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Relationship between Intracellular Ph and Calcium Uptake in the Skeletal Muscle During Sepsis

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RELATIONSHIP BETWEEN INTRACELLULAR pH AND CALCIUM UPTAKE IN THE SKELETAL MUSCLE DURING SEPSIS

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sepsis is a common and complicated syndrome among patients in intensive care units in hospitals(1). It is not a single disease; several etiologic factors may contribute to it. The clinical symptoms and signs may be recognized well before the results of cultures of body fluids for bacteria are available. Although sepsis may be caused by different etiologic factors including both gram-negative and gram-positive bacteria, fungi, and viruses, the gram-negative bacteria have been shown to be the most frequent causative agents in hospitalized patients (2). In spite of the use of antibiotics and advances in critical care medicine, gram-negative sepsis results in a mortality rate of 20-50%.

The most striking changes in gram-negative bacteria induced sepsis are the metabolic disturbances. Metabolic changes observed during sepsis include alterations in carbohydrate, protein, and lipid metabolism(4). The characteristics of carbohydrate metabolism in sepsis include an initial hyperglycemia followed by hypoglycemia and accompanied by increased glucose utilization(78). Although basal muscle glucose utilization is increased , insulin-stimulated muscle glucose utilization is often depressed in the septic state(78). Alterations in glucose utilization may

be due to changes in certain rate-limiting enzymes, especially pyruvate dehydrogenase(PDH) and phosphofructose kinase(PFK)(78). As for protein metabolism, there is inhibition of skeletal muscle protein synthesis in sepsis(6) and increased muscle protein degradation in endotoxemic rats(7). The intracellular signals responsible for these metabolic alterations remain unresolved. Some investigators have proposed that elevated intracellular calcium plays an important role in this alteration(11), although the exact mechanism is still controversial(17, 28).

It has been suggested that intracellular H^+ functions as a synergistic messenger and serves to integrate metabolic activities mediated by other messengers(67). Portoles et al.(8) reported a decrease in intracellular pH in isolated rat hepatocytes exposed to Escherichia coli endotoxin. Some reports have related altered calcium fluxes to the intracellular pH change(8). Most of these experiments were carried out using non-skeletal muscle tissue or cell lines.

This study was designed to investigate changes in skeletal muscle intracellular pH, and the relationship between the intracellular pH and Ca^{2+} alterations during sepsis in rats. The intracellular pH changes were estimated by quantitating the uptake of an intracellular pH marker, ^{14}C-benzoic acid, by skeletal muscle. The administration of the Ca^{2+} channel blocker, diltiazem, to animals followed by measurements of ^{14}C-benzoic acid and $^{45}Ca^{2+}$ uptake by

skeletal muscle, was used to assess a relationship between intracellular pH and Ca^{2+}.

CHAPTER II

LITERATURE REVIEW

A. Protein metabolism in skeletal muscle during sepsis

The metabolic responses to sepsis or trauma include marked changes in skeletal muscle protein metabolism and nitrogen balance with a resultant muscle wasting. Indeed, increased net protein degradation is a prominent metabolic disturbance in skeletal muscle in sepsis(10, 11). Hasselgren and Fischer et al.(13) found that proteolysis in isolated soleus muscles was increased by 36% at 8 hours and 42% at 16 hours after cecal ligation and puncture (CLP). In contrast, in isolated extensor digitorum longus muscle(EDL) protein breakdown was not significantly increased until 16 hours after CLP. No significant differences in protein synthesis were found between septic and control rats. Vary and Siegel(6) reported inhibition of skeletal muscle protein synthesis, in vivo, during intraabdominal sepsis induced by implantating fecal-agar pellets containing both Escherichia coli and Bacteroides fragilis into the abdomens of rats. Measuring the release of tyrosine and 3-methylhistidine as indicators of breakdown of tissue protein and myofibrillar protein,

respectively, Hasselgren et al. reported that the rate of tyrosine release was increased with sepsis by 58%, but the corresponding figure for 3-methylhistidine was 103% , which indicated a preferential breakdown of myofibrillar protein(13).

B. Potential mechanisms of protein breakdown during sepsis

No single factor solely contributes to the increased protein breakdown in sepsis. Different factors may play a major role in increased protein degradation in different animal models of sepsis and under different experimental conditions.

Goldberg and Baracos reported that prostaglandin E_2 (PGE_2) is an important regulator of muscle proteolysis(11). They incubated rat skeletal muscles with arachidonate and found increased protein breakdown and a shift in protein balance to a more negative state. Aspirin and indomethacin markedly reduced this proteolytic effect. In contrast, the experimental results of Hasselgren et al. do not support a role for PGE_2 in the accelerated muscle proteolysis in the CLP model; addition of indomethacin to the incubation medium reduced PGE_2 release from both septic and nonseptic muscles, however, the muscle protein breakdown rate was unaffected(21). The importance of interleukin 1(IL 1) in increasing protein breakdown was also investigated by Goldberg et al.(11). They incubated the rat soleus muscle at 37 ^{o}C with purified human IL 1, and found

that the IL 1 stimulated net protein degradation by 66-118%(11). Although IL 1 increased proteolysis dramatically, it did not reduce overall protein synthesis in the muscles(14). In addition, IL 1 dramatically stimulated the synthesis of PGE_2 in muscle. Addition of indomethacin with IL 1 prevented PGE_2 synthesis and prevented the increase in protein breakdown. Thus IL 1 acts directly on skeletal muscle to increase the production of PGE_2 and thereby promotes protein catabolism. IL 1 may also act on the hypothalamic center to initiate fever and thus further augment protein breakdown(11).

Tumor necrosis factor(TNF) which is secreted by macrophages during sepsis was reported to contribute to increased protein breakdown(11). However Hall-Anger and Fischer(15), studying extensor digitorun longus(EDL) muscles incubated with TNF, reported that TNF did not increase muscle protein turnover rate. When TNF was administered together with corticosterone, total and myofibrillar protein breakdown rates were increased. Fischer et al.(15) found that when corticosterone alone or in combination with TNF was injected into adrenalectomized rats, TNF did not increase plasma corticosterone levels or muscle protein breakdown rates. They suggested that muscle catabolism induced by in vivo administration of TNF is mediated by glucocorticoids.

The effects of catabolic hormones on protein turnover and amino acid uptake have also been investigated. Infusion of the catabolic hormones(glucagon,epinephrine and cortisol) for 16

hours resulted in elevated plasma glucose and lactate levels, reduced plasma concentration of most amino acids, and in accelerated protein breakdown(16). The catabolic hormones did not reduce muscle protein synthesis and amino acid uptake -- processes which are probably signaled by other substances and/or mechanisms.

In the cecal ligation and puncture(CLP) model, Hasselgren and Dimlich found an approximately 50% increase in the activity of myofibrillar proteinase, a nonlysosomal proteolytic enzyme, and an approximately 30% increase in the activity of cathepsin B, a lysosomal proteinase. Treatment of septic rats with the mast cell degranulating compound 48/80 or the lysosomal protease inhibitor, leupeptin, significantly reduced the myofibrillar proteinase and cathepsin B activities, but did not affect protein breakdown rates. They suggested that increased protein breakdown in septic skeletal muscle may be associated with but not caused by myofibrillar proteinase or cathepsin B activity(17). Some physical factor, such as elevated temperature has been implicated in protein degradation(18). At the molecular level, the influence of sepsis on transcription of myofibrillar protein in skeletal muscle was studied in rats(19). In the CLP model, 16 hours later, the muscle levels of mRNA for myofibrillar protein were determined by using cDNA probes specific for transcripts for alpha-actin and myosin heavy chain. Sepsis resulted in a 2-6 fold decrease in alpha-actin mRNA level and an even more

pronounced reduction in myosin heavy chain mRNA level; however, the changes in mRNA levels may be the result of other contributing factors instead of the cause of these changes.

In earlier work using a rat muscle preparation, the insulin-induced inhibition of proteolysis decreased in the presence of plasma from injured patients. But the work of Mitchell and Norton(20) found that insulin reduced degradation an average of 6% in soleus muscle and 10% in extensor digitorum longus muscle(EDL) incubated in normal plasma, which is not different to the 10% in soleus and 8% in EDL incubated in septic rat plasma.

C. The role of calcium in septic injury

Calcium is an important intracellular second messenger in mediating metabolic changes and Ca^{2+} dyshomeostasis in septic states has been extensively investigated. Sayeed et al.(22, 23) reported that intracellular exchangeable calcium was increased in hepatocytes in endotoxic shock; but the response of hepatocytes to epinephrine(NE) stimulation was impaired . The epinephrine-induced cytosolic Ca^{2+} increase was less than in the control condition. In vascular smooth muscle, Litten and Roth(24) reported that calcium influx was unaltered under basal conditions, but that the abilities of norepinephrine(NE) to augment influx and efflux were significantly depressed in rat aorta during sepsis. They suggested that the decreased

influx and efflux in response to NE may be related to the depressed aortic contractility observed in sepsis.

