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THE UNIVERSITY OF ALBERTA

AMINO ACID METABOLISM DURING ENDOTOXIN INDUCED FEVER IN SHEEP

BY

BONITA GAYE SOUTHORN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

Animal Biochemistry

Department of Animal Science

EDMONTON, ALBERTA

FALL, 1986

ISBN 0-315-32310-8

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Bonita Gaye Southorn

TITLE OF THESIS AMINO ACID METABOLISM DURING ENDOTOXIN INDUCED FEVER IN SHEEP

DEGREE FOR WHICH THESIS WAS PRESENTED MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED FALL, 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled AMINO ACID METABOLISM DURING ENDOTOXIN INDUCED FEVER IN SHEEP submitted by Bonita Gaye Southorn in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in Animal Biochemistry.

J.R. Thompson
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Date August 28, 1978

ABSTRACT

Experiments were conducted to determine the effect of endotoxin induced fever on amino acid metabolism in sheep. In the first experiment, seven adult wethers were each subjected to two treatments: saline and endotoxin (*E. coli* serotype 055:B5) injection. Endotoxin injection caused a fever lasting from 0.75 to 6.25 h postinjection with a maximum temperature rise of 2.3°C. The plasma concentration of most individual plasma amino acids and glucose decreased ($P<0.05$) during the fever or shortly thereafter. Plasma serine concentration showed the most dramatic change, dropping to 14% of preinjection values during fever. The concentration of some amino acids such as, glutamate, serine, and histidine were still depressed ($P<0.05$) 24 h after fever had abated. Plasma glucose concentration in the endotoxin treated sheep remained depressed ($P<0.05$) below the concentration in control animals at 55 h postinjection. Plasma insulin concentration was elevated ($P<0.05$) in the endotoxin treated sheep from 4.5 to 13.0 h postinjection. These changes in plasma concentrations indicated that there was a response to fever in glucose and amino acid metabolism in sheep during and following endotoxin-induced fever, but the results were inconclusive as to the actual mechanisms involved.

The second experiment was conducted to determine the

alterations in hepatic metabolism of serine and alanine which could have led to the changes in their plasma concentrations observed in the first experiment. Six adult wethers were used in this experiment with the same treatment protocol as in experiment one. Liver biopsies were obtained at six hours postinjection and incubated with U-^{14}C-alanine or U-^{14}C-serine. The incorporation of carbon from ^{14}C-alanine into protein increased ($P<0.05$) by 72% and into glycogen increased by 325% following endotoxin treatment. The incorporation of carbon from ^{14}C-serine into CO_2 increased ($P<0.05$) by 62%, into protein increased by 73%, and into glycogen increased by 275% in response to endotoxin injection.

The results of these studies indicate that sheep alter amino acid metabolism during fever induced by endotoxin and that these changes may not be completely analogous to those observed in monogastric species under similar circumstances.

ACKNOWLEDGEMENTS

The experiments described herein were supported by the Agricultural Research Council of Alberta and The Natural Sciences and Engineering Research Council of Canada.

I wish to thank the staff at the Metabolic Unit, University of Alberta farm for their valuable assistance in animal care and their advice. The laboratory assistance of Mr. Gary Sedgwick and Mrs. Margret Drury is gratefully acknowledged. The surgical assistance of Dr. Richard Early, John Kelly and Alicia Strelkov is much appreciated.

Scholarship support from the Province of Alberta during a portion of these studies is gratefully acknowledged.

I also wish to thank my fellow graduate students for their constant support and advice. A special thank you is extended to my supervisor, Dr. J.R. Thompson for his understanding, support and advice throughout the course of my studies.

Finally, I wish to thank my family for their support of all my endeavours.

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I. INTRODUCTION

Infectious disease and the associated fever have potentially deterimental effects on protein deposition in livestock. However, few studies have been conducted to determine the effects of fever and infection on the metabolism of agricultural animals. In humans and rodents, fever and infection are accompanied by loss of body protein (Beisel, 1966; Powanda et al., 1972). Following clinical recovery from disease, recovery of lost body protein is not immediate and may require several weeks.

