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A PROGRAM FOR STUDY OF SKELETAL MUSCLE CATABOLISM  
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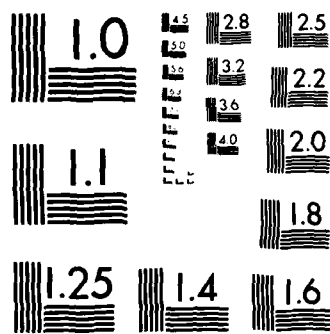
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A PROGRAM FOR STUDY OF SKELETAL MUSCLE  
CATABOLISM FOLLOWING PHYSICAL TRAUMA

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(August 1981-September 1982)

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br><br>To determine if the ketonemia following injury contributed to the increased<br>glucogenesis associated with this catabolic disorder, glucose production and<br>arterial substrates were measured before and after infusion of Na-DL- $\beta$ -<br>hydroxybutyrate ( $\beta$ -OHB, 20 Mol/kg $\cdot$ minute) in fed, fasted, and fasted-<br>infected sheep. Following three hours of $\beta$ -OHB infusion in the awake,<br>conditioned animals, -OHB and acetoacetate blood concentrations more than<br>doubled. With infusion, blood glucose and alanine concentrations decreased |                       |                                                                                                  |

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To determine the effect of the endorphine system on post-traumatic/septic metabolic responses, Naloxone (2 mg, I.V.) was administered to four sheep, before and after infection. In these normotensive animals, no major alteration in substrate concentration was noted. The endorphine system does not appear to exert major metabolic regulatory effects in this model.

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# SUMMARY

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal/Resources, National Academy of Sciences-National Research Council.

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A. THE EFFECTS OF KETONE BODY ADMINISTRATION ON GLUCONEOGENESIS AND PROTEIN CATABOLISM, AND MEDIATION OF PROTEIN SPARING WITH KETONE INFUSION.

INTRODUCTION

Following a variety of stresses, hyperglycemia and increased gluconeogenesis usually occur.<sup>1</sup> With the stress of infection<sup>2,3</sup> or injury<sup>4</sup> the serum concentrations of  $\beta$ -OHB and AcAc remain low, even during caloric deprivation. It has been hypothesized that the failure to develop hyperketonemia during these stress states is causally related to the accelerated proteolysis and increase glucose production that characterize the metabolic responses following major injury and severe infection.<sup>5,6</sup> In this study, we examined the relationship among ketosis, glucose production, and protein catabolism in the fed, fasted, and fasted-infected state. Infusion of exogenous ketones ( $\beta$ -OHB) into fed and fasting animals suppressed glucose production, but this did not occur in the non-ketotic infected animals.

METHODS AND MATERIALS

Sheep were selected as the experimental model because of the major contributions of long chain and ketone acids to energy pathways during fasting with or without pregnancy.<sup>7,8</sup> In addition, an extensive data base describing ruminant metabolism is available, and methodology for performing similar studies has been described.<sup>9,10</sup> Because ruminants do not absorb glucose from the gastrointestinal tract, they constantly produce new glucose, primarily in the liver from glucose precursors arising from the rumen in the fed state or mobilized from substrate stores during fasting.<sup>11</sup> Conditioned, non-pregnant, non-lactating ewes or rams of mixed breeds, weighing 24-38 kilograms were studied. The non-sheared animals were housed indoors in the individual pens in constant temperature (23°C) with an intermittent light cycle (lights on approximately 12 hours during the day and off at night). The pens were cleaned in the morning of each day, and at noon the sheep were fed generous portions of hay and molasses-coated mixed grains (Omolene, Ralston Purina Co., St. Louis, MO). Water and a salt lick were always available.

At least 10 days prior to an experiment the animals were anesthetized (sodium pentobarbitol, Abbott Laboratories, North Chicago, Ill., administered intravenously, 20-30 mg/kg), prepared for a sterile operative procedure, a small groin incision made, and catheters (polyethylene tubing O.D. 2.80 mm with silastic tips) directed into the distal aorta and inferior vena cava via the femoral vessels. In 6 animals laparotomy was performed and an additional catheter was inserted into a branch of the mesenteric vein and directed into the portal vein. The tip of this catheter was placed at the porta hepatis, as previously described.<sup>11</sup> The distal ends of the catheters were tunneled subcutaneously to the flank and exteriorized via a stab wound. The groin incision was closed and a blunt tip needle fitted with an intermittent injection cap (Jelco®, Jelco Laboratories, Raritan, NJ) glued to each exteriorized catheter. The needles were supported and protected on the flank by adhesive tape placed around the girth. Procaine penicillin (Wyeth Laboratories, Inc., Philadelphia, PA, 600,000 units) was given intramuscularly. In the postoperative period the animals were weighed daily, the catheters irrigated with saline, and rectal temperature monitored. The sheep studied in the protocols requiring prolonged infusions were conditioned by transporting them to the laboratory in a small, portable restraint pen where they remained in the study environment for two to four hours per day. After four to six such training periods, the animals were accustomed to the laboratory and personnel. Usually the sheep were conditioned and studied in pairs.

Soft tissue infection was established by implanting two 10x10 cm gauze sponges contaminated with canine fecal flora into the thigh muscle and subcutaneous tissue of one leg<sup>12</sup> This was accomplished by anesthetizing the animal (intravenous thiopentol, Abbott Laboratories, North Chicago, Ill., 20-30 mg/kg), shaving a small area of the non-catheterized hindlimb, and making a 6-8 cm skin incision on the lateral aspect of the thigh. Sharp dissection was carried down through subcutaneous fat and fascia, and the muscle fibers were split using blunt dissection. One gauze sponge containing approximately 15 grams of fecal residue was placed deep within the muscle and the fascia tightly closed, utilizing 3-0 nylon suture. A second contaminated sponge was placed in the subcutaneous pocket and the skin tightly closed. Procain penicillin

(600,000 units) was given intramuscularly at the time of operation. This antibiotic was administered in this manner based on previous reports of beneficial effects on survival with penicillin administration to ruminants with contaminated extremity wounds.<sup>13</sup>

While developing this infected sheep model, we found that the administration of the anesthetic alone, anesthesia plus the implantation of sterile gauze sponges, or anesthesia plus contamination with a smaller innoculum did not produce clinical or biochemical responses associated with soft tissue infection. We quantitated the bacteria in the innoculum and attempted to define dose responses to infection, but the biochemical alterations were highly variable to known quantities of bacteria. Hence, we utilized this model which most consistently provided both clinical and biochemical alterations to infection, but usually did not cause overwhelming sepsis and death. For example, of the 24 animals infected during this entire study, two died, both on the second day following infection. These two animals were eliminated from the study. All others survived for at least 6 days until sacrifice or operative drainage of the infected leg. Serial blood cultures obtained daily from the arterial catheter yielded at least one positive blood culture on the second to the fourth days following infection in all 8 infected animals cultured. The bacteremia subsequently cleared with resolution and drainage of the soft tissue infection. E. coli, Pseudomonas aeruginosa, and Klebsiella, all gram negative organisms, were identified in the blood cultures. Serial blood cultures in five control animals did not grow microorganisms. For the purposes of this study then, we have simply classified animals as "infected" and compared them with non-infected sheep.