In cardiomyocytes, some studies showed that there was deranged calcium uptake by sarcoplasmic reticulum, which may contribute to the damaged cardiac relaxation and filling. In contrast, McDonough(25) reported that in the early hyperdynamic stage of sepsis, the rate of uptake of $^{45}Ca^{2+}$ by sarcoplasmic reticulum(SR) was not depressed compared to control, but in fact was elevated. The maximum calcium accumulation by SR and calcium-stimulated ATPase activity were similar in both control and septic hearts.

In skeletal muscle, several observations have suggested an important metabolic role for calcium-activated protease in muscle protein breakdown. For example, Kameyama and Etlinger(26) showed that treatment of muscles with calcium ionophore, A23187, increased protein breakdown. The calcium ionophores, A23187 and ionomycin, increased overall proteolysis by 50-100% without any significant alteration in protein synthesis(27). This effect may reflect an activation of a calcium-activated protease(28, 29, 30); although calcium also influences many other cell enzymes(e.g. protein kinases). Because dystrophic and denervated muscles contain high levels of calcium, a stimulation of proteolysis by increased calcium under these conditions has been emphasized(31). In control muscles not treated with ionophores, a lack of calcium in the extracellular medium decreased protein breakdown by 20-

30%(27), and also prevented the stimulation of protein catabolism by PGE_2 and arachidonate. One of the interesting findings by Goldberg et al.(27) was that the calcium-activated protease (CAP) was not essential for the activation of protein breakdown by ionophores. They found that the sulfhydryl blocking agent, mersalyl, could completely inactivate the CAP in the incubated muscles without reducing overall protein breakdown. Furthermore, in the mersalyl-treated muscle, A23187 stimulated proteolysis. By contrast, leupeptin and Ep-475, which inhibit cathepsin B in the muscle, decreased the stimulation of proteolysis by the calcium ionophores, just as they reduced the response of muscle to PGE_2. Thus, they suggested that the increased proteolysis induced by calcium, like that by PGE_2 , occurred within the lysosomes.

Westfall and Sayeed(33) used an intraperitoneal bacteria(Escherichia coli) injection model, and found that bacteremia had no effect on steady-state exchangeable calcium, but it significantly reduced the time required to reach half-maximal uptake compared with controls. This suggested an enhanced rate of inward calcium movement in bacteremic rat muscle. Results similar to those observed in bacteremic rat muscle were reported by Borle et al.(34), who showed a decrease in the time to half-maximal calcium uptake without a change in steady-state exchangeable calcium content in monkey kidney cells treated with metabolic inhibitors. Borle(34) postulated that an initial decrease in calcium uptake by

cellular organelles resulted in elevated cytoplasmic free calcium, and increased intracellular calcium was responsible for the stimulation of inward calcium movement across the plasma membrane. Increased cytosolic free calcium then stimulated calcium influx via an intracellular sodium-dependent and ATP-dependent mechanism in excitable cells(35).

In the endotoxemic model, an increase in skeletal muscle exchangeable calcium was found(36). Although endotoxic injury has some features in common with bacteremia, endotoxic animals exhibit cardiovascular and metabolic changes over a shorter period of time compared to bacteremic animals(37). One characteristic of the calcium alteration in bacteremic rats is that diltiazem could prevent the increased inward movement of calcium (33). Studies of ischemia-induced injury suggest that calcium blockers protected cardiac tissue against injury when the cardiac cell plasma membrane calcium channels were in the activated state(38).

D. Intracellular pH changes during sepsis

Many workers have demonstrated that cells undergo substantial intracellular pH changes with metabolic transitions occurring during differentiation and growth. They have suggested that the pH change plays a role as an effector of metabolic integration(39).

Moore et al.(40), using DMO(5,5-dimethyl-2,4-oxazolidinedione), a weak acid, measured the intracellular

pH(pH_i) changes in frog skeletal muscle, and found that insulin alkalinized the intracellular pH by 0.1-0.2 pH unit. They indicated that the changes in pH_i by insulin was due to activation of the Na^+/H^+ exchange system. The pH_i change by insulin may affect the activity of phosphofructose kinase(PFK), the rate-limiting enzyme of glycolysis, and thereby affect the rate of insulin-dependent glycolysis.

L'Allemain and Pouyssegur(41) measured changes of intracellular pH in G_0/G_1 -arrested Chinese hamster lung fibroblasts(CC139) treated with the growth factors, alpha-thrombin and insulin. They used the equilibrium distribution of benzoic acid, and found an increase in cytoplasmic pH by 0.2-0.3 pH unit upon addition of the growth factors. However, CC139-derived mutant cells, lacking the Na^+/H^+ exchange activity, were unable to regulate pH_i and did not respond to growth factors by cytoplasmic alkalinization. They suggested that cytoplasmic alkalinization after addition of growth factors is Na^+ -dependent and sensitive to amiloride, the Na^+/H^+ antiport inhibitor(41).

There are a number of experimental results that indicate a major role of intracellular pH in controlling the rate of protein synthesis(42, 43). Winker et al.(44) measured the influence of changes in intracellular pH on the synthesis of proteins in sea urchin eggs. They used Ca^{2+}-Mg^{2+}-EGTA buffers to maintain Ca^{2+} at low level, and measured rate of ^3H-valine incorporation as an indicator of protein synthesis

at varying pH_i. At a pH_i of 6.9 the rate of valine incorporation was very low, almost close to zero. As the intracellular pH increased, the rate of valine incorporation was also increased. The optimum pH_i was 7.40 at which the incorporation rate was highest. At higher pH_i, the rate of valine incorporation began to decrease.

England and Mitch et al.(45, 46) also reported the effect of intracellular pH on protein degradation and synthesis. They used the BC_3H_1 myocytes, which have characteristics of both skeletal muscle and smooth muscle. The intracellular pH was determined by the measurement of cellular uptake of benzoic acid. The pH_i was varied by altering extracellular pH. They found that both protein degradation and protein synthesis were decreased at a lower pH range. This response was associated with intracellular acidification and was blunted by insulin but not affected by glucocorticoids.

Some products of arachidonate metabolism also influence the intracellular pH. Sumimoto and Minakami(47) found that leukotriene B_4 induced a biphasic change in the cytoplasmic pH of human neutrophils -- an initial rapid acidification followed by an alkalinization. Only a few reports have evaluated pH_i changes in sepsis. In cultured hepatocytes, Portoles et al.(8) reported that the intracellular pH of Escherichia coli endotoxin-treated hepatocytes was lower than the controls.

E. The relationship between intracellular pH and calcium

A discussion of intracellular pH requires considering the intracellular calcium. There are membrane transport systems which regulate pH_i, such as the Na^+/H^+ antiport. The intracellular H^+ buffering systems also play a role. The mechanism underlying changes in intracellular pH and calcium are not entirely understood, but it is apparent that many of the conditions which affect one of these also modify the other(52). Siffert and Akkerman(53) reported that experimental alkalinization of the cytosolic compartment induced calcium uptake across the plasma membrane of endothelial cells, lymphocytes, and smooth muscle cells. Furthermore, inhibition of Na^+/H^+ exchange reduced calcium influx. In cardiac muscle, increased intracellular pH increased the calcium release from intracellular sources, and increased the affinity of troponin C with calcium(54).

Nakanishi and Takao(56) reported that during respiratory acidosis, myocardial diastolic and systolic calcium increased, and intracellular pH decreased. These changes corresponded with acidosis-related damage in cardiac function. Portoles and Pagani(8) found increased intracellular calcium and decreased intracellular pH in isolated and cultured hepatocytes incubated with Escherichia coli endotoxin(100 microgram/ml).

Rosoff et al.(57) used the chemically transformed murine pre-B lymphocyte cell line 70Z/3 as a model system for the study of the initiation of cellular differentiation. They

reported increased intracellular calcium and intracellular pH in 70Z/3 cells exposed to LPS(10 microgram/ml). In this experiment, they suggested increased intracellular calcium may stimulate the sodium-hydrogen exchange system and contribute to the increased intracellular pH. There is no data specifically showing the potential relationship between the intracellular calcium and pH of skeletal muscle in a septic animal model.

F. Septic animal models

There are several animal models of sepsis available for the study of sepsis and mechanisms of its pathogenesis. One of these models is the endotoxicosis model. This involves intraperitoneal or intravenous injection of a high dose of LPS(5-20 mg/Kg) without any supportive therapy. Some of the advantages of using LPS are: 1) LPS is a stable and purified compound, and 2) LPS may be stored in a lyophilized form. But, there are some questions about the clinical relevance of endotoxicosis. Clinical studies failed to show significant blood elevations of endotoxin in more than half of patients with documented gram-negative sepsis(59). Endotoxin lethal dose of gram-negative bacteria is in the microgram range, but the dose in milligram range is needed to produce endotoxin shock in animals(60). Human volunteers rendered tolerant to LPS by repeated injections still manifest signs of fever and the acute syndrome when infected with viable gram-negative

organisms(61).

There are also some models in which sepsis is induced by inoculating animals with bacteria. In one such model, live gram-negative bacteria, such as Escherichia coli, are infused into animals in massive doses. The massive infusion of bacteria into animals is quite unlike the low and variable quantities of bacteria released into the circulation of patients with gram-negative sepsis. Septic patients rather harbor a septic focus which intermittently showers the body with bacteria(62). Another bacterial model is the cecal ligation and puncture perforation(CLP) developed by Wichterman et al.(63).The advantage of the CLP model is its simplicity. Difficulties in controlling the magnitude and the mortality are its drawback.