A substantial cost of disease to livestock producers is the increased time required to raise animals to market weight due to a slower rate of growth. This portion of economic loss due to disease is usually not taken into account when estimates of the costs of animal disease to the livestock industry are calculated. Most such estimates include only calculations of the cost of animal disease based on the worth of animals which died due to disease and the cost of veterinary supplies and labour. A few authors have accounted for the costs of slower growth rates due to disease, but the estimates are not very accurate and the methods used are not well explained (eg. Muller and Abbott, 1986). The reason for this ommission is that there have been no experimental studies on the extent of reduced efficiency of livestock production due to disease of surviving animals. In the past, a few reports have

presented theoretical estimates of the metabolic cost of disease. For example, Loew (1974) used a value for increased metabolic rate in humans due to malaria and applied this to cattle based on an estimate of the number of febrile days in cattle in Canada per year. Through the use of estimates of digestible energy requirements of cattle and the cost of grain to supply the excess energy required for maintenance of fever, he determined the theoretical cost of fever to cattle in Canada. The necessity of using these general assumptions and extrapolations to make an estimate of the cost of disease illustrates the need for more in depth studies into the consequences of fever on the growth of livestock species.

A. What is fever?

Fever has classically been described as an elevation of an animal's hypothalamic temperature set point (Dinarello and Wolff, 1982). This means that through behavioural and/or metabolic alterations, the animal will thermoregulate about a higher body temperature than normal. Fever may be produced in the animal by many stimuli, the most common being the invasion of all or part of the body by microorganisms, a condition referred to as infection. A serious infection involving the whole body, which is often fatal, is called sepsis. Injection of a variety of chemicals and some bacterial products will also cause fever to develop. Whether or not the development of

fever in response to infection is beneficial to the animal is still the subject of debate. The adaptive value to the animal of developing fever is dependent on the length and severity of the illness and the nutritional state of the animal, as well as other factors (Dinarello and Wolff, 1982).

When a fever causing stimulus enters the body, the mononuclear phagocytes become activated by phagocytosis of the foreign antigen. Once activated, the cells synthesize a 15000 dalton protein called interleukin-1. Interleukin-1 is released into the blood and mediates a large number of host responses including increased leukocyte number, decreased plasma iron and zinc concentrations, increased muscle protein degradation and increased body temperature. The effect of interleukin-1 on body temperature is dependent on prostaglandin formation in the hypothalamus (Dinarello and Wolff, 1982). A 4000 dalton fragment of interleukin-1 which is thought to be a product of its metabolism will also stimulate muscle protein degradation (Loda et al., 1984). A summary of the metabolic events mediated by interleukin-1 which follow infection are shown in Figure I.1.

B. Endotoxins

Endotoxins are microbial products which will induce fever when injected into animals. Endotoxin injection into animals is used as an experimental model of infection or

sepsis because of the short-term response it provides which is not complicated by other factors that may be present during infection. The effects of endotoxin injection are acute when compared with infection. A fever which develops following infection with live organisms may last days or weeks. In contrast, endotoxin injection will produce a fever which lasts only a few hours.

Gram negative bacteria differ from gram positive bacteria in the structure of the cell wall. The gram positive cell wall is only a single thick layer of peptidoglycan whereas the gram negative cell wall has a thin layer of peptidoglycan which is covered with an outer membrane. The outer membrane is semi-permeable and can be removed from the cells by extraction with phenol/water (Luderitz et al., 1982). The lipopolysaccharide from the outer membrane which is removed by this procedure is the endotoxin.

It is difficult to use endotoxin to mimic the fever of infection because the response is short in duration and repeated doses cause the development of tolerance (Cakala, 1965a; van Miert and Frens, 1968; van Miert, 1973; Greisman et al., 1969), however endotoxin injection will elicit most of the acute phase responses associated with infection. Repeated injections of endotoxin within a day cause progressively shorter and smaller fever curves in the sheep. Cakala (1965a) has shown that three injections within twelve hours will cause complete attenuation of the

febrile response. Similarily, daily injections of endotoxin into goats will cause complete absence of the febrile reaction within three days (van Miert and Frens, 1968). Neither of these authors, however, examined the length of time that the observed tolerance was sustained.

