by skeletal muscle; however, in vitro experiments by Filkin, et al., in 1977, using hemidiaphragms of rats do not support a direct insulin-like action. No explanation for this discrepancy is apparent, unless differences in methodology between this present in vivo study and their in vitro preparation is involved.

While these data clearly demonstrate that endotoxin directly increased glucose uptake by skeletal muscle, the involvement of this mechanism in the progressive, long-term hypoglycemia associated with endotoxin shock in the dog is uncertain. Studies by Zlydaszyk and Moon (1976), Buchanan and Filkins (1976), and Starzecki, et al., (1967) have shown that endotoxin is taken up rapidly by the reticuloendothelial system (RES) -- the plasma half-life averaging between 8 to 60 minutes. It has also been reported by these investigators that approximately 80% of

the injected endotoxin is removedwithin the first 45 minutes.

In the typical experimental model in which endotoxin is injected as a bolus, endotoxin whold likely be taken up rapidly by the RES and hence the plasma concentration of endotoxin would progressively decrease, thereby contributing less to the direct action of endotoxin on skeletal muscle glucose uptake causing a dminished direct effect in the production of hypoglycemia of endotoxin shock. On the other hand, during gram-negative septic shock in the experimental animal or man, the bacteria are being continually destroyed and thereby are continually releasing endotoxin into the blood; the plasma concentration of endotoxin would be continually increasing and might contribute significantly to the progression of hypoglycemia. This would become particularly significant as the RES is overwhelmed and becomes less effective as septic shock progresses (Berry, 1972).

Experiments in which gracilis muscle blood flow was held constant while the animals were in endotoxin shock (2mg/kg, Series III) showed that muscle glucose uptake was maintained at control levels during 6-hour protocols. Although muscle glucose uptake was not significantly altered during endotoxin shock, a tendency for glucose uptake to increase was noticed at the first thirty minute time interval post-endotoxin administration. These data supply enidence that, although endotoxin exerted direct

"insulinomimetic" effect in causing muscle glucose uptake when given locally, its insulin-like effect when given intravenously was without effect. It should be mentioned that the concentration of endotoxin given intravenously (2mg/kg) was equal to approximately 0.38 mg/min/ 100 grams reaching the gracilis muscle. This is based on the assumption that all the endotoxin given itravenously would remain in the plasma; however, as stated earlier, most of the endotoxin given intravenously is removed early via the RES, thereby decreasing the plasma levels of endotoxin. Since the first determination of glucose uptake was made 30 minutes after endotoxin was given intravenously, it is likely that any direct effect of endotoxin on glucose uptake would be missed. It is conceivable that early after endotoxin administration (approximately 1-5 minutes), the concentration of endotoxin reaching the gracilis muscle would be sufficient to increase gracilis muscle glucose uptake. During constant flow conditions, endotoxin plays a very minor role (< 30 minutes) in contributing to the hypoglycemia of shock when given intravenously. Also, prior to 30 minutes, endotoxin administration usually produces an increase or no change in plasma glucose concentration.

Glucose uptake by the gracilis muscle increased during endotoxin shock under natural flow conditions (Figure 5) and occurred in the presence of a decreased arterial plasma glucose concentraiton. Many reports have cited evidence that, during endotoxin shock in a variety of animal

species, plasma insulin concentration is depressed (via decreased arterial glucose concenteration and increased catecholamine levels). Based on previous data from the literature concerning the levels of plasma insulin during shock, it does not seem likely that enhanced skeletal muscle glucose uptake is mediated through an increased plasma insulin secretion. Direct effects of endotoxin, as shown in Series I, could produce the increased glucose uptake seen in Series II.