Nakatani(64) in 1984 proposed a peritonitis model using a defined bacteria inoculum which permits control over the type and number of infecting bacteria. The rats are implanted intraperitoneally with a pellet consisting of sterilized rat feces, agar, and a known number of Escherichia coli and/or Bacteroides fragilis. This model results in body weight loss, severe leukopenia, fever, increased lactate levels in blood, blood glucose changes(64), and protein degradation(6). This model results in the induction of a chronic or subacute septic state of a graded severity which is somewhat similar to septic state occurring in humans.

CHAPTER III

THE HYPOTHESIS/OBJECTIVES

The literature survey shows that there are few data concerning intracellular pH change during the septic state. Portoles et al. have measured pH_i in liver cells exposed to endotoxin in vitro and found an endotoxin-induced decrease in pH_i. This result, however, can not be taken to indicate changes in pH_i in cells in intact animals with an endotoxic or septic insult. The effect of endotoxin in vitro could be due primarily to high concentrations of endotoxin used in the in vitro study(8). Such high concentration of endotoxin(100 mg/ml) is not likely to be present during endotoxicosis in vivo. The findings of Rosoff et al.(57) are in contrast to those of Portoles et al.(8). They found that exposure of pre-B lymphocytes to endotoxin increased both intracellular Ca^{2+} and pH_i. Some other studies focusing on the relationship between the intracellular Ca^{2+} and H^+ have shown that alterations in intracellular pH are associated with altered Ca^{2+} uptake by the cells(53, 54). An alteration in Ca^{2+} uptake is known to occur in the skeletal muscle during

sepsis(33). Furthermore, the alteration in Ca^{2+} uptake was prevented when septic animals were treated with the Ca^{2+} channel blocker, diltiazem.

The aforementioned findings support the concept that pH_i changes could occur with sepsis and that such changes could plausibly be related to altered regulation of Ca_i.

This study addressed the following questions:

1) Is there a change in the intracellular pH in skeletal muscle during intraabdominal sepsis?

2) If there is a significant change of pH_i in the skeletal muscle, could it be potentially related to an alteration in intracellular calcium regulation?

To ascertain the relationship between pH_i and intracellular calcium regulation, this study assessed changes in Ca^{2+} uptake by skeletal muscle. The calcium blocker, diltiazem, was then used to prevent alteration in Ca^{2+} regulation, as has been shown in previous studies(33, 36). It was hypothesized that calcium blocker treatment of septic rats could be used to establish a link between altered Ca^{2+} regulation and pH_i in the skeletal muscle during sepsis.

CHAPTER IV

MATERIALS AND METHODS

A. Animal model of sepsis

Male Sprague Dawley rats(220-280g, Harlan Inc., Indianapolis, Ind.) were used in all experiments. The rats were acclimated for a minimum of 4 days in light-dark cycle, and kept at an ambient temperature, 22^{o}C. Rats were provided with Purina Rat Chow and water. Freshly collected rat feces were ground in 0.9% sodium chloride saline to a semi-solid consistency and then packed into 1 cm^{3} pellets. The pellets were autoclaved(30 min., 120^{o}C) and then impregnated with Escherichia coli(50 - 100 CFU) and Bacteroides fragilis(8 x 10^{3} - 1 X 10^{4} CFU). The bacteria loaded pellets were then coated with 1% agar and used for septic intraabdominal implantation.

The rats were intraperitoneally anesthetized with pentobarbital(35 mg/kg), and placed in supine position. The abdomen was shaved and disinfected with betadine and 70% alcohol. A 1.5 cm lower midline incision was made, and either a sterile or a bacteria-containing pellet was placed into the right lower abdominal cavity. The abdomen was closed by

separately suturing the muscle and skin layers. After the operation, the animals were returned to the cages and allowed free access to chow and water. The rectal temperature and survivability were determined prior to implantation(day 0) and from day 1 to day 4 post-implantation.

The use of two gram-negative organisms to produce sepsis in rats was originally described by Nakatani et al.(64). They reported that PO_2 in the pus of intra-abdominal abscess in the rat model was nearly zero. The increased replication of facultative Escherichia coli in the abscess is presumed to cause the decrease in PO_2 and to result in an inhibition of Escherichia coli proliferation. The resulted anaerobic condition could promote the growth of the anaerobic Bacteroides fragilis in the abscess (72). Furthermore, intra-abdominal sepsis in humans is often caused by mixed infections with Escherichia coli and enterococcus as well as anaerobic organisms such as Bacteroides fragilis and Fusobacterium varium (70,71). Several intra-abdominal peritonitis animal models with mixed infections have been developed. Many of these models use colonic content as the inoculum. Although these inocula contain normal mixed flora(63), it is usually difficult to control the strains and numbers of bacteria in the inocula.

B. The uptake of ^{14}C-benzoic acid(BA)

The measurement of the distribution of the labeled weak

acid, benzoic acid, between the intracellular and extracellular compartments was used to estimate the intracellular pH. This method has been used in a variety of cell systems(67). The procedures used in this study is similar to that used by L'Allemain(41) and others(45, 68, 69).

The epitrochlearis muscles of rats were isolated at day 2 after the implantation and placed on stainless steel holders to maintain them at resting lengths. The muscles were preincubated for 30 minutes at 37°C in 2 ml oxygenated(95%O_2-5%CO_2) Krebs-Ringer bicarbonate(KRB) buffer, containing 1 mM $CaCl_2$ and 5 mM glucose. The medium pH was adjusted at 7.40. Muscles were then incubated for varying times in fresh KRB medium(2 ml) at the same temperature and pH, containing 2 μ Ci of ^{14}C-benzoic acid(BA). A 20 μ l aliquot of the medium was collected and counted to determine the ^{14}C-BA specific activity after the incubation. After incubation, the muscles were removed from the medium, and slightly blotted with filter paper to remove the water adherent to the muscle surface. The wet weight of the muscles were measured. The muscles were digested overnight in the tissue solubilizer TS-1(Research Products International) and the BA radioactivity was measured in a liquid scintillator counter. Tissue radioactivity was converted into BA contents(nmol) after dividing by medium BA specific activity (dpm/nmol).

C. Estimation of extracellular water and intracellular water

Extracellular water was determined by measuring epitrochlearis muscle uptake of ^{3}H-inulin. The experiments to determine the muscle uptake were carried out separately, but similarly to those of muscle BA uptake. The medium specific radioactivity of ^{3}H-inulin was 1 μCi/ml. To determine dry weights, muscles were incubated in a vacuum oven for 16 hours at 95^{o}C. Total tissue water(ml/g) was calculated for each muscle from its wet weight to dry weight ratio. The extracellular(inulin) space was calculated using following equation:

$$ECW(ml/g) = \frac{\text{Inulin DPM/g wet weight}}{\text{Inulin DPM/ml medium}}$$

The intracellular water was calculated as the difference between the total tissue water and the extracellular water.

D. Calculation of intracellular pH(pH_i)

The intracellular pH was calculated with the equation used by L'Allemain(41).

$$pH_i = pH_o + \log \frac{Bi}{B_o} \qquad (1)$$

where B_o is specific radioactivity of benzoic acid in the external medium, B_i(dpm/ml) is the intracellular benzoic acid radioactivity. pH_o is the pH in the external medium(7.40).

The calculation of B_i depends upon two assumptions:

(1) The radioactivity of BA in the muscle is that distributed in the extracellular water(ECW) and intracellular water(ICW), and not bound to tissues.

(2) The specific radioactivity of BA in the extracellular water is equal to that of the external medium.

Therefore :

BA radioactivity in ECW of the muscle(dpm)

= muscle weight(g) X ECW(ml/g) X B_o(dpm/ml) (2)

BA radioactivity in ICW of the muscle(dpm)

= radioactivity of the muscle(dpm) -

radioactivity in ECW(dpm) (3)

$$B_i(\text{dpm/ml}) = \frac{\text{Radioactivity in ICW of muscle(dpm)}}{\text{Muscle weight(g) X ICW (ml/g)}} \quad (4)$$

E. Measurement of pH_i with "warm ischemia"

To test whether or not the BA uptake by muscle indeed assessed alterations in intracellular pH, pH_i assessments by this method were carried out in muscles of rats subjected to "warm ischemia" --a condition which is known to affect pH_i. To produce ischemia in the muscles, rats were decapitated and left at room temperature for one hour before the

epitrochlearis muscles were removed for assessment of the ischemic effect on muscles. Muscles removed immediately after the decapitation of rats were used as nonischemic controls.