Post-operatively, the animals were allowed free access to water and salt, but no feed. On the first day post-infection, swelling and tenderness were present in the infected limb, and the animals were frequently hemo-concentrated. In some animals, physiologic saline (500 ml) was given intravenously on that day only, to restore the hematocrit towards pre-infection levels. When required, fluid was administered after the morning measurements were performed. Thirty-six to forty-eight hours after operation all animals were drinking water. Throughout the post-infection period, metabolic substrate, body weight, and rectal temperature were monitored daily.

### Studies of Glucose Production

Preliminary studies demonstrated that established infection in fed animals resulted in a rapid reduction of food intake; spontaneous feeding resumed with resolution of the infection. Because of the variation in energy intake with infection, fed animals were infected as previously described and then fasted for the subsequent three days. The metabolic response of these animals (fasted-infected) was compared to non-infected, three-day fasted sheep. Measurements were also made in normal but previously catheterized fed sheep. In this last group of fed animals, food was removed from their pens the evening before the glucose turnover study.

On the morning of the glucose turnover study, the animals were transported to the laboratory and allowed to rest for at least one hour in the restraint pens. A primed constant infusion of D(6-<sup>3</sup>H(N)) glucose (New England Nuclear, Boston, MA) was delivered into the venous catheter by a constant infusion pump (Harvard Apparatus Company, Inc., Millis, MA); a prime of 1.5 mci/kg was given followed by an infusion of 20 nci/kg · minute. After allowing one hour to reach isotopic steady state, samples were drawn from the arterial catheter at 15-minute intervals over the next hour for determination of glucose specific activity and concentrations of glucose,  $\beta$ -OHB, and AcAc. During the last withdrawal, samples were also taken for whole blood alanine, lactate, pyruvate, blood gases and pH, and plasma free fatty acids. Blood pressure was monitored throughout the study by a transducer (Stratham) attached to the arterial line and pressure recorded on a Sanborn Recorder (770 series). Rectal temperature was measured at the end of each study.

### Infusion of Beta-Hydroxybutyrate

To determine if a causal relationship existed between glucose production and elevated blood ketone concentrations, animals from the fed, fasted, and fasted-infected groups were studied before and after an acute elevation in the  $\beta$ -OHB. In addition to the fasted control group, fed animals were also selected for this study because they demonstrated low concentrations of ketones and rates of glucose production comparable to the fasted-infected group. Moreover, the fasted animals were already ketotic, and infusing a comparable quantity of  $\beta$ -OHB into these sheep elevated  $\beta$ -OHB concentrations above levels usually observed during fasting alone.

At the end of the glucose turnover protocol outlined above (short term studies), the tritiated glucose infusion was continued and sodium DL- $\beta$ -OHB (Sigma Chemical Co., St. Louis, MO) infused. The  $\beta$ -OHB was prepared in a manufacturing pharmacy where the compound was dissolved in sterile water and the solution passed through a 0.22 micron membrane filter (Millipore Corporation, Bedford, MA), the concentration being varied to give a delivery rate of 65 ml of solution per hour and 20  $\mu$ Mol of  $\beta$ -OHB/kg body weight  $\cdot$  minute via an infusion pump (IMED Corporation, San Diego, CA). All solutions were tested for bacterial growth and pyrogenicity before infusion. No  $\beta$ -OHB prime was given. After two hours of  $\beta$ -OHB infusion, the samples for glucose specific activity and arterial substrates were obtained at 15-minute intervals as outlined in the glucose turnover protocol. The effect of  $\beta$ -OHB infusion was studied in 11 fed, 8 three-day fasted, and 9 fasted-infected animals.

To evaluate the effects of  $\beta$ -OHB infusion on insulin elaboration, three fed and four fasted-infected animals with previously implanted portal vein catheters were studied. Additional blood samples were drawn from the arterial and portal venous catheter for insulin determinations at the end of the control and infusion periods.

To evaluate the effects of sodium infusion and/or alkalosis associated with  $\beta$ -OHB administration, sodium bicarbonate (1 mEq/ml) was substituted for the  $\beta$ -OHB and infused at a rate of 0.82 ml/minute in two fed and two fasted-infected animals. The same sampling protocol was followed as the  $\beta$ -OHB infusion protocol previously described.

In all of the investigations outlined above, no animal was subjected to an infusion study on more than one occasion every 10 days. No more than 80 ml of whole blood were required for any individual study. After all fasting studies, at least 10 days of recovery were allowed before re-study. After hindlimb infection, the animal was dropped from the study.

### Chemical Analysis

All samples were immediately chilled in their respective collecting tubes. Samples of whole blood were placed into tubes containing oxylate and fluoride and analyzed in duplicate for glucose concentration by the glucose oxidase/peroxide method (Sigma Chemical Co.).<sup>14</sup>  $\beta$ -OHB, AcAc, and alanine were precipitated with 3.3 M perchloro-acetic acid and analyzed in duplicate by the methods of Williamson and Mellanby.<sup>14</sup> Plasma free fatty acids were measured using the method of Dole,<sup>15</sup> and blood lactates and pyruvates by the Bohringer techniques.<sup>14</sup> Insulin was determined by the immunoassay method of Soeldner.<sup>16</sup>

Tritiated glucose specific activity was determined by first precipitating plasma proteins with barium hydroxide and zinc sulfate (Somugin) and passing the supernatant through an anion-exchange column (Amberlite IRA 400 CP, Mallinckrodt Chemical Corporation, St. Louis, MO). The elutant was evaporated to dryness in a vacuum oven and re-dissolved in 1 ml of water and 10 ml Aquasol. Emission was counted on a Searle Mark III liquid scintillation spectrophotometer. Plasma and elutant glucose concentrations were determined in triplicate on a Beckman glucose analyzer. An infusate sample from each experiment was diluted and also counted. Hematocrits were determined in triplicate and arterial blood gases measured using a Corning Model #168 pH and Medical Blood Gas Analyzer.