To test the hypothesis that endotoxin directly stimulated glucose uptake of skeletal muscle in shock, a third series of experiments was conducted (Series III). These experiments utilized the gracilis muscle preparation under constant flow conditions and endotoxin was given I.V. (2 mg/kg/(LD_{100}) to induce shock. Metabolic alterations of this series are summarized graphically in Figures 7 and 9. These results provided evidence that, although endotoxin shock stimulated glucose uptake by the gracilis muscle during natural flow conditions, it had no effect during constant flow. These data show that endotoxin does not exert an insulin like effect during endotoxin shock, and that the numerous metabolites reported to be released during endotoxin shock (i.e., histamine and bradykinin) also do not stimulate glucose uptake during this condition when muscle blood flow is held constant. This point is interesting because the study by Nies, et al., (1968) demonstrated the release of kinins during endotoxin shock. Haberland (1972)

demonstrated insulin-like acitons of kinins in increasing skeletal muscle glucose uptake. The inference from these two reports was that kinins, released during endotoxin shock, caused increased skeletal muscle glucose uptake. This speculation, along with the results of Haberland, has been the subject of much debate over the years. As previously mentioned, endotoxin removal by the RES -- decreasing the concentration of circulating plasma endotoxoin to such a low level that it may not have any insulin-like actions to increase skeletal muscle glucose uptake. The results from this present study, however, do not support the concept that any metabolite released during shock has a direct effect in stimulating glucose uptake by skeletal muscle.

Differences in glucose uptake by the gracilis muscle during constant and natural flow conditions seem to imply local muscle metabolism. Obvious local differences include decreased oxygen uptake and skeletal muscle hypoxia in the natural flow series, whereas these changes were prevented by maintaining constant flow. Morgan, et al. (1959) and Rendle and Smith (1958) provided evidence that skeletal muscle glucose uptake increases substantially during hypoxia and that this increase is independent of insulin. Since the gracilis muscle was hypoxic during natural flow shock experiments, a possible mechanism for increased muscle glucose uptake during shock is muscle hypoxia.

Changes in systemic arterial blood gases and pH

include an elevated PO_2 and decreased PCO_2 and pH. The increases in arterial PO_2 and decreased in PCO_2 can be attributed to an increased respiratory rate, as was reported by Raymond and Emerson (1978), Parker and Emerson (1977) and others (Gilbert, 1960). Although respiratory rate was not recorded in this study, many of the animals, if not all, showed early and maintained increased respiratory rates following the intravenous infusion of endotoxin. Decreased systemic arterial pH has been reported by Emerson and Gill in 1968 and Emerson and Kelly in the same year.

The decrease in gracilis venous oxygen content and increase in PCO_2 most probably is ascribed to the low muscle flow which would cause an elevated "lag-time" for oxygen extraction and CO_2 production. Similar reasoning could also account for a decreased gracilis venous pH but this may also be produced by an increased anaerobic metabolism by the muscle during reduced oxygen uptake. During endotoxin shock, many reports have indicated an increase in venous lactate concentration and concommitant increase in the lactate/pyruvate ratio. These findings were interpreted as reflecting a generalized increase in anaerobic metabolism. Therefore, the decrease in gracilis venous pH probably resulted both from an increase in PCO_2 and an accumulation of lactic acid via anaerobic glycolysis. Substantiation of anaerobic glycolysis during natural flow whock experiments (Series II) is seen by an increase in muscle glucose uptake.

To test the hypothesis that local muscle hypoxia

and/or ischemia may be responsible for the increased muscle glucose uptake, gracilis muscle blood flow was reduced by decreasing the rate of the perfusion pump. This muscle ischemia (Series IV) is independent of metabolic and hemodynamic changes normally associated with endotoxin shock and gives a beter understanding of local control mechanisms regulating skeletal muscle metabolic changes associated with low flow states. Glucose uptake and gracilis venous PO_2 were similar to that produced during shock induction in the naturally perfused gracilis muscle (Figures 5 and 10). These data confirm the hypothesis that local muscle hypoxia and/or ischemia cause increases in glucose uptake. To differentiate between gracilis muscle ischemia or hypoxia in causing the increased muscle glucose uptake, two series of experiments were conducted (Series V and VI). During constant flow gracilis muscle perfusion, local hypoxemia in the inflowing blood stimulated the muscle to increase glucose uptake. Increased glucose uptake was maintained throughout the 6-hour hypoxemic, constant flow experiments. Also, muscle vascular resistance decreased during the period of local muscle hypoxia. Therefore, the increased glucose uptake by the gracilis muscle could have resultaed simply from an increased muscle surface area. To answer this question, acetylcholine was infused (lug/min) behind the perfusion pump during constant flow conditions. Initially, acetylcholine was infused for one hour and glucose uptake was determined (Figure 14). During this period, neither