F. $^{45}Ca^{2+}$ uptake experiments

The method used for $^{45}Ca^{2+}$ uptake is similar to that described by Westfall and Sayeed(33). The muscles were preincubated in a metabolic shaker bath(37°C) for 30 min. in 2 ml of oxygenated(95% O_2 - 5% CO_2) Krebs-Ringer-bicarbonate(KRB) containing 1 mM $CaCl_2$ and 5 mM glucose. Muscles were then incubated at 37°C and pH 7.40 in 2.0 ml of the oxygenated KRB containing 1 mM $CaCl_2$, 5 mM glucose, and 2 μ Ci ^{45}Ca-$CaCl_2$ (Amersham). A 20 μ l aliquot of the medium was collected to determine $^{45}Ca^{2+}$ specific activity. The extracellular Ca^{2+} was removed by incubating muscles for an additional 60 min. in 2.0 ml solution at pH 7.40, which contained 134 mM $NaCl_2$, 4.0 mM KCl, 1.0 mM $MgCl_2$, 20 mM HEPES, 5 mM glucose, and 2 mM $LaCl_3$. After incubation, the muscles were blotted with filter paper, and then the wet weight was measured. The muscles were digested overnight by tissue solubilizer (Research Products International) at 55°C, and then the radioactivity was measured in a liquid scintillator counter(LKB 1209 Rackbeta). Calcium uptake was calculated with the following equations:

$$^{45}Ca^{2+} \text{ Specific activity} = \frac{\text{DPM/ml medium}}{\text{NMOL } Ca^{2+}\text{/ml medium}}$$

$$Ca^{2+} \text{ uptake} = \frac{\text{DPM/g wet weight}}{^{45}Ca^{2+} \text{ specific activity}}$$

G. Diltiazem treatment of rats

Diltiazem(2.0 mg/Kg in saline) was injected intraperitoneally 8 hours after the implantation of pellets into rats. The effects of diltiazem on $^{45}Ca^{2+}$ uptake and ^{14}C-benzoic acid uptake were measured with the same methods as described above.

H. Statistics

The results of change in body temperature(Fig. 1) and benzoic acid uptake by septic and sterile rat muscles(Fig. 2, table 3) were analyzed with 2-way ANOVA and post-hoc Newman-Keuls tests. The results of effects of diltiazem on $^{45}Ca^{2+}$ uptake(Fig. 3) and benzoic acid uptake(Fig. 4) were analyzed with one-way ANOVA and post-hoc Newman-Keuls tests. The results of change of intracellular pH(table 4, table 6), BA uptake by ischemic and nonischemic rat muscles(table 6), and water content change of the muscles(table 2, table 5) were analyzed with independent 2-tailed Student's T tests. Chi-square test was used to analyze the mortality rate(table 1). $P < 0.05$ was considered statistically significant.
All results are expressed as mean $\pm$ SEM.

CHAPTER V

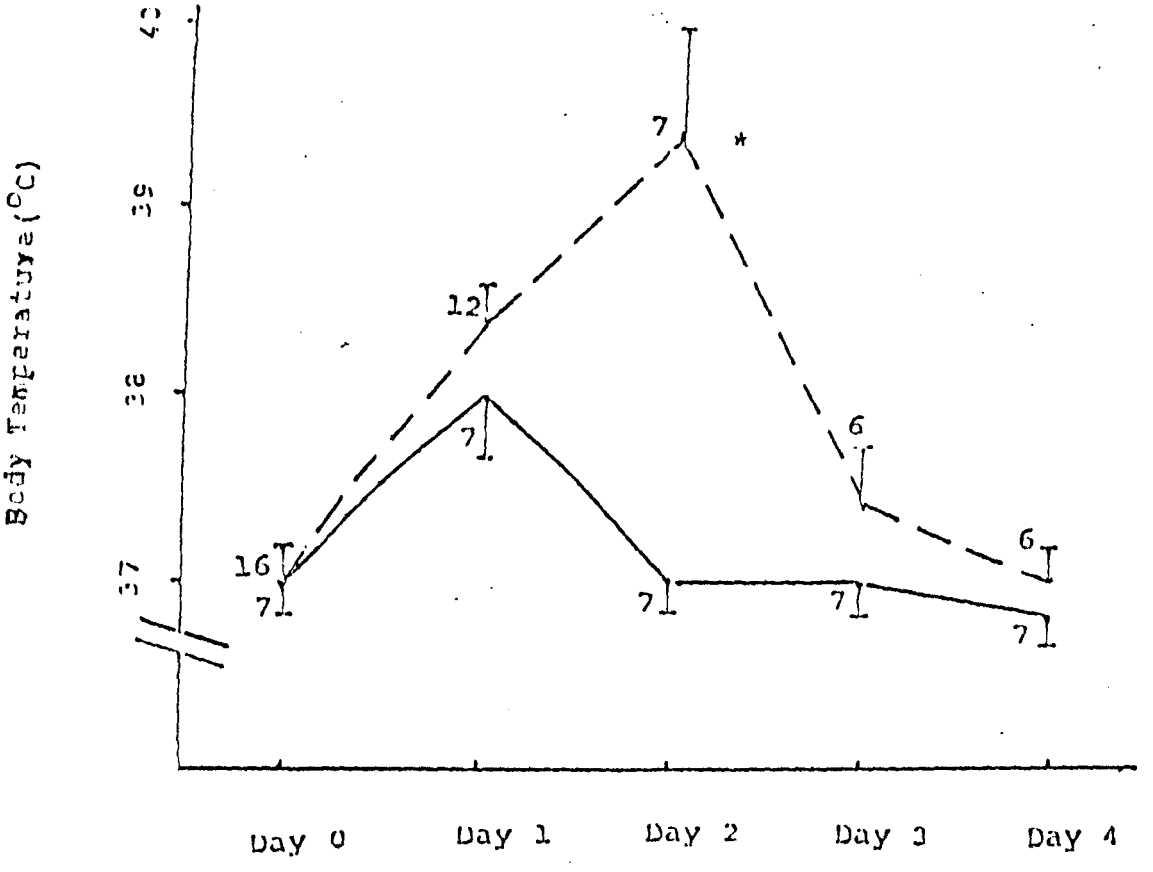
RESULTS

A. Body temperature and mortality

The rats regained consciousness three hours after they were implantated with pellets with or without Escherichia coli and Bacteroides fragilis. On day 1 after the operation, septic animals were weak and lethargic. In the later part of day 1, some septic rats exhibited piloerection , bloody exudate around both eyes, and decreased sensitivity to sound and mechanical stimulation. Diarrhea was noticed usually before the death of the septic rats. Body temperature increased on day 1 in both septic and sterile rats(Fig. 1). The increase in body temperature in septic rats was not significantly different from that occurring in the sterile rats. For sterile rats the body temperature began to drop back toward the normal range by the end of the first twenty-four hours. The mortality in septic rats was 25% by day 1 and 56% by day 2. No sterile-implanted rats died during the whole observation period of the experiment. In septic rats, the body temperature increased on day 2 from that found on day 1; it was significantly higher than in sterile-implanted rats(Fig. 1).

Fig. 1

Body Temperature After Implantation
with E. coli and B. fragilis



Days After Implantation

* $p < 0.01$ compared to the sterile group. N = 6-16
Solid line represents sterile groups, dash line represents septic groups.
The numbers indicate the number of rats in each group.

On day 3, the body temperature dropped back to normal levels in the septic rats. No deaths occurred in septic rats after on day 4 (table 1).

Table 1

Mortality Rate in Rats After Sterile- and Septic*-Implantations

Groups	Day 0	Day 1	Day 2	Day 3	Day 4
Sterile	0/7(0%)	0/7(0%)	0/7(0%)**	0/7(0%)**	0/7(0%)**
Septic	0/16(0%)	4/16(25%)	9/16(56%)	10/16(62%)	10/16(62%)

* Septic-implanted rats recieved pellets impregnated with *Escherichia coli* and *Bacteroides fragilis*.

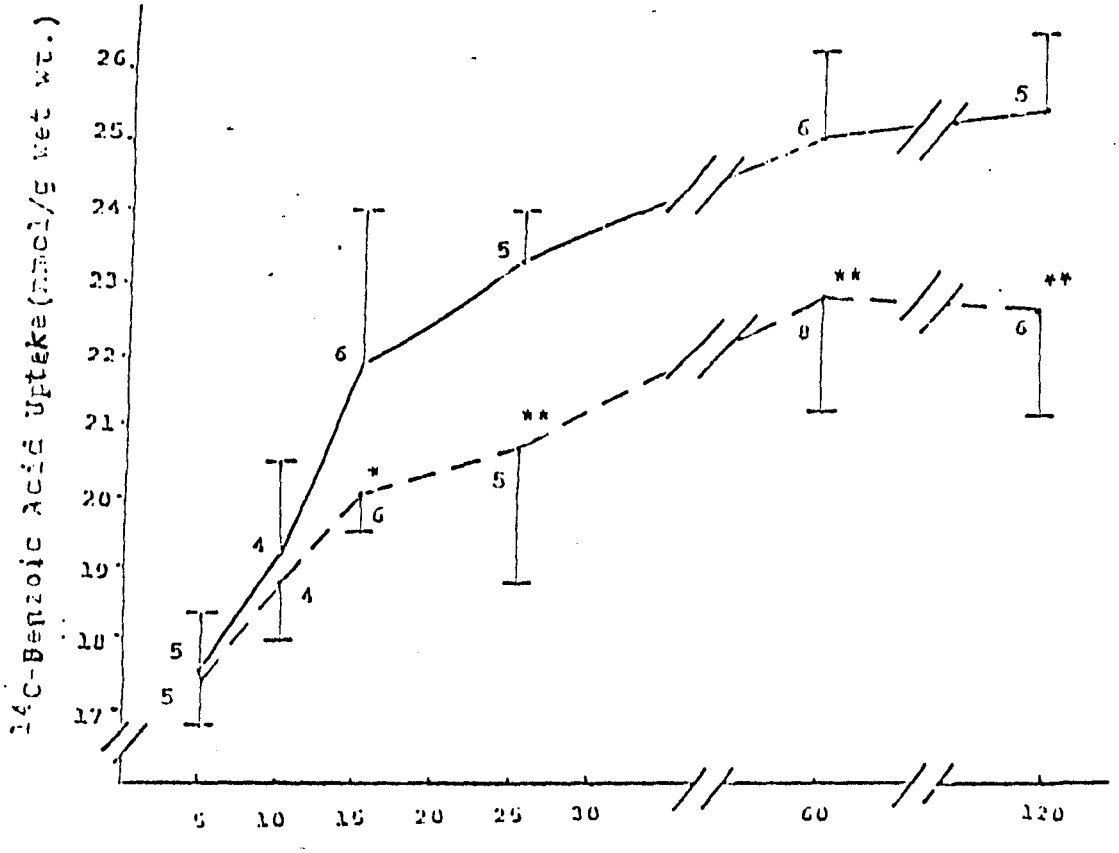
** P <0.05 compared with septic groups.