Details for the calculation of glucose turnover have been described by Steele.<sup>17</sup> In all cases, an equilibrium in glucose specific activity was achieved. In this situation, the rate of glucose turnover is determined by dividing the isotope infusion rate by the specific activity. We have tested the accuracy of this approach in dogs in which all sources of endogenous glucose production were removed. In the steady state situation, the isotopic technique enabled the determination of the rate of appearance of unlabeled glucose within a maximum error of 5%.<sup>18</sup> The method has been applied successfully in sheep by Bergman and associates.<sup>19</sup> All group values are expressed as mean  $\pm$  standard error of the mean and differences between groups were determined using unpaired t-tests and analyses of variance. The effect of  $\beta$ -OHB infusion was determined by comparing control and infusion data using paired t-tests. The level of significance selected was  $p < 0.05$ .

### Studies of Glucose Production

Body temperature and arterial substrate concentrations in the fed sheep were similar to measurements obtained during the initial period of the longitudinal studies presented in last year's Annual Report. The responses to three days of fasting were similar to those noted on day 3 of the longitudinal fasting experiment previously described. When the infected animals studied on the third day of fast were compared to three-day fasted, non-infected controls there was a significant elevation in body temperature and blood lactate and pyruvate levels (Table I). With infection there was a significant decrease below fasting controls in concentrations of  $\beta$ -OHB, AcAc, and alanine. There was no difference in blood glucose concentrations between these two groups, but blood glucose in fasted and fasted-infected animals was significantly below levels observed in the fed sheep. There was no difference between the three groups in arterial hematocrit, pH, or partial pressure of oxygen or carbon dioxide.

In all animals, an isotopic plateau in glucose specific activity was achieved (for representative examples see Figure 1). The condition of the animal did not affect the ability to achieve isotopic steady state. Therefore, the use of the steady state Steele equation was justified. Glucose production in the fed animals was  $10.2 \pm 2.9$   $\mu\text{Mol/kg} \cdot \text{min}$  or approximately 3.3 g/hour in a 30-kg animal. With fasting, glucose production fell significantly to  $5.56 \pm 2.22$   $\mu\text{Mol/kg} \cdot \text{min}$ , levels which were 40%-50% below those observed during feeding. These rates of glucose production are comparable to previous reports of glucose kinetics in fed and fasted sheep. In contrast to the fasted control animals, glucose production in the fasted-infected sheep was elevated to  $9.5 \pm 1.11$   $\mu\text{Mol/kg} \cdot \text{min}$ , indistinguishable from rates observed in fed animals. In spite of the elevated glucose production rates in the infected sheep, blood glucose concentrations were comparable to those observed in fasted animals.

### Effect of Beta-Hydroxybutyrate Infusion

To determine the interrelationship between elevated blood ketone concentrations and the rate of glucose production,  $\beta$ -OHB was infused into animals from the three groups previously described. The initial arterial blood substrate concentrations (Table II) were similar in all three groups to levels previously reported (see last year's Annual Report).

With the infusion of  $\beta$ -OHB, blood concentrations of this ketoacid rose to near plateau levels in the third hour (Figure 2). In the fed and fasted-infected animals these concentrations were comparable to levels observed during fasting alone. In the fasted animals with elevated basal blood ketones,  $\beta$ -OHB concentrations rose above 2 mM/L.

In the fed animals,  $\beta$ -OHB infusion resulted in an elevation of blood AcAc, and a fall in levels of blood glucose and alanine (Table II). Lactate levels remained unchanged. With  $\beta$ -OHB infusion in fasting sheep, concentrations of arterial substrate were altered in a similar manner; AcAc increased, glucose and alanine decreased, and lactate did not change. However, similar responses were not observed in the fasted-infected animals. With  $\beta$ -OHB infusion, blood concentrations of  $\beta$ -OHB and AcAc rose to levels similar to those observed in the fed group (Figure 2, Table II). However, during  $\beta$ -OHB infusion there were no alterations in concentration of blood glucose, lactate or alanine.

With  $\beta$ -OHB infusion, glucose production was significantly reduced in the fed sheep from  $10.11 \pm 1.33$  to  $8.44 \pm 1.05$   $\mu\text{Mol/kg} \cdot \text{min}$  ( $p < 0.01$ ) and in the fasted animals from  $5.05 \pm 0.28$  to  $4.11 \pm 0.33$   $\mu\text{Mol/kg} \cdot \text{min}$  ( $p < 0.01$ ). In contrast, glucose production was not altered in the fasted-infected animals ( $9.50 \pm 1.83$  versus  $9.11 \pm 1.44$   $\mu\text{Mol/kg} \cdot \text{min}$ , NS by paired t-test). The reduction of glucose production in the fed and fasted sheep was not large (approximately 15%-20%), although inhibition occurred in all animals of these two groups.  $\beta$ -OHB infusion into the fasted-infected animals did not lower glucose production to levels observed in the fasted animals (approximately 5  $\mu\text{Mol/kg} \cdot \text{min}$ ), and, in fact, even a modest inhibitory effect of ketone infusion on glucose production was not observed. The direction of change of blood glucose concentrations following the  $\beta$ -OHB infusion correlated with the alterations in glucose production rate in these three groups of animals; concentrations and production fell in the fed and fasted groups and neither changed in the fasted-infected group.

Arterial concentrations of insulin were comparable in the fed and fasted-infected animals studied ( $11 \pm 2$   $\mu\text{U/ml}$  versus  $12 \pm 1$ ), and these concentrations did not change with  $\beta$ -OHB infusion ( $12 \pm 1$  and  $11 \pm 1$ ). Portal insulin concentrations were elevated above arterial levels in the two groups of animals ( $16 \pm 2$  and  $20 \pm 2$ ), and did not change significantly with  $\beta$ -OHB infusion ( $18 \pm 3$  and  $15 \pm 2$ ).



To determine if the effects observed were due to alterations in sodium load or an elevation in pH associated with the Na-DL- $\beta$ -OHB infusion,<sup>20</sup> similar studies were performed in two fed and two fasted-infected sheep, with sodium bicarbonate infused rather than  $\beta$ -OHB. Similar blood pH and pCO<sub>2</sub> alterations occurred with infusion (Table III), but no consistent alterations were observed in glucose turnover or arterial substrate concentrations (Table IV).

### DISCUSSION

Infected animals fasted for three days did not develop ketosis, as demonstrated by the short term studies. On the third day, however, glucose production rates were elevated above those observed in fasting controls; glucose turnover was comparable to that found in the fed animals. Because of the previous relationship between ketosis and rate of glucose production observed in the fed and fasted animals, we tested the hypothesis that the failure of glucose production to fall during fasting in the fasted-infected animals resulted from the failure of the ketogenic response. While  $\beta$ -OHB infusion reduced blood concentrations of glucose and alanine and diminished rates of glucose production in fed and fasted animals, these effects were not observed in fasted-infected animals. In other words, the development of ketosis with  $\beta$ -OHB infusion in the fasted-infected animals did not result in metabolic alterations which allowed these animals to be comparable to non-infected, fasted controls. That is, these metabolic responses to trauma/infection were not dependent on the hypoketonemic state.