glucose uptake nor gracilis venous PO_2 varied from control. During hypoxemic conditions along with acetylcholine infusion and under constant flow, gracilis muscle glucose uptake increased substantially and remained elevated. When blood perfusing the gracilis muscle was made hypoxemic, gracilis vascular perfusion pressure did not decrease substantially (1Ø mmHg), indicating that acetylcholine infusion nearly maximally dilated the gracilis muscle vasculature. These results have shown that vasodilation per se has no effect in increasing muscle glucose uptake above control levels, but hypoxia, together with maxial vasodilation, greatly increased glucose uptake of skeletal muscle. An explanation for these data is that under normal aerobic conditions, skeletal muscle, like cardiac muscle, utilized primarily fatty acids as an energy source and use only trivial amounts of glucose. Under hypoxic conditions, however, glucose transport and uptake by skeletal muscle increases. Increased glycolysis in the absence of oxygen is termed the Pasteru effect, and explains the increase in glucose uptake by the gracilis muscle during natural flow shock, local ischemia, and in Series V, where flow was held constant but arterial PO_2 was decreased. Since it is well known that tissue hypoxia stimulated glycolysis (Randle and Smith, 1958), the effect of ischemia, independent of hypoxia in regulating glucose uptake is vague. Because endotoxin shock causes muscle ischemia together with hypoxia, experiments were completed to determine what role a

decreased substrate delivery via ischemia would have on glucose metabolism under normoxic and hyperoxic conditions (Series VI). Induction of severe ischemia of the gracilis muscle, together with local hyperoxia, did not result in increased uptake of glucose by the gracilis muscle -- in fact, glucose uptake decreased during the hyperoxic state (increased gracilis venous PO_2). As mentioned previously, skeletal muscle metabolism is similar to cardiac metabolism.

Under aerobic conditions, skeletal and cardiac muscle preferentially utilize fattly for energy production. While skeletal muscle utilized only small amounts of glucose during control situations, increases in oxygen delivery or increases in tissue PO_2 would result in a greater efficient utilization of fatty acids rather than glucose (White, et al., 1973). The arterio-venous difference of glucose across the gracilis muscle remained unchanged during ischemic-hyperoxia which resulted in a calculated decreased in glucose uptake (Pasteur effect).

SUMMARY AND CONCLUSIONS

The purpose of this study was to define the role played by skeletal muscle in regulating glucose metabolism during endotoxin shock. It was found that E. coli endotoxin had insulinomimetic activity by increasing glucose uptake of the gracilis muscle when infused locally. Direct insulin-like actions of endotoxin were not noted when endotoxin was given intravenously to induce shock. Intravenously administered lethal doses of E. coli endotoxin did cause a sharp fall in gracilis muscle blood flow resulting in tissue hypoxia. During constant flow conditions, no oxygen differences were seen. In experiments where blood flow was held constant, endotoxin shock did not alter glucose metabloism of the gracilis muscle, which provided evidence that metabolites, released as a consequence of shock induction also failed to alter glucose metabolism in the gracilis muscle, which provided evidence that metabolites, released as a consequence of shock induction also failed to alter glucose metabolism in the gracilis muscle. A possible explanation for endotoxin not

causing an increase in muscle glucose uptake during constant flow is related to the fact that the half-life of endotoxin in plasma is short -- approximately 8 to 45 minutes. The first measurement of glucose uptake during constant flow shock was made at 30 minutes post-endotoxin infusion. Any glucose uptake that may have occurred earlier than 30 minutes would have been missed.

Local muscle hypoxia was found to be the major, if not the only, mechanism for increased glucose uptake in shock, and ischemia, alone, did not cause an elevated glucose uptake, provided muscle tissue was not hypoxic. In fact, glucose uptake decreased during ischemic hyperoxic condtions.