B. BA uptake and pH_i changes in septic and sterile rat muscles

The time course of the benzoic acid uptake by septic and sterile rat muscles is shown in Fig. 2 and table 3. During the early phase of incubation(5 - 10 min.), there was no significant difference between the sepsis and sterile groups. Significant differences were found in benzoic acid(BA) uptake between sepsis and sterile groups at 15 min. after incubation; BA uptake in septic rat muscles was lower compared to the sterile rat muscle ($P< 0.05$). As incubation time increased,

Fig. 2

Benzoic Acid Uptake by Epitrochlearis Muscle in Sterile and Septic Rats



* $P < 0.05$ compared to the corresponding sterile group.
** $P < 0.01$ compared to the corresponding sterile group.
Solid line represents sterile groups, dash line represents septic groups. M ±4-8.
The numbers indicate the number of muscles in each group.

Table 2

Water Contents of Sterile- and Septic-implanted Rat Muscles

Group	TTW	ECW	ICW
Sterile	0.800 ± 0.013 (N = 8)	0.250 ± 0.002 (N = 8)	0.550 ± 0.013 (N = 8)
Septic	0.799 ± 0.012* (N = 8)	0.249 ± 0.002* (N = 8)	0.550 ± 0.012* (N = 8)

The incubation time is 90 minutes.
N = numbers of muscles.
Water content is expressed as mean ± SEM(ml/gram wet weight).
* P > 0.05 compared to the corresponding group.

Table 3

Benzoic Acid Uptake by Epitrochlearis Muscles in Sterile and Septic Rats

Incubation time[a]	Sterile[b]	Septic
5	17.54 ± 0.89 (N = 5)	17.45 ± 0.99 (N = 5)
10	19.19 ± 1.33 (N = 4)	18.72 ± 0.87 (N = 4)
15	21.81 ± 2.20 (N = 6)	20.02 ± 0.59* (N = 6)
25	23.31 ± 0.77 (N = 5)	20.72 ± 2.0** (N = 5)
60	25.03 ± 1.25 (N = 6)	22.93 ± 1.70** (N = 8)
120	25.52 ± 0.99 (N = 5)	22.72 ± 1.62** (N = 6)

a Incubation time is expressed as minutes.
b Uptake is expressed as nanomole per gram of wet muscle.
* $P < 0.05$ compared to the corresponding sterile group.
** $P < 0.01$ compared to the corresponding sterile group.

Table 4

Intracellular pH in the Skeletal Muscle of Sterile- and Septic-implanted Rats

Group	N	Intracellular pH
Sterile	6	7.40 ± 0.01
Septic	8	7.33 ± 0.02*

* $P < 0.05$ compared to the sterile group.

N = numbers of muscles; values are mean ± SEM.

larger differences were found in the uptake of benzoic acid. Significant differences($P< 0.01$) between sepsis and sterile groups were found at 60 and 120 min.. Within the sterile groups, the differences at 60 minute and at 120 minute were not significant ($P>0.05$). Also, there were no significant differences between the 60 and 120 minute uptake values in the septic group($P>0.05$). Therefore, at 60 minute, the uptake of benzoic acid by both septic and sterile muscles appeared to be in an equilibrium state.

Table 2 shows the water contents of sterile and septic rat epitrochlearis muscles. The total tissue water of sterile rat muscle was 0.800 ± 0.005 (ml/gm) and 0.799 ± 0.004(ml/gm) in the septic rat muscles. There is no significant difference between these groups ($P>0.05$). As for extracellular water and intracellular water, there were no significant differences between the septic and sterile rat muscles($P>0.05$).

The intracellular pH in epitrochlearis muscle, after equilibrium BA uptake at 60 minute, is shown in table 4. With the same external medium pH(7.40), the intracellular pH of septic rat muscle(7.33 ± 0.02) was significantly lower than the intracellular pH in the sterile muscle(7.40 ± 0.01, $P<0.05$).

C. Benzoic acid uptake and pH_i in the skeletal muscle of rats subjected to "warm ischemia"

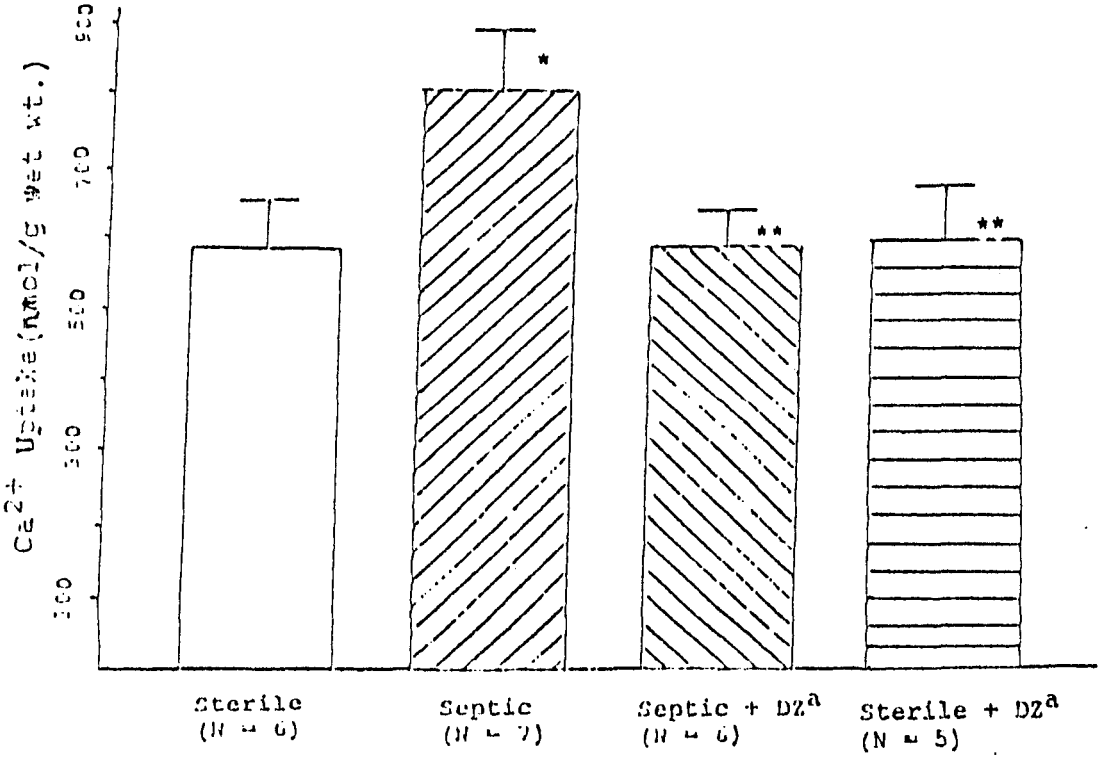
Table 5 shows water contents of epitrochlearis muscles removed from rats before and after a 60 min. period of warm ischemia. The results of the BA uptake by ischemic and nonischemic muscles, and calculated pH_i values are shown in table 6. A significant change in BA uptake and intracellular pH occurred after a 60 min. of "warm ischemia". Previous studies reported a significant decrease in pH_i under hypoxic and/or ischemic conditions(9, 5, 3, 32). The mechanism responsible for the ischemia-related decrease in pH_i is increased anaerobic metabolism(12).