Why should the infusion of exogenous  $\beta$ -OHB suppress the glucose production rate in fed and fasted sheep and fail to elicit this effect in infected animals? One explanation would be that infection interferes with ketone disposal or intracellular metabolism of this substrate. This appears not to be the case, for blood concentrations of  $\beta$ -OHB rose to similar levels in the fed and fasted-infected animals during comparable rates of  $\beta$ -OHB infusion. This suggests that the fasted-infected animals were clearing the  $\beta$ -OHB from the bloodstream at rates comparable to those observed in the fed animals.

One indication of  $\beta$ -OHB utilization is its conversion to AcAc. In this regard, the concentrations of AcAc were comparable in both the fed and fasted-infected animals during the  $\beta$ -OHB infusion. Thus, it appears there is no impairment in tissue uptake of the  $\beta$ -OHB or its conversion to AcAc in the fasted-infected animals.

Another explanation for the lack of  $\beta$ -OHB glucosuppressive effect in infected animals would be that  $\beta$ -OHB is ineffective as a substrate to diminish net skeletal muscle proteolysis or conserve glucose utilized in the central nervous system. In these studies, the concentrations of alanine, quantitatively the most important gluconeogenic amino acid precursor, decreased in the fed and fasted animals during ketone infusion, but did not change in the fasted-infected animals. Alterations in blood alanine concentrations may reflect an effect of  $\beta$ -OHB on skeletal muscle amino acid release, as suggested by Sherwin.<sup>20</sup> Studies in our laboratory support this hypothesis;  $\beta$ -OHB infusion decreased alanine and glutamine efflux from the hindquarter of fed sheep by approximately 50%, while exerting minimal effects on hindquarter nitrogen release from animals with front limb infection (Table V). In these same experiments, uptake of  $\beta$ -OHB across the hindquarter was comparable in both groups of animals. Because the mechanisms which accelerate net skeletal muscle proteolysis during infection are unknown, the exact role of  $\beta$ -OHB in the protein biochemistry in control and infected sheep remains to be elucidated. Moreover, the contribution of exogenous  $\beta$ -OHB as an alternate fuel for the central nervous system of the sheep is unknown.

Other investigators have reported that infusion of ketones stimulates insulin elaboration;<sup>20,21</sup> insulin serves as the signal for decreasing proteolysis and gluconeogenesis. Insulin release is often blunted by a variety of stresses,<sup>22</sup> and the lack of insulin elaboration to  $\beta$ -OHB infusion in the fasted-infected sheep would account for the observed results. However, arterial and portal venous insulin concentrations were similar in the basal state and did not increase in either group during the ketone infusion, suggesting that insulin did not play this regulatory role in these experiments. Changes in other glucoregulatory hormones such as glucagon and catecholamines may have been involved in eliciting the observed responses. However, changes in tissue responsiveness to hormones in stress states make the physiological significance of static concentrations of these hormones difficult to interpret.<sup>23</sup> In addition, the role of endogenous pyrogen on these responses is unknown.

We feel that the effects of  $\beta$ -OHB infusion could not be explained solely by the effects of this substrate on energy metabolism. The quantity of energy administered via the infused  $\beta$ -OHB is relatively small when compared to the overall energy demands of the animal, and probably accounts for less than 5% of the basal metabolic requirements. In another set of experiments we infused 1/60th the dose of  $\beta$ -OHB reported in this study into three fasted animals.  $\beta$ -OHB concentration rose minimally from  $0.79 \pm 0.04$  mM/L to  $0.95 \pm 0.06$ , and AcAc concentrations were  $0.13 \pm 0.01$  versus  $0.14 \pm 0.01$  post-infusion. However, in all three

animals, blood glucose concentration decreased from  $2.50 \pm 0.10$  to  $2.18 \pm 0.07$  mM/L, glucose production rate fell ( $6.33 \pm 0.17$  to  $5.35 \pm 0.27$   $\mu\text{Mol/kg} \cdot \text{min}$ ), and blood alanine was reduced ( $0.13 \pm 0.01$  to  $0.11 \pm 0.02$  mM/L). These alterations occurred in a quantitative way similar to those changes observed during the larger dose infusions reported in this study. These data suggest that  $\beta$ -OHB serves as an important regulatory signal in the fasting, non-infected state which either directly or indirectly influences glucose production.

However, the acute effect of the  $\beta$ -OHB infusion on glucose production was not major; in the fed and fasted animals, glucose production rates decreased only 15%-20%. Moreover, in the time frame of these infusion studies, glucose production in the fed animals was not reduced to the rate observed in the fasting animals, despite comparable blood concentrations of ketoacids. It may, therefore, not be unexpected that this moderate inhibitory effect of  $\beta$ -OHB on glucose production in fed or fasted animals fails to exert a demonstrable effect in infected sheep, for this stress state is characterized by major systemic stimuli which favor increased gluconeogenesis.<sup>1</sup> Similar competition between systemic stimulatory and local inhibitory regulation of glucose production has been demonstrated in an infected, small animal model,<sup>2,4</sup> with major systemic stimulatory effects dominating. In the small animal model, a sustained increase in glucose production was observed in vivo, but the hepatic gluconeogenic capacity of the liver was decreased in vitro. This suggests that minor inhibitory influences which affect glucose production in vitro are over-ridden by stronger in vivo factors such as hormonal stimuli. These same systemic influences may affect glucose regulation in our infected animals and, hence, over-ride the minimal inhibitory effect of ketone acids on glucose production. Whatever the mechanism of  $\beta$ -OHB induced glucose sparing in the fed and fasted states, it appears clear that the accelerated rate of gluconeogenesis is not a consequence of the hypoketonemic state associated with infection.

This concept is important, for the hypothesis has been advanced that the catabolism associated with the stress of infection and injury is a consequence of failure of ketoadaptation. It has been suggested that dietary manipulation<sup>5</sup> or the administration of exogenous ketoacids<sup>6</sup> (or substrates with ketogenic potential) be utilized to diminish these catabolic responses. However, the metabolic responses to injury and infection appear stimulated by hormones which favor gluconeogenesis and proteolysis.<sup>1</sup> This same hormonal environment most probably accounts

for the blunted ketogenic response observed in these stress states. When dietary carbohydrate is restricted, some stressed patients may develop hyperketonemia, but in control studies this metabolic event appears to have minimal impact on the catabolic response to injury. In addition, these sheep studies demonstrate that the exogenous administration of  $\beta$ -OHB has no impact on glucose production following infection. These data then do not support the hypothesis that the metabolic responses to stress are related to a failure to develop the hyperketonemic state. Further research should be directed toward an understanding of the mediators of these catabolic states so that these mediators may be altered in order to modulate catabolic responses.