In conclusion, it is felt that occurrence of hypoglycemia associated whit endotoxin shock arises from two major sources. Hepatic alteration in gluconeogenesis have been well defined in shock. Although it is depressed, the amount of glucose produced is still above control levels; however, increased peripheral utilizatjion of glucose by muscle may be the key factor which causes plasma glucose to decrease early following endotoxin shock in the dog.

BIBLIOGRAPHY

Alican, F.M.; Dalton, M.L., Jr.; and Hardy, J.O.: Experimental endotoxin shock. Am. J. Surg. 103:702, 1962.

Aust, J.B.: Distribution kinetics of various substances in endotoxin shock. Fed. Proc. 18:5, 1959.

Archer, L.T.: Hypoglycemia in conscious dogs in live Escherichia coli septicemia: achronic study. Circ. Shock 3:93, 1957.

Balis, J.V.; Rapport. E.S.; Gerber, L.; and Buddengh, F.: Continuous endotoxemia in Rhesus monkeys as a clinically relevant model for shock-lung. Am. J. Pathol. 74:90, 1974.

Benjamin, B.; Alaupovic, P.; Wang, C.S.; and Hinshaw, L.B.: Relationship of chemical structure to pathophysiologic properties of endotoxin from serratia marcescens. Circ. Shock 1:61-69, 1974.

Berk, J.L.; Hagen, F.F.; Beyer, W.H.; and Gerber, M.J.: Hypoglycemia of shock. Ann. Surg. 171:400, 1970.

Berry, L.J.: Metabolic effects of bacterial endotoxins. Page 165 in S. Kadis, G. Weinbaum, and S.J. Ajl, eds. Microbial Toxins. Vol. V. Academic Press, New York. 1971.

Berry, J.L.: Factors influencing the function of the reticuloendothelial. J. Reticuloendothelial Soc. 11:450, 1972.

Binkley, F; Perlman, E.; andf Goebel, W.F.: jThe chemical degradation of the specific antigen of type Z Shigella paradysenteriae (Flexner) J. Exptl. Med. 81:331-347, 1945.

Blackwood, J.M.; Hsieh, J.; Fewel, J.; and Rush, BlF., Fr.: Tissue metabolites in endotoxin and hemorrhagic shock. Arch. Surg. 107:181, 1973.

Blattberg, B. and Levy, M.N.: Sites of extra hepatic pooling in early response to endotoxin. Am. J. Physiol. 220:1267, 1971.

Boho. C.T.; Turbow, M.E.; and Kolmen, S.N.: Improvement of coronary blood flow in endotoxin shock without improvement of myocardial conrtractility. Physiologist 16:268, 1973.

Boivin, A.; Mesrobeanu, I.; and Mesrobeanu, L.: Extraction d'un complexe toxique et antigenique a partir du bacile d'aertrycke. Compt. Rend. Soc. Biol. 114:307, 1933.

Boivin, A.; Mesrobeanu, I.; and Mesrobeanu, L.: Technique pour la preparation des polysaccharides microbiens specifiques. Soc. Biol. (Paris) 113:490, 1933a.

Boroff, D.A.: Study on toxins and antigens of shigella dysenteriae I. toxicity and antigenicity of whole organisms and various fractions of shigella dysenteriae. J. Bact. 57:617-632, 1949.

Brochman, S.K.; Thomas, C.S., Jr.; and Vasko, J.S.: The effect of Escherichia coli endotoxin on the circulation. Surg. Gynec. Obstet. 125:763, 1967.

Brand, E.D. and Lefer, A.M.: Myocardial depressant factor in plasma from cats in irreversible post-oligenic shock. Proc. Soc. Exptl. Biol. Med. 122:220, 1966.

Chien, S.; Chang, C.; Dellenback, R.J.; Vsami, S.; and Gregersen, M.I.: Hemodynamic changes in endotoxin shock. Am. J. Physiol. 210:1401, 1966.