D. Effect of diltiazem on $^{45}Ca^{2+}$ uptake and benzoic acid uptake

Fig.3 shows $^{45}Ca^{2+}$ uptake by epitrochlearis muscles of sterile and septic rats. The muscle incubation time was 25 minutes. As can be seen, there was a significant increase in $^{45}Ca^{2+}$ uptake of the septic rat epitrochlearis muscle (800.5 $\pm$ 90.68 nmol/g wet wt.) compared to that of sterile muscles (592.07 $\pm$ 70.88 nmol/g wet wt.). In septic rat muscle, after an intraperitoneal injection of diltiazem (2.0 mg/Kg) 8 hours after implantation, the $^{45}Ca^{2+}$ uptake was lower than in the untreated septic rats($P<0.01$). In contrast, the $^{45}Ca^{2+}$ uptake(586.90 $\pm$ 77.00 nmol/g wet wt.) by sterile muscles with diltiazem treatment was not significantly different than sterile muscles without diltiazem treatment(592.07 $\pm$ 70.90 nmol/g wet wt., $P>0.05$). The effect

Fig. 3

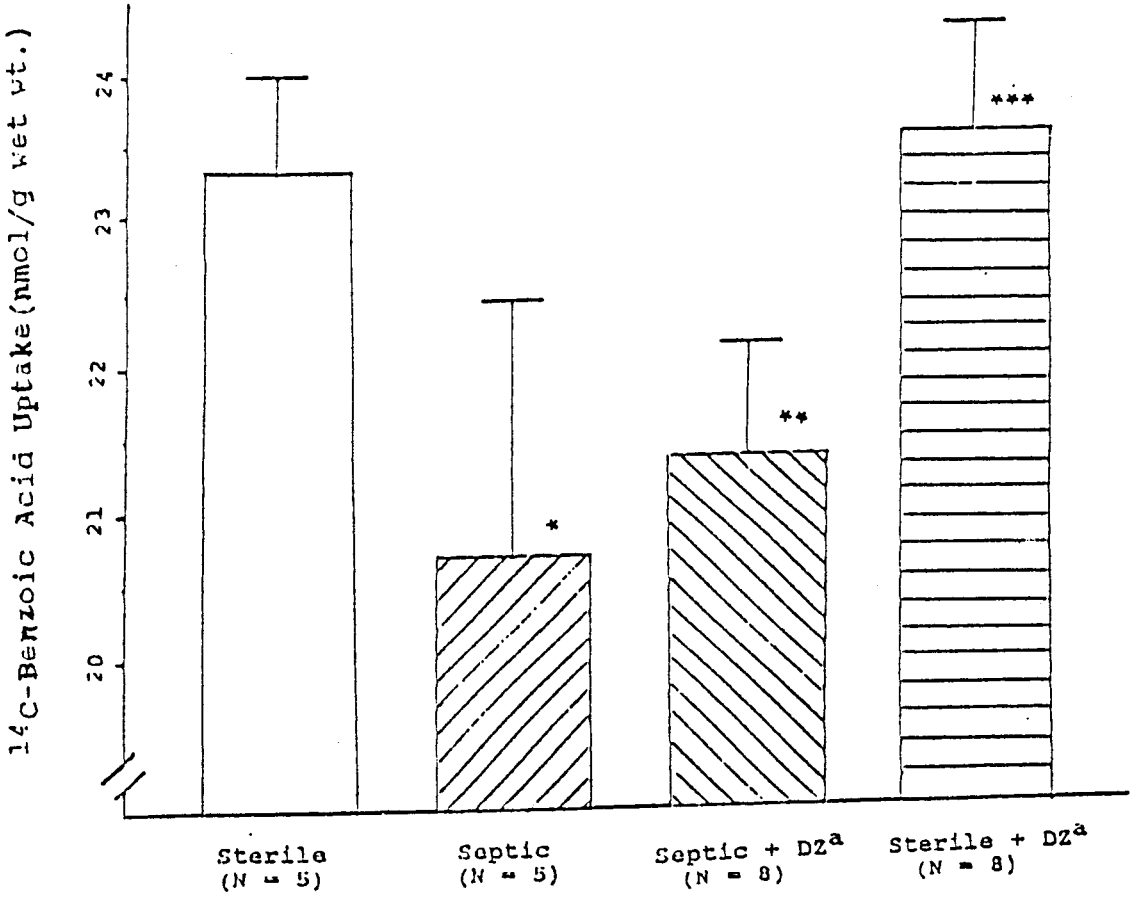
Calcium Uptake by Epitrochlearis Muscle
in Sterile- and Septic-Implanted Rats



* P < 0.01 compared to the sterile group.
** P > 0.05 compared to the sterile group,
but < 0.01 compared to the septic group.
a Diltiazem intraperitoneal injection 2.0 mg/Kg,
0 hours after implantation.

Fig. 4

Effect of Diltiazem on Benzoic Acid Uptake
by Epitrochlearis Muscles



* P < 0.05 compared to the sterile group.
** P > 0.05 compared to the septic group.
*** P > 0.05 compared to the sterile group.
a Diltiazem intraperitoneal injection 2.0 mg/Kg, 8 hours after implantation.

Table 5

Water Contents of Rat Epitrochlearis Muscles before and after a Period of Warm Ischemia

Group	TTW	ECW	ICW
Nonischemic	0.806 ± 0.004 (N = 6)	0.250 ± 0.002 (N = 6)	0.551 ± 0.014 (N = 6)
Ischemic	0.801 ± 0.002* (N = 6)	0.250 ± 0.004* (N = 6)	0.552 ± 0.006* (N = 6)

TTW = total tissue water.
ECW = extracellular water.
ICW = intracellular water.
* P > 0.05 compared to the corresponding group.
Values are mean ± SEM, and have units of ml/gram wet weight.

Table 6

Effect of Warm Ischemia on pH_i and BA Uptake in the Skeletal Muscle

Group	N	BA uptake(nmol/g wet wt.)**	pH_i
Ischemic	12	21.56 ± 0.32*	7.31 ± 0.01*
Nonischemic	12	24.46 ± 0.30	7.39 ± 0.01

N = numbers of muscles; values are mean ± SEM

* $P < 0.01$ compared to the nonischemic group.

** measured after 60 min. incubation of muscles.

of diltiazem on ^{14}C - BA uptake in epitrochlearis muscle is shown in Fig.4. The muscle incubation time was the same as for $^{45}Ca^{2+}$ uptake experiment(25 minute).With diltiazem treatment, there was no significant difference in BA uptake of the septic group(2.0 mg/Kg) compared to the untreated septic group (21.50 $\pm$ 0.80 nmol/g wet wt. vs. 20.72 $\pm$ 2.0 nmol/g wet wt., P> 0.05). BA uptake in the diltiazem-treated sterile group was also not significantly different from that in the untreated sterile group(23.55 $\pm$ 0.76 nmol/g wet wt. vs. 23.31 $\pm$ 0.76 nmol/g wet wt., P > 0.05).

Previous investigators have measured benzoic acid(BA) uptake by cultured cells to assess intracellular pH(41, 45, 67, 68). Benzoic acid is a weak acid with a pK_a value of 4.2(41). The intracellular to extracellular proton concentration gradient determines the distribution ratio of the uncharged benzoic acid across the plasma membrane. In the present experiments, we studied BA uptake by the epitrochlearis muscle. This is a thin muscle and is thus presumed to allow a rapid and a near-complete equilibration of BA across the cells. Nesher et al.(73, 74) reported that the epitrochlearis muscle is composed of approximately 10 - 15 % slow - twitch red, 20% fast-twitch red, and 65% fast-twitch white muscle fibers.

The time course of benzoic acid uptake by rat epitrochlearis muscle represents uptake into both the interstitial and intracellular compartments. The uptake into the interstitial compartment in all probability occurs during the initial phase of muscle incubation. On the other hand, transport of benzoic acid through the sarcolemma into the intracellular compartment is a slower process and therefore would contribute to uptake during the later period of incubation. The time course of benzoic acid uptake by septic and nonseptic muscles showed no difference during the initial phase. This may be due to lack of effect of the septic injury process on the initial uptake of benzoic acid into the interstitial compartment. The decreased level of uptake of

benzoic acid during the later incubation periods(beyond 10 minutes) in septic muscle(Fig. 2, table 3) may be due to a decrease in intracellular space or to a decreased uptake into intracellular compartment. Since this study showed an absence of a change in the tissue intracellular space with septic injury(table 2), the observed decrease in the septic rat muscles is likely due to a decrease in the uptake into the intracellular compartment in accordance with the extra to intracellular proton concentration gradients in these muscles.

The calculation of intracellular pH based on the benzoic acid distribution ratio shows a lower intracellular pH in septic rat muscles than the sterile rat muscles under the same external pH. This finding is similar to that of Portoles et al.(8) who found the intracellular pH of Escherichia coli endotoxin-treated hepatocytes to be lower than control hepatocytes. In contrast to these findings, Rosoff et al.(57) found increased pH_i of the pre-B lymphocytes(cell line 70Z/3) after they were incubated with a low dose of endotoxin. England and Mitch et al.(45) also used the ^{14}C-benzoic acid method to measure the intracellular pH in BC_3H_1 cells which have the characteristics of the skeletal muscle cells. They found that when they lowered the extracellular pH, the intracellular pH also decreased. In BC_3H_1 cells, the degradation of proteins was increased and the synthesis was decreased when the extracellular pH was lowered. Therefore decreased intracellular pH may contribute to deranged

intracellular metabolism of proteins.