B. EFFECT OF ENDORPHINE BLOCKADE ON THE POST-TRAUMATIC-SEPTIC SHEEP METABOLISM.

The endorphine system plays a major role in the hypotension associated with systemic injury and sepsis. The activity of this system on the metabolic response to injury/infection has not been determined. The purpose of this study was to utilize the injured-infected sheep model to evaluate alterations in metabolism following blockade of the endorphine system.

METHODS AND MATERIALS

Four conditioned sheep with chronic implanted arterial cannulas were studied. The initial investigation was carried out following overnight withdrawal of food. The animals had free access to water and a salt lick during this time. The animals were housed in small restraint pens in the laboratory. After an hour of acclimatization to the study area, three basal samples were drawn at 15-minute intervals for substrate determinations. Naloxone (2 mg I.V.) was then administered, and blood was taken for analysis at 15-minute intervals for the next one hour.

Several days later the animals were anesthetized and soft tissue infection of the hindlimb created as previously described. The animals were then fasted for three days and subsequently re-studied as outlined above.

Blood chemical analysis was carried out as previously described for determination of glucose, lactate, pyruvate,  $\beta$ -hydroxybutyrate, acetoacetate, alanine, and free fatty acids. Because of the small sample size, statistical evaluation was carried out by non-parametric analysis. An increase or decrease in substrate concentration was determined by analyzing all data points to see if a statistically significant slope in the plot of concentration versus time could be obtained.

## RESULTS

The fed sheep weighed 32.75 kg on the morning of study. Average rectal temperature was 102.8°F. Initial concentrations of blood substrates were similar to those concentrations previously observed in previously studied fed animals. With Naloxone administration, no alterations in concentrations of glucose,  $\beta$ -hydroxybutyrate, acetoacetate, alanine, pyruvate or free fatty acids were noted. The only significant change observed was a fall in lactate concentration that occurred with time (see Figure 3).

With fasting and infection the four animals lost weight to  $28.75 \pm 1.2$  kg. Body temperature was 104.8°F at the time of study, but remained stable throughout the study period. No fluctuations were noted after Naloxone administration. Differences between groups in blood substrate concentration were consistent with observations made in the previous study. The administration of Naloxone did not alter concentrations of glucose,  $\beta$ -hydroxybutyrate, acetoacetate, alanine, lactate, pyruvate, or free fatty acids (Table VI).

No clinical effect of Naloxone administration was observed in either group; no changes in vital signs or body temperature occurred during the study period.

## DISCUSSION

Naloxone administration does not appear to affect substrate metabolism in the injured-infected state when hypotension is not present. Representative examples of substrates representing metabolism of carbohydrate, lipid, and amino acids were monitored serially with time, and no change was observed in the fasted-infected animals with Naloxone administration. It should be realized, however, that these animals were not hypotensive and were not in shock. Metabolic changes such as correction of acidosis and reduction in lactic acid and pyruvate elevations may have occurred with restoration of perfusion had the animals demonstrated hypotension. Under the conditions of study (high flow sepsis) no changes associated with treatment occurred.

In contrast to the fasted-infected sheep, one major change was observed in the control animals. With the administration of Naloxone there was a fall of blood lactate from 0.8 mMol to 0.35 mMol. All four animals demonstrated this alteration.

In the fed animals, lactate is generated in the rumen and is maintained at elevated concentrations. Lactate falls to low levels during fasting when the ruminal contents diminish. One speculation is that Naloxone administration alters visceral bloodflow, and hence this would affect ruminal absorption and/or hepatic lactate extraction. However, ketone bodies are also absorbed from the rumen in the fed state, and no change was observed in their concentration following Naloxone administration. Therefore, the precise cause of this concentration change remains unknown.

Previous investigations have demonstrated that substrate turnover is closely correlated with concentration change, and hence when no alteration is observed in concentration one can state with reasonable assurance that turnover rate has not changed. We feel, then, that these studies fail to demonstrate a change in alteration of metabolism of the substrates studied; Naloxone does not appear to exert major metabolic regulatory effects in normotensive, injured/infected animals.

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TABLE I

CHARACTERISTICS, SUBSTRATE CONCENTRATIONS, AND GLUCOSE TURNOVER  
RATES IN FED, THREE-DAY FASTED, AND FASTED-INFECTED SHEEP  
DESCRIBED IN THE SHORT-TERM STUDIES  
(MEAN  $\pm$  S.E.)

|                                                      | <u>Fed</u>                    | <u>Fasted<br/>3-Days</u> | <u>Fasted<br/>Infected<br/>3-Days</u> |
|------------------------------------------------------|-------------------------------|--------------------------|---------------------------------------|
| N                                                    | 16                            | 15                       | 13                                    |
| Weight (kg)                                          | 30.9 $\pm$ 1.9                | 28.8 $\pm$ 1.1           | 29.0 $\pm$ 2.0                        |
| Rectal Temperature ( $^{\circ}$ C)                   | 39.7 $\pm$ 0.1 <sup>!</sup>   | 39.3 $\pm$ 0.1           | 40.7 $\pm$ 0.1 <sup>**</sup>          |
| Blood Glucose (mM/L)                                 | 3.28 $\pm$ 0.11 <sup>*!</sup> | 2.33 $\pm$ 0.06          | 2.44 $\pm$ 0.11                       |
| Lactate (mM/L)                                       | 0.60 $\pm$ 0.11 <sup>*</sup>  | 0.31 $\pm$ 0.03          | 0.85 $\pm$ 0.15 <sup>**</sup>         |
| Pyruvate (mM/L)                                      | 0.09 $\pm$ 0.01               | 0.06 $\pm$ 0.01          | 0.10 $\pm$ 0.01 <sup>**</sup>         |
| $\beta$ -OHB (mM/L)                                  | 0.31 $\pm$ 0.06 <sup>*</sup>  | 0.77 $\pm$ 0.06          | 0.44 $\pm$ 0.07 <sup>**</sup>         |
| AcAc (mM/L)                                          | 0.03 $\pm$ 0.01 <sup>*!</sup> | 0.10 $\pm$ 0.01          | 0.06 $\pm$ 0.01 <sup>**</sup>         |
| Alanine (mM/L)                                       | 0.17 $\pm$ 0.01 <sup>!</sup>  | 0.13 $\pm$ 0.01          | 0.08 $\pm$ 0.01 <sup>**</sup>         |
| Glucose Production<br>( $\mu$ Mol/kg $\cdot$ minute) | 10.20 $\pm$ 2.9 <sup>*</sup>  | 5.56 $\pm$ 2.22          | 9.50 $\pm$ 1.11 <sup>**</sup>         |

\* Different than fasted,  $p < 0.05$ .