Chien, S.; Dellenback, R.J.; Vsami, S.; Treitel, K.; Chang, C.; and Gregersen, M.I.: Blood volume and its distributjion in endotoxin shock. Am. J. Physiol. 210:1411, 1966.

Chien, S.; Sinclair, D.G.; Dellenback, R.J.; Chang, jC.; Peric, B.; Vsami, S.; and Gregersen, M.I.: Effect of endotoxin upon capillary permeability to macromolecules. Am. J. Physiol. 207:518, 1964.

Clowes, G.H.A., Jr.; O'Donnell, T.F., Jr.; Ryan, N.T.; and Blackburn, G.L.: Energy metabolism in sepsis: Treatment based on different patterns in shock and high output state. Ann. Surg. 179:684, 1974.

Cryer, P.E.; Coran, A.G.; Sode, J.; Herman, C.M.; andf Horwitz, D.L.: Lethal Escherichia coli septicemia in the baboon: Alpha adremergic inhibition of insulin secretion and its relationship to the duration of survival. J. Lab. Clin. Med. 79:622, 1972.

Cryer, P.E.; Herman, C.M.; and Sode, J.: Carbohydrate metabolism in the baboon subjected to gram-negative (E. coli) septicemia. I. Hyperglyceria with depressed (plasma) insulin

concentrations. Ann. Surg. 174:91, 1971.

Dedichen, H. and Schenk, W.G.: Hemodynamics of endotoxin shock in the dog. Arch. Surg. 95:1013, 1967.

Drucker, W.R., and Dekiewiet, J.C.: Glucose uptake by diaphragms from rats subjected to hemorrhagic shock. Am. J. Physiol. 206:317, 1964.

Ebert, R.V., Borden, C.W., Hall, W.H., and Gold, D.: A study of hypotension (shock) produced by meningecoccus toxin. Circ. Res. 3:378, 1955.

Emerson, T.E., Jr., and Gill, C.C.: Effect of slow intravenous infusjion of endotoxin in dogs. J. Appl. Physiol. 22:874,1967.

Emerson, T.E., Jr., and Kelly, K.C.: Effect of living E. coli cells on hemodynamics and mortality in the dog. J. Appl. Physiol. 23:609, 1967.

Engel, F.L.: Metabolic aspects of the shock problem. J. Mt. Sinai Hosp. 12:152, 1946.

Epstein, R.B., Waxman, F.J., Bennett, B.T., and Andersen, B. R.: Pseudomonas septicemia in neutropenic dogs. I. Treatment with granulocyte transfusions. Transfusion 14:51, 1974.

Felig, P., and Wahren, J.: Amino acid metabolism in exercinsing man. J. Clin Invest. 50:2703, 1971.

Ferguson, W.W., Glenn, T.M., and Lefer, A.M.: Mechanisms of production of circulatory shock factors in isolated perfused pancreas. Am. J. Physiol. 222:450, 1972.

Filkins, J.P., and Buchanan, B.J.: In vivo vs. in vitro effects of endotoxin on glycogenolysis, gluconeogenesis and glucose utilization. Proc. Soc. Exptl. Biol. Med. 155:216, 1977.

Filkins, J.P., Buchanan, B.J. and Cornell, R.P.: Hepatic carbohydrate metabolism alterations during endotoxin and traumatic shock. Circ. Shock 2:129, 1979.

Filkins, J.P. and Cornell, R.P.: Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. Am. J. Physiol. 227:778, 1974.

Freedberg, A.S., Haimovici, H., and Glumgart, H.L.: The cardiovascular dynamics in shock dur to infection. J. Clin. Invest. 23:930, 1944.

Guenter, C.A., Fiorica, V., and Hinshaw, L.B.: Cardiorespiratory and metabolic responses to live E. coli and endotoxin in the monkey. J. Appl. Physiol. 26:780, 1969.

Haberland, G.L.: Selected references in G.L. Haberland and J.W. Rohen, eds. Kininogenases, first symposium on physiological properties and pharmacolocical retionale. F.K. Schattauer Verlag, New York, 1972.