The increased Ca^{2+} uptake by epitrochlearis muscles from septic rats compared to sterile rat muscles(Fig. 3) suggested an enhancement of Ca^{2+} flux during sepsis. The altered Ca^{2+} movement in soleus muscle has also been shown during bacteremic shock(33). The increased Ca^{2+} flux could be due to increased mobilization of Ca^{2+} from the intracellular store. After the intraperitoneal injection of diltiazem into rat, the increased Ca^{2+} flux in septic epitrochlearis muscle was restored to the control level. This confirms the effect of Ca^{2+} channel blocker in preventing the increased Ca^{2+} flux in skeletal muscle during septic state as was shown by Sayeed and Westfall (33, 65). Diltiazem did not affect $^{45}Ca^{2+}$ uptake by the muscles from sterile-implanted rats -- which may reflect different sensitivities of calcium channels in septic and sterile states to this calcium channel blocker. Previous studies have presented evidence that Ca^{2+} blockers protected cardiac tissue against injury only when the tissue was depolarized and thus sensitive to calcium blockers(38). The observed calcium blocker efficacy in septic-implanted rat skeletal muscle could also be related to the depolarized state of the skeletal muscle during sepsis(50). The insensitivity of sterile-implanted rat muscle to diltiazem may be due to an absence of depolarization in this state. In BA uptake experiment, the dose of diltiazem which blocked the increase in Ca^{2+} uptake, did not have a significant effect on BA

uptake. This suggests the Ca^{2+} channel blocker do not have an effect on the proton concentration gradient in the septic rat muscles. Also, there was no significant effect of diltiazem on BA uptake by sterile rat muscle. Previous data have shown that diltiazem administered to bacteremic rats prevented bacteremia-induced changes in skeletal muscle glucose transport (77). Another report (66)has shown that the calcium channel blockers, verapamil and nimodipine, may correct the decreased extracellular pH in ischemic rat brains without altering cerebral blood flow suggesting a metabolic rather than a hemodynamic effect of the calcium channel blockers. Rosoff et al.(57) suggested increased intracellular Ca^{2+} may stimulate the Na^{+}/H^{+} exchange antiport system, and may increase the pHi. Insulin may also increase pH_i by stimulating the Na^{+}/H^{+} exchange system (40). Taking these findings into consideration, the altered intracellular pH in septic skeletal muscle may be due to increased intracellular proton production and/or damaged Na^{+}/H^{+} exchange system under the pathophysiological condition. The pH_i change may be related to deranged protein metabolism(44, 45, 46).

CHAPTER VII

CONCLUSIONS

1) The rat intraabdominal sepsis model is a suitable animal model for the studies of the pathogenesis of sepsis.

2) Intraabdominal sepsis causes skeletal muscle metabolic changes, such as a decrease in intracellular pH and an increase in calcium uptake.

3) Treatment of the septic rats by calcium channel blocker, diltiazem, prevents the change in skeletal muscle calcium uptake but not the change in intracellular pH.

4) Although both increased calcium uptake and decreased intracellular pH may be related to sepsis-induced disturbance in protein metabolism, they appear to produce this effect independently.

BIBLIOGRPHY

1. Martin, M., and R. Wenzel. Gram-negative bacterial sepsis in hospitals in the United States-natural history in the 1980s. IN: Bacterial Endotoxins. New York: A John Wiley & Son Inc,1991. pp. 111-119.

2. Hinshaw, L., and P. Peduzzi. Effect of high dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. N. Engl. J. Med. 317:659-665, 1987.

3. Soussi, B.I., D.I.P. Bylund-Fellenius and A.C. Seherst. Dynamics of skeletal muscle energetics during ischemia and reperfusion assessed by in vivo ^{31}P NMR. NMR Biomed. 3:71-77, 1990.

4. Mizock, B.. Septic shock: a metabolic perspective. Arch. Int. Med. 144:579-585, 1984.

5. Crumrine, R.C., J.C. LaManna and W.D. Lust. Regional changes in intracellular pH determined by neutral red histophotometry high energy metabolites during cardiac arrest and following resuscitation in the rat. Metab. Brain Dis. 6:145-55,1991.

6. Vary, T., and J.H. Siegel. Inhibition of skeletal muscle

protein synthesis in septic intra-abdominal abscess. J.of Trauma 28:981-988, 1988.

7. Jepson, M., and J. Pell. The effects of endotoxemia on protein metabolism in skeletal muscle and liver of fed and fasted rats. Biochem. J. 235:329-336, 1986.

8. Portoles, M.T., M.J. Ainaga and R. Pagani. Intracellular calcium and pH alterations induced by E. coli endotoxin in rat hepatocytes. Biochimica et Biophysica Acta 1092:1-6, 1991.

9. Shee, C.D., I.R. Cameron. The effect of pH and hypoxia on function and intracellular pH of the rat diaphragm. Respir. Physiol. 79:57-68,1990.

10. O'Donnel, T.F., and G. H. A. Clowes. Proteolysis associated with a deficit of peripheral energy fuel substrates in septic man. Ann. Surg. 80:192-200 1976.

11. Goldberg, A.L., and V. Baracos. Control of protein degradation in muscle by prostaglandins, Ca^{2+}, and leukocytic pyrogen(interleukin-1). Fed. Proc. 43:1301-1306, 1984.

12. MacLarty, J.L., D.R. Campion and G.J. Hausman. Effect of fetal decapitation on the composition and metabolic characteristics of pig skeletal muscle. Biology of the Neonate 45:142-9, 1984.

13. Hasselgren, P.O., and J. H. Benson. Total and myofibrillar protein breakdown in different types of rat skeletal muscle: effects of sepsis and regulation by

insulin. Metabolism 38:634-640, 1989.

14. Baracos, V.E., H P Rodemann and A. L. Goldberg. Human leukocytic pyogen(interleukin-1) stimulates protein degradation and prostaglandin synthesis in skeletal muscle. N. Engl. Med. 308:553-558, 1988.

15. Hall-Anger, M., and J.E. Fischer. Interaction between corticosterone and tumor necrosis factor stimulated protein breakdown in rat skeletal muscle, similar to sepsis. Surgery 108:460-466, 1990.

16. Hasselgren, W.B., and J E Fischer. Effect of catabolic hormone infusion and protein turnover and amino acid uptake in skeletal muscle. Am. J. Surg. 159:295-300, 1990.

17. Hall-Anger, M., R. V. Dimlich and J. E. Fischer. Myofibrillar proteinase, cathepsin B and protein breakdown rates in skeletal muscle from septic rats. Metabolism 40:302-306, 1991.

18. Hasselgren, P.O., and J. E. Fischer. Effect of elevated temperature on protein breakdown in muscle from septic rat. Am. J. Physiol. 258:589-592, 1990.

19. Hasselgren, P.O., J. V. Tricoli and J. E. Fischer. Reduced levels of mRNA for myofibrillar proteins in skeletal muscle from septic rats. Life Sci. 49:756-760, 1991.

20. Mitchell L. and L. W. Norton. Insulin protects against muscle proteolysis induced by septic plasma. Arch.

Surg. 125:396-398,1990.

21. Hasselgren, P.O., B.W. Warner and J.E. Fischer. Further evidence that accelerated muscle protein breakdown during sepsis is not mediated by prostaglandin E_2. Ann. Surg. 07:399-403, 1988.

22. Sayeed. M.M., and S. R. Maitra. Cellular Ca^{2+} dyshomeostasis in the liver in endotoxic shock. Advances in Experimental Medicine and Biology 232:125-129, 1988.

23. Sayeed, M.M., M. Zhu and S. R. Maitra. Alterations in cellular calcium and magnesium during circulatory/septic shock. Magnesium 8(3-4):179-189, 1989.

24. Littern, R.Z., and B. L. Roth. Alterations in bidirectional transmembrane calcium flux occur without changes in protein kinase C levels in rat aorta during sepsis. Circ. Shock 25:123-130, 1988.

25. McDonough, K.H.. Calcium uptake by sarcoplasmic reticulum isolated from hearts of septic rats. Circ. Shock 25:291-297, 1988.

26. Kameyama, T., and J. D. Etlinger. Calcium dependent regulation of protein synthesis and degradation in muscle. Nature(London) 279:344-346, 1979.

27. Rodemann, H.P., and A. L. Goldberg. The stimulation of protein degradation by Ca^{2+} is mediated by PGE_2 and does not require the calcium-activated protease. J. Biol. Chem. 257:8716-8723,1982.

28. Ishiura, S.. Calcium-dependent proteolysis in living cells. Life Sci. 29:1079-1087, 1981.

29. Lewis, S.E.M., and D. F. Goldspink. The effects of calcium on protein turnover in skeletal muscles of the rat. Biochem. J.204:257-264, 1982.

30. Sugden, P.H.. The effects of calcium ions, ionophore A23187 and inhibition of energy metabolism on protein degradation in rat diaphragm and epitrochlearis muscles in vitro. Biochem. J. 190:593-603, 1980.

31. Joffe, M., N. Savage and H. Issacs. Increased muscle calcium. Biochem. J. 196:663-667, 1981.

32. Vanheel B., L. Leybaert, A De Hemptinne and I. Leusen. Simulated ischemia and intracellular pH in isolated ventricular muscle. Am. J. Physiol. 257:C365-C376,1989.

33. Westfall, M.V., and M.M. Sayeed. Skeletal muscle calcium uptake in bacteremic rats. Am. J. Physiol. 256:R201-R206, 1989.