\*\* Different than fasted,  $p < 0.05$ .

! Different than fasted-infected,  $p < 0.05$ .

(Differences determined by analysis of variance.)

TABLE II

THE EFFECTS OF  $\beta$ -OHB INFUSION ON ARTERIAL SUBSTRATE  
CONCENTRATION AND GLUCOSE TURNOVER  
(MEAN  $\pm$  S.E.)

|                                                      | Fed<br>(N=11)    |                  | Fasted 3 Days<br>(N=8) |                  | Fasted-Infected<br>(N=9) |                  |
|------------------------------------------------------|------------------|------------------|------------------------|------------------|--------------------------|------------------|
|                                                      | Before           | After            | Before                 | After            | Before                   | After            |
| Body Weight (kg)                                     | 26.4 $\pm$ 1.12  | -                | 27.9 $\pm$ 0.35        | -                | 26.7 $\pm$ 1.9           | -                |
| Rectal Temperature ( $^{\circ}$ C)                   | -                | 39.4 $\pm$ 0.1   | -                      | 38.8 $\pm$ 0.1   | -                        | 40.4 $\pm$ 0.2   |
| $\beta$ -OHB (mM/L)                                  | 0.46 $\pm$ 0.08  | 1.26 $\pm$ 0.13* | 0.81 $\pm$ 0.10        | 2.42 $\pm$ 0.19* | 0.46 $\pm$ 0.5           | 1.18 $\pm$ 0.11* |
| AcAc (mM/L)                                          | 0.04 $\pm$ 0.01  | 0.11 $\pm$ 0.02* | 0.11 $\pm$ 0.02        | 0.52 $\pm$ 0.03* | 0.05 $\pm$ 0.01          | 0.13 $\pm$ 0.01* |
| FFA ( $\mu$ Eq/L)                                    | 684 $\pm$ 93     | 381 $\pm$ 46*    | -                      | -                | 722 $\pm$ 86             | 469 $\pm$ 72*    |
| Blood Glucose (mM/L)                                 | 3.17 $\pm$ 0.17  | 2.89 $\pm$ 0.11* | 2.22 $\pm$ 0.06        | 1.94 $\pm$ 0.06* | 2.33 $\pm$ 0.17          | 2.44 $\pm$ 0.11  |
| Glucose Production<br>( $\mu$ Mol/kg $\cdot$ minute) | 10.11 $\pm$ 1.33 | 8.44 $\pm$ 1.05* | 5.05 $\pm$ 0.28        | 4.11 $\pm$ 0.33* | 9.50 $\pm$ 1.83          | 9.11 $\pm$ 1.44  |
| Lactate (mM/L)                                       | 0.69 $\pm$ 0.15  | 0.84 $\pm$ 0.15  | 0.34 $\pm$ 0.04        | 0.39 $\pm$ 0.08  | 0.89 $\pm$ 0.15          | 1.61 $\pm$ 0.62  |
| Alanine (mM/L)                                       | 0.18 $\pm$ 0.02  | 0.14 $\pm$ 0.01* | 0.13 $\pm$ 0.01        | 0.10 $\pm$ 0.01* | 0.08 $\pm$ 0.01          | 0.08 $\pm$ 0.01  |

\* Different from before infusion by paired t-test.  $p < 0.05$ .

TABLE III  
EFFECT OF  $\beta$ -OHB AND  $\text{NaHCO}_3$  INFUSION ON  
pH AND PARTIAL PRESSURE BLOOD GASES  
(MEAN  $\pm$ S.E.)

|                        | <u>Fed</u>      |                 | <u>Fasted-Infected</u> |                 |
|------------------------|-----------------|-----------------|------------------------|-----------------|
|                        | Before          | After           | Before                 | After           |
| pH                     |                 |                 |                        |                 |
| $\beta$ -OHB           | 7.50 $\pm$ 0.01 | 7.56 $\pm$ 0.01 | 7.52 $\pm$ 0.01        | 7.58 $\pm$ 0.01 |
| $\text{NaHCO}_3$       | 7.48            | 7.58            | 7.52                   | 7.61            |
| $\text{pO}_2$ (mm Hg)  |                 |                 |                        |                 |
| $\beta$ -OHB           | 86.2 $\pm$ 3.4  | 87.7 $\pm$ 3.7  | 83.8 $\pm$ 4.1         | 85.6 $\pm$ 2.6  |
| $\text{NaHCO}_3$       | 93              | 91              | 82                     | 80              |
| $\text{PCO}_2$ (mm Hg) |                 |                 |                        |                 |
| $\beta$ -OHB           | 35.0 $\pm$ 1.5  | 35.3 $\pm$ 2.3  | 34.0 $\pm$ 1.6         | 34.1 $\pm$ 1.0  |
| $\text{NaHCO}_3$       | 32.5            | 33              | 32.5                   | 35              |

TABLE IV  
EFFECT OF NaHCO<sub>3</sub> ON ARTERIAL SUBSTRATE  
AND GLUCOSE TURNOVER  
(MEAN)

|                                          | Fed<br>(N=2) |       | Fasted-Infected<br>(N=2) |       |
|------------------------------------------|--------------|-------|--------------------------|-------|
|                                          | Before       | After | Before                   | After |
| β-OHB (mM/L)                             | 0.23         | 0.28  | 0.39                     | 0.35  |
| Blood Glucose (mM/L)                     | 2.78         | 2.88  | 2.72                     | 2.50  |
| Glucose Production<br>(μMol/kg · minute) | 10.3         | 10.0  | 9.11                     | 9.89  |
| Alanine (mM/L)                           | 0.19         | 0.17  | 0.08                     | 0.06  |

TABLE V  
THE EFFECT OF KETONE INFUSION ON HINDLIMB RELEASE OF  
AMINO ACIDS IN FED AND INFECTED ANIMALS

|                        | Before Ketone Infusion                       |                        |                              | After Ketone Infusion                        |                        |                              |
|------------------------|----------------------------------------------|------------------------|------------------------------|----------------------------------------------|------------------------|------------------------------|
|                        | Arterial<br>Concentration<br>$\mu\text{M/L}$ | A-V<br>$\mu\text{M/L}$ | Bloodflow<br>$\text{ml/min}$ | Arterial<br>Concentration<br>$\mu\text{M/L}$ | A-V<br>$\mu\text{M/L}$ | Bloodflow<br>$\text{ml/min}$ |
| Fed Sheep <sup>*</sup> |                                              |                        |                              |                                              |                        |                              |
| Alanine                | 158±21 <sup>*</sup>                          | -34±6                  | 700±107 <sup>**</sup>        | 117±12                                       | -21±6                  | 719±69                       |
| Glutamine              | 265±45 <sup>**</sup>                         | -44±3                  |                              | 265±37                                       | -15±4                  |                              |
| Infected Sheep         |                                              |                        |                              |                                              |                        |                              |
| Alanine                | 65±11 <sup>*</sup>                           | -34±8                  | 904±98 <sup>**</sup>         | 61±16.4                                      | -28±5.2                | 901±119                      |
| Glutamine              | 202±25 <sup>*</sup>                          | -43±7                  |                              | 203±30                                       | -32±9                  |                              |