Haddy, F.J., Overbeck, H.W., and Daugherty, R.M.: Peripheral Vascular Resistance. Annu. Rev. Med. 19:167, 1968.

Hall, R.C., and Hodge, R.L: Vasoactive hormones in endotoxin shock: A comparative study in cats and dogs. J. Physiol. (London) 213:69, 1971.

Halnagyi, D.F.J., Broell, J., Gump, F.E., and Kinney, J.M.: Hypermetabolism is sepsis: correlation with fever and tachycardia. Surgery 75:763, 1974.

Haskins, W.T., Landy, M., Milner, K.C., and Ribi, E.: Biological properties of parent endotoxins and lipid fractions, with kinetic study of acid-hydrolyzed endotoxins. J. Exper. Med. 114:665-684, 1961.

Herion, J.C., Walder, R.I., Herring, W.B., and Palmer, J.G.: Effects of endotoxin and nitrogen mustarf on leukoctye kinetics. Blood 25:522, 1965.

Herlihy, B.L., and Lefer, jA.M.: Alteratjions in pancreatic acinar cell organelles during circulatory shock. Circ. Shock. 2:143, 1975.

Hinshaw, L.B.: The role of glucose in endotoxin shock. Circ. Shock 3:1, 1976.

Hinshaw, L.B., Archer, L.T., Beller, B.K., White, G.L., Schroeder, T.M., and Holmes, D.D.: Glucose utilization and tole of blood in endotoxin shock. Am. J. Physiol. 233:E 71, 1977.

Hinshaw, L.B., Archer, L.T., Black, M.R., Elkins, R.C., Brown, P.P., and Greenfield, L.J.: Myocardial function in shock. Am. J. Pysiol. 226:357, 1974.

Hinshaw, L.B., Archer, L.T., Greenfield, L.J., and Guenter, C.A.: Effects of endotoxin on myocardial hemodynamics, performance and metabolism. Am. J. Physiol. 221:504, 1971.

Hinshaw, L.B., Archer, L.T., Greenfield, L.J., Miller, J.A., and Guenter, C.A.: Effects of endotoxin on myocardial performance. J. Trauma. 12:1056, 1972.

Hinshaw, L.B., Beller, B.K., Archer, L.T., and White, G.L: Associated leukocyte responses in the lethal aspects of E. coli shock. Proc. Soc. Exptl. Biol. Med. 155:179, 1977.

Hinshaw, L.B., Benjamin, B., Coalson, J.J., Elkins, R.C., Taylor, F.B., Jr., Price, J.T., Smith, C.W., and Greenfield, L.J.: Hypoglycemia in lethal subhuman primate septic shock. Circ. Shock 2:197, 1975.

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

Hinshaw, L.B., Gilbert, R.P., Kuida, H., and Visscher, M.B.: Peripheral resistance changes and blood pooling after endotoxin in evicerated dogs. Am. J. Physiol. 195:631, 1958.

Hinshaw, L.B., Greenfield, L.J., Owens, W.E., Archer, L.T., and Guenter, C.A.: Cardiac response to circulating factors in endotoxin shock. Am. J. Physiol. 222:1047, 1972.

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

Hinshaw, L.B, and Owens, S.E.: Correlation of pooling and resistance changes in the canine forelimb in septic shock. J. Appl. Physiol. 30:331, 1971.

Hinshaw, L.B., Peyton, M.D., Archer, L.T., Black, M.R., Coalson, J.J., and Greenfield, L.: Prevention of death in endotoxin shock by glucose administration. Surg. Gynec. Obstet. 139:851, 1974.

Hinshaw, L.B., Vick, J.A., Jordan, M.M., and Wittmers, L.E.: Vascular changes associated with development of irreversible endotoxin shock. Am. J. Physiol. 202:103, 1972.

Horwitz, D.L., Ballantine, T.V.N., and Herman, C.M.: Acute effects of septic shock on plasma and red cell volumes in baboons. J. Appl. Physiol. 33:320, 1972.