34. Borle, A.B.. Pitfalls of the $^{45}Ca^{2+}$ method. Cell Calcium 2:187-196, 1981.

35. Dipolo, R.. Calcium influx in internally dialyzed squid giant axons. J. Gen. Physiol. 73:91-113, 1979.

36. Dudley, C.M., S.R. Martra and M.M. Sayeed. Effect of diltiazem on cellular calcium alterations in skeletal muscle during endotoxic shock. In: Perspectives in Shock Research, edited by R. F. Bond. New York: Liss, 264, pp.373-378, 1988.

37. Westfall, M.V., and M.M. Sayeed. Basal and insulin-stimulated skeletal muscle sugar transport in endotoxic and bacteremic rats. Am. J. Physiol. 254:R673-R679, 1988.

38. Cheung, J.H., and J. V. Bonventer. Mechanism of protection by verapamil and nifedipine from anoxic injury in isolated cardiac myocytes. Am. J. Physiol. 246:C323-C329, 1984.

39. Busa, W.B.. The proton as an integrating effector in metabolic activation. In: Na^+/H^+ exchange, intracellular pH, and cell function, edited by P.S. Aronson and W.F. Baron. Academic Press, Inc. pp.291-305, 1986.

40. Moore, R.D.. The role of intracellular pH in insulin action and in diabetes mellitus. In: Na^+/H^+ exchange, intracellular pH, and cell function, edited by P.S. Aronson and W.F. Baron. Academic Press, Inc. pp.291-305, 1986.

41. L'Allemain, G., G Paris and J Pouyssegur. Growth factor action and intracellular pH regulation in fibroblasts. J. Biol. Chem. 259:5809-5815, 1984.

42. Shen, S.S., and R. A. Stainhardt. Direct measurement of intracellular pH during metabolic depression at fertilization and ammonia activation of the sea urchin egg. Nature(London) 272:253, 1978.

43. Epel, D., T Humpreys and D. Mazia. An analysis of the

partial metabolic derepression of sea urchin eggs by ammonia: the existence of independent pathways. Dev. Biol. 40:245, 1974.

44. Winker, M.M.. Regulation of protein synthesis in sea urchin eggs by intracellular pH. In: Intracellular pH: its measurement, regulation, and utilization in cellular functions, edited by R. Nuccitelli and D. W. Deamer. New York: Liss, pp.325-340, 1982.

45. England, R.K., J. L. Chastain, and W. E. Mitch. Abnormalities in protein synthesis and degradation induced by extracellular pH in BC_3H_1 myocytes. Am. J. Physiol. 260:C277-C282, 1991.

46. Mitch, W.E., R. C. May and W. Druml. Protein and amino acid metabolism in uremia: influence of metabolic acidosis. Kidney International 36:S205-S207, 1989.

47. Sumimoto, H., M. Satah and S. Minakami. Cytoplasmic pH change induced by leukotriene B_4 in human neutrophils. Biochimica et Biophysica Acta 970:31-38, 1988.

48. Miller, R.G., M. D. Bosku, P. J. Carson and M. W. Weiner. ^{31}P nuclear magnetic resonance studies of high energy phosphates and pH in human muscle fatigue: comparison of aerobic and anaerobic exercise. J. Clin. Invest. 81:1190-1196, 1988.

49. Rothe, G., and G. Valet. Flow cytometric parameters of neutrophil function as early indicators of sepsis-or-

trauma related pulmonary or cardiovascular organ failure, J. Lab. Clin . Med. 115:52-61, 1990.

50. Trunkey, D.D., H. Illner, I.Y. Wagner and G.T. Shires. The effect of septic shock on skeletal muscle action potentials in the primate. Surgery 85:638-643, 1979.

51. Jacobs, D.O., T. Kobayashi, and D. W. Wilmore. Sepsis alters skeletal muscle energetics and membrane function. Surgery 110:318-326, 1991.

52. Grinstain, S., and S Cohen. Cytoplasmic free Ca^{2+} and the intracellular pH of lymphocytes. In: Cell calcium and the control of membrane transport, edited by L. J. Mandel and M. Eaton. New York: The Rockefeller University Press. pp.215-228, 1987.

53. Siffert, W., and J. W. Akkerman. Sodium-hydrogen exchange and calcium influx. Febs. Letters 259:1-4, 1989.

54. Achicke F.I., and S. Dai. Influence of pH changes on the action of verapamil and cardiac excitation-contraction coupling. Europ. J. Pharmacol. 196:77-83, 1991.

55. Tsunoda, Y., and Y. Tashiroi. Cytosolic acidification leads to calcium mobilization from intracellular stores in single and populational parietal cells and platellets. Exp. Cell Res. 193:356-363, 1991.

56. Nakanishi, T., M. Seguchi and A. Takao. Effect of acidosis on intracellular pH and calcium concentration in the newborn and adult rabbit myocardium. Circ. Res.

67:111-123, 1990.

57. Rosoff, P.M.. B lymphocyte differentiation: role of phosphoinositides, C kinase, and sodium-hydrogen exchange. In: Sodium-hydrogen exchange, intracellular pH, and cell function, edited by P. S. Aroson and W.F. Baron. Academic Press, Inc, pp.193-199, 1986.

58. Mitchell, P.F., and S. O. Heard. Laboratory models of sepsis and septic shock. J. Surg. Res. 49:186-196,1990.

59. Stumacher, R.T., M.T. Kovnot and W.R. McCabe. Limitation of the usefulness of the limulus assay for endotoxin. N. Engl. Med. 288:1261, 1973.

60. Postel, J., and D. Furtado. Pathophysiological alterations during bacterial infusion for the study of bacteremic shock. Surg. Gynecol. Obstet. 141:638, 1975.

61. Greisman, S.E., and T.E. Woodward. The role of endotoxin during typhoid fever and tularemia in mice. J. Clin. Invest. 48:61, 1969.

62. Pass, L.T., and W.R. Drucker. Cardiopulmonary response of the rat to gram-negative bacteremia. Am. J. Physiol. 246:H344-H356, 1984.

63. Wichterman, K.A., and I. H. Chandry. Sepsis and septic shock-a review of laboratory models and a proposal. J. Surg. Res. 29:189-201, 1980.

64. Nakatani, T., T. Sato, and T. H. Siegel. Hepatic and systemic metabolic responses to aerobic and anaerobic intra-abdominal abscesses in a highly reproducible

chronic rat model. Circ. Shock 13:271-294, 1984.

65. Sayeed, M.M.. Diltiazem prevents endotoxin-induced disturbances in intracellular Ca^{2+} regulation. In: Second Vienna shock forum. Alan R. Liss, Inc. pp.1053-1063, 1989.

66. Berger, L., and A. M. Hakins. Calcium channel blockers correct acidosis in ischemic rat brain without altering cerebral blood flow. Stroke 19:1257-1261, 1988.

67. Roos, A., and W.F. Baron. Intracellular pH. Physiol. Rev. 61:296-434, 1981.

68. De Bongioanni, L.C., and E. H. Ramos. Determination of intracellular pH by the distribution of benzoic acid in S. Cerevisiae amino acid transport and proton gradient. Revista Argentina De Microbiologia 20:1-15, 1988.

69. Sies, H., and K.H. Halder. Carbon-dioxide concentration and the distribution of monocarboxylate and hydrogen ions between intracellular and extracellular spaces of hemoglobin-free perfused rat liver. Eur. J. Biochem. 38:247-258, 1973.

70. Bartlett, J.G., and S.L. Gorbach. An animal model of intra-abdominal sepsis. Scand. J. Infect. Dis.(suppl) 19:26-29, 1979.

71. Onderdonk, A.B., J. G. Bartlett and S.L. Gorbach. Microbial synergy in experimental intra-abdominal abscess. Infect.Immunol. 13:22-26, 1976.

72. Fry, D.E., R.N. Garrison and J. D. Richardson. An

experimental model of intra-abdominal abscess in the rat. Adv. Shock Res. 7:7-11, 1982.

73. Nesher, R., and D.M. Kipnis. Epitrochlearis muscle I: mechanical performance, energetics,and fiber composition. Am. J. Physiol. 239:E454-E460, 1980.

74. Nesher, R., and D.M. Kipnis. Epitrochlearis muscle II: metabolic effects of contraction and catecholamines, Am. J. Physiol. 239:E461-E467,1980.

75. Bagby, G.T., C.H. Lang and J.J. Spitzer. Increased glucose metabolism by epitrochlearis muscle removed from endotoxin-treated rats. Circ. Shock, 20:171-179, 1986.

76. Karlstad, M.D., and M.M. Sayeed. Alpha-amino isobutyric acid transport in rat soleus muscle during endotoxic shock. Am. J.Physiol. 248:R142-R146, 1985.

77. Westfall, M.V., and M.M. Sayeed. Concentration of cellular Ca^{2+} uptake to altered sugar transport in skeletal muscle during E. coli bacteremia(abstract). Circ. Shock 24:256, 1988.

78. Wolfe, R.R., D. Elahi and J.J. Spitzer. Glucose kinetics in dogs following a lethal dose of endotoxin. Metabolism 26:847-850,1977.

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APPROVAL SHEET

The thesis submitted by Gin Mo has been read and approved by the following committee:

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

4/5/93
Date

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