<sup>\*</sup> n=5

<sup>\*\*</sup> n=3

TABLE VI

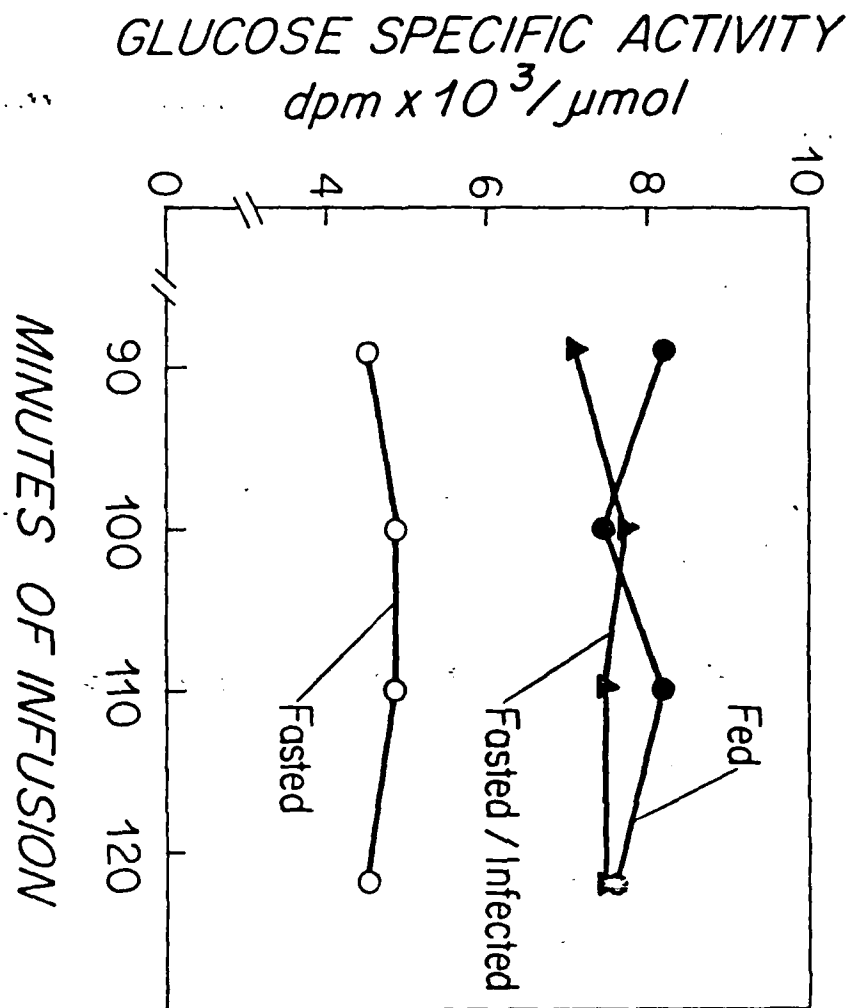
EFFECTS OF NALOXONE ADMINISTRATION (2 mg I.V.)  
ON BLOOD SUBSTRATE CONCENTRATIONS  
(MEAN  $\pm$  S.E.M.)

|                             | <u>"Normal" Substrate<br/>Concentration<br/>For Sheep</u> | <u>Initial<br/>Concentration</u> | <u>Final Concentration<br/>(1 Hr. Post-Admin.)</u> |
|-----------------------------|-----------------------------------------------------------|----------------------------------|----------------------------------------------------|
| <u>Control</u>              |                                                           |                                  |                                                    |
| Glucose<br>(mg/100 ml)      | 57 $\pm$ 3                                                | 45 $\pm$ 2                       | 46 $\pm$ 2                                         |
| $\beta$ -OHB (mM/L)         | 0.46 $\pm$ 0.08                                           | 0.42 $\pm$ 0.02                  | 0.43 $\pm$ 0.02                                    |
| Acetoacetate<br>(mM/L)      | 0.04 $\pm$ 0.01                                           | 0.02 $\pm$ 0.01                  | 0.03 $\pm$ 0.01                                    |
| Alanine (mM/L)              | 0.18 $\pm$ 0.02                                           | 0.20 $\pm$ 0.02                  | 0.19 $\pm$ 0.1                                     |
| Lactate (mM/L)              | 0.69 $\pm$ 0.15                                           | 0.80 $\pm$ 0.20                  | 0.35 $\pm$ 0.17                                    |
| Pyruvate (mM/L)             | ---                                                       | 0.07 $\pm$ 0.01                  | 0.08 $\pm$ 0.01                                    |
| Free Fatty Acids<br>(mEq/L) | 684 $\pm$ 93                                              | 553 $\pm$ 48                     | 656 $\pm$ 111                                      |
| <u>Infected</u>             |                                                           |                                  |                                                    |
| Glucose<br>(mg/100 ml)      | 40 $\pm$ 1                                                | 42 $\pm$ 4                       | 38 $\pm$ 3                                         |
| $\beta$ -OHB (mM/L)         | 0.46 $\pm$ 0.05                                           | 0.37 $\pm$ 0.14                  | 0.38 $\pm$ 0.15                                    |
| Acetoacetate<br>(mM/L)      | 0.05 $\pm$ 0.01                                           | 0.03 $\pm$ 0.01                  | 0.02 $\pm$ 0.01                                    |
| Alanine (mM/L)              | 0.08 $\pm$ 0.01                                           | 0.08 $\pm$ 0.01                  | 0.07 $\pm$ 0.01                                    |
| Lactate (mM/L)              | 0.89 $\pm$ 0.15                                           | 1.38 $\pm$ 0.41                  | 1.25 $\pm$ 0.58                                    |
| Pyruvate (mM/L)             | ---                                                       | 0.11 $\pm$ 0.02                  | 0.10 $\pm$ 0.03                                    |
| Free Fatty Acids<br>(mEq/L) | 722 $\pm$ 86                                              | 645 $\pm$ 84                     | 585 $\pm$ 24                                       |