Kinney, J.M.: Energy expenditure and tissue fuel in the septic patient. Page 145 in B.K. Forscher, R.C. Lillehei, and S.S. Stubbs, eds. Shock in Low and High-Flow States. Excerpta Medica, Amsterdam.

Kraneberg, G., and Sandritter, W.: Zur toxikoligie der Bakterienendotoxine. Arch. exper. Path. u. Pharmakol. 218:111, 1953.

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

LaNoue, K., Mason, D.D., Jr., and Daniels, J.: The impairment of gluconeogenesis by gram-negative infection. Metabolism 17:606, 1968.

Lansing, A.M.: Septic shock. J. Canad. Med. Assoc. 89:583, 1963.

Lecocq, F.R., Mebane, D., and Madison, L.L.: The acute effect of hydrocortisone on hepatic glucose output and peripheral glucose utilization. J. Clin, Invest. 43:237, 1964.

Lefer, A.M.: Role of myocardial depressant factor in the pathogenesis of circulatory shock. Fed. Proc. 29:1836, 1970.

Lefer, A.M.: Role of a myocardial depressant factor in shock states. Modern Con. Cardiovasc, Dis. 42:59, 1973.

Lefer, A.M., and Spath, J.A., Jr.: Pancreatic hypoperfusion and production of a myocardial depressant factor in hemorrhagic shock. Ann. Surg. 179:868, 2974.

Lillehei, R.C., Longerbeam, J.K., Block, F.H., and Manax, W.G.: The nature of irreversible shock: Experimental and clinical observations, Ann. Surg. 160:682, 1964.

Long, C.L., Spencer, F.L., Gump, F.E., and Kinney, J.M.: Hypermetabolism in sepsis: correlation with fever and tachycardi. Surgery. 75:763, 1974.

Lundgren, O.J., Lundwall, J., and Mellander, S.: Range of sympathetic discharge and reflex vascular adjustment in skeletal muscle during hemorrhagic hypotension. Acta. Physiol. Scand. 62:380, 1964.

Lundsgaard-Hansen, P., Pappova, E., Urbaschek, B., Heitmann, L., Leaderach, A., Manes, N., Oroz, M., and Wirth, V.: Circulatory deterioration as the determinant of energy metabolism in endotoxin shock. J. Surg. Res. 13:282, 1972.

Mack, E., and Egdahl, R.H.: Adrenal blood flow and corticosteroid secretion in hemorrhagic shock. Surg. Gynec. Obset. 131:65, 1970.

Madison, L.L., Combes, B., Strickland, W., Unga, R., and Adams, R.: Evidence for a direct effect of insulin on heptic glucose output. Metabolism. 8:469, 1959.

Marchick, J.B., Finley, R.J., Groves, A.C., Wolfe, L.I., Holliday, R.L., and Duff, J.H.: Catabolic hormones and substrate patterns in septic patients. J. Surg. Res. 23:177, 1977.

Marlis, E.B., Aoki, T.T., Toews, C.J., Felig, P., Cannon, J.J., Kyner J., Huckabee, W.E., and Cahill, G.F.: Amino acid metabolism in lactic acidosis. Am. J. Med. 52:474, 1972.

Martin, D.S., Cassisi, N.J., and Pickens, J.L.: Endotoxin shock: a collective review. Rev. Surg. 311, 1965.

McCallum, R.E., and Berry, L.J.: Effects of endotoxin on gluconeogenesis, glycogen synthesis and liver glycogen synthase in mice. Inf. Immunity. 7:642, 1973.

Mela, L., Bacalzo, L.U., and Miller, L.D., Defective oxidative metabolism in rat liver mitochondria in hemorrhagic and endotoxin shock, Am. J. Physiol. 220:571, 1971.

Mellander, S., and Lewis, D.H.: Effect on hemorrhagic shock on the reactivity of resistance and capacitance vessels and on capillary filtration transfer in cat skelltal muscle. Circ. Res. 13:105, 1963.

Menten, M.L., and Manning, H.M.: Blood sugar studies on rabbits infected with organisms of the enteritidfis-parathyphoid B group. J. Med. Res. 44:675, 1924.