Tolerance to the effects of endotoxin appears to exist in two phases (Greisman et al., 1969). A single injection of endotoxin into rabbits will cause a refractory period lasting one day. On the second day after injection the endotoxin tolerance is nonapparent. From the third day on, however tolerance to a single injection of endotoxin reappears. The persistence of this second phase of tolerance is not known. The tolerance during the first day is not specific to the species of origin of endotoxin and it is not associated with a high antibody titer. The later tolerance which is developed is very species-specific and is associated with the development of an antibody titer specific for the endotoxin used.

C. Infection and Nitrogen Balance

One of the most simply measured parameters of protein metabolism in the whole animal is nitrogen balance. In the normal growing animal nitrogen balance is positive, ie. intake is greater than excretion. In the healthy adult, nitrogen balance is approximately zero. Negative nitrogen balance has been shown to occur in adult human subjects following the appearance of clinical symptoms of infection

(Beisel, 1966). The daily balance returns to normal at the time of or shortly after the remission of fever. The total nitrogen loss is not recovered however, until long into convalescence (Beisel, 1966; Beisel et al., 1967) and reachs a maximum only after clinical symptoms are no longer apparent. The length of time required for the complete recovery of lost body nitrogen depends on many factors such as, the type of infectious agent and the length of illness (Beisel, 1966).

The negative nitrogen balance observed in infected humans is at least partially due to reduced dietary protein intake resulting from anorexia. Beisel (1966), in a pair-feeding trial of healthy men and subjects with experimentally induced tularemia, showed that the negative nitrogen balance in response to reduced feed intake was of smaller magnitude and shorter duration than that induced by tularemia. In these balance studies, total fecal nitrogen excretion was not reduced. In a similar study, Beisel et al. (1967) interpreted this finding to mean that there was no impairment of intestinal absorption during infection. However, if this were the case, there should have been a lower total fecal nitrogen excretion, as less nitrogen was being ingested. Similarily, Powanda et al. (1972) observed a significant increase in nitrogen excretion in septic rats when compared to their growing pair-fed controls. The nitrogen balance however failed to become negative in these animals. The supply of exogenous substrates for the body

must be an important component of negative nitrogen balance as parenteral feeding will reverse it in septic patients (Leverve et al., 1984).

Negative nitrogen balance may be due to a direct effect of the higher body temperature associated with fever; a hypothesis which is supported by the coincident timing of the onset of the negative nitrogen balance and fever. Beisel et al. (1968) subjected healthy volunteers to a period of environmentally induced hyperthermia which mimicked the rectal temperature response during acute tularemia. The pattern of cummulative nitrogen loss was similar in shape to that of the diseased state, but of smaller magnitude and shorter duration.

These observations suggest that a major portion of the loss of body nitrogen measured during infection is due to a combination of reduced voluntary feed intake and high body temperature. These two components are not mutually exclusive because higher temperature alone tends to cause anorexia (Beisel et al., 1968). Other factors, such as, glucocorticoid levels and antibiotics have been investigated to determine their roles in the development of negative nitrogen balance during fever. Administration of glucocorticoids to mimic the plasma concentration pattern in people with tularemia, to healthy adult humans failed to cause negative nitrogen balance (Beisel, 1966). Similarily, antibiotic therapy did not alter the nature of negative nitrogen balance observed during tularemia or Q

fever in human subjects (Beisel, 1966). It is possible that changes in the concentrations of other hormones such as, growth hormone and thyroid hormones may be partially responsible for negative nitrogen balance during infection, however, hormone concentrations may be also directly affected by anorexia and hyperthermia.

Some short term models of infection do not cause negative nitrogen balance. Vaccination of adult human subjects to typhoid-