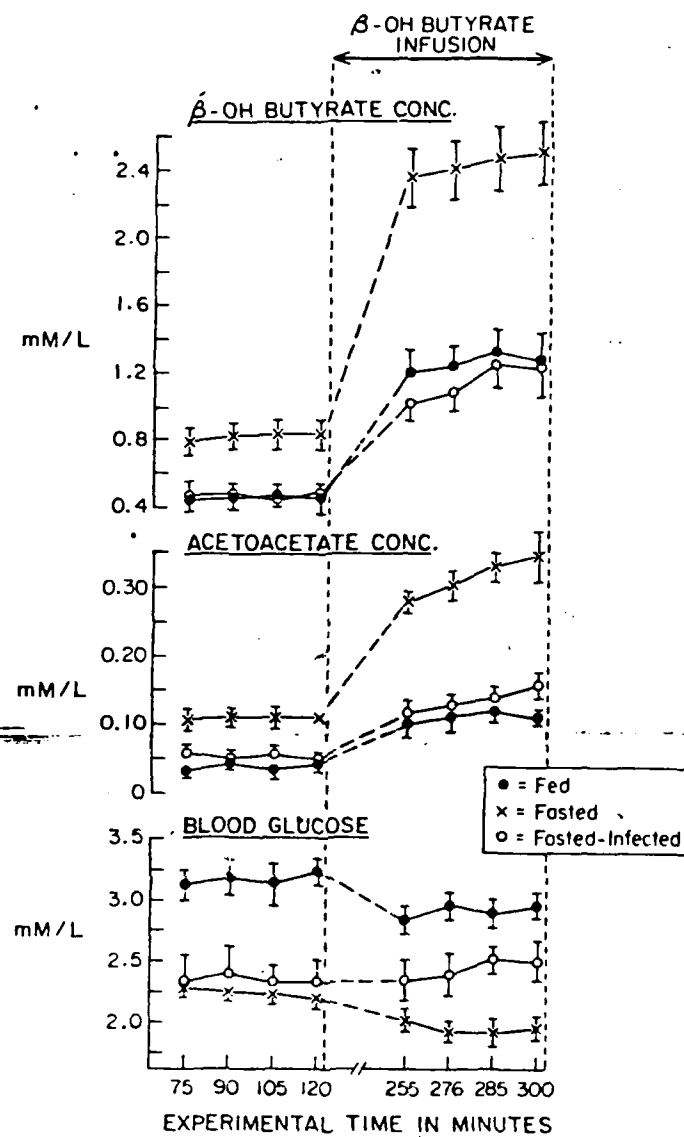


- Figure 1: Representative examples of glucose specific activity in fasted (0-0), fed (●-●), and fasted and infected sheep (Δ-Δ). The isotope infusion rates in the three experiments were 46,065, 47,864, and 39,130 dpm/kg · min, respectively.
- Figure 2: The effect of  $\beta$ -OHB infusion on substrate concentrations in fed, fasted, and fasted-infected sheep.
- Figure 3: Substrate concentration before and after Naloxone administration (mean  $\pm$  SEM). Naloxone was administered at time 0.

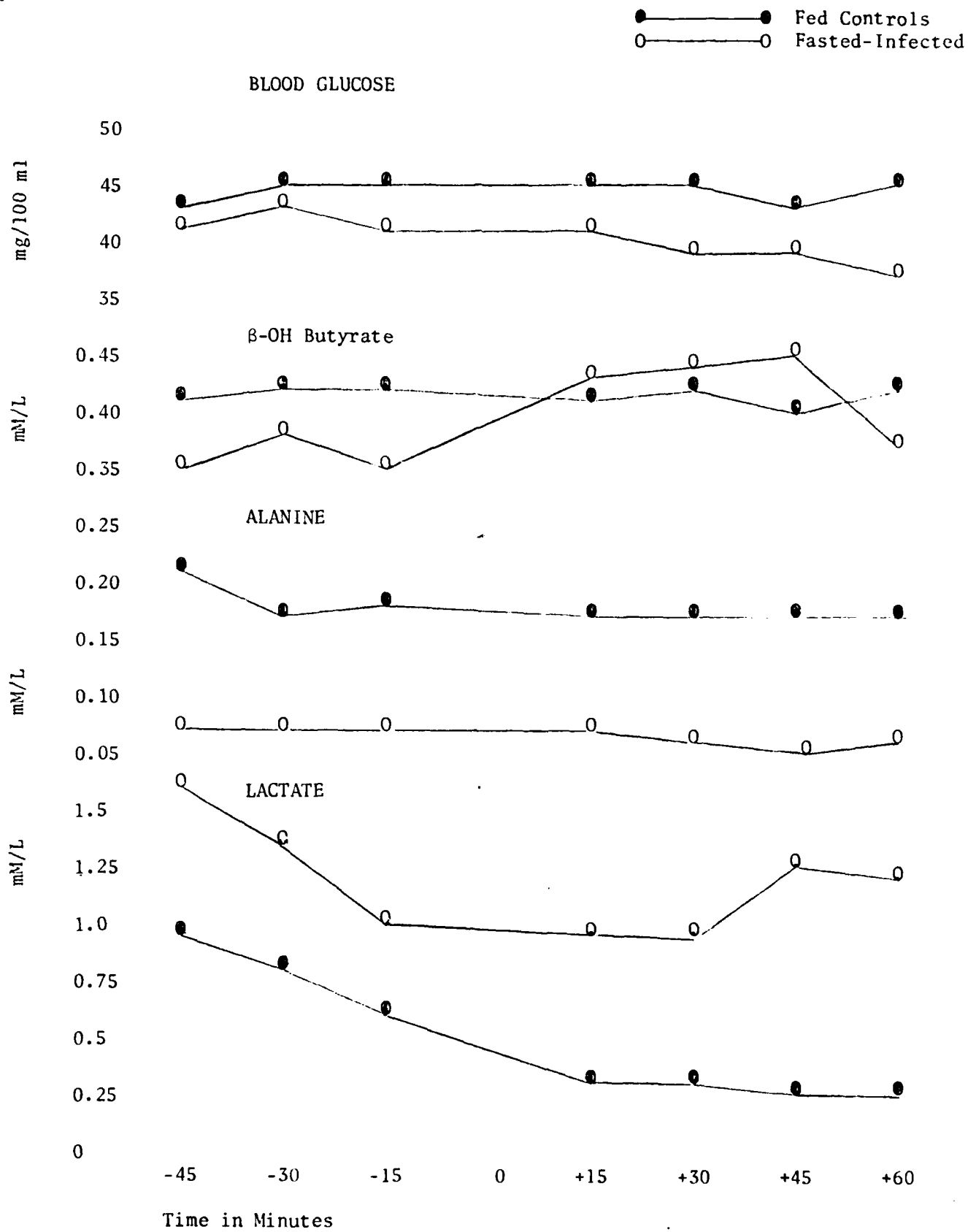
Figure 1



-30-  
Figure 2



-31-  
Figure 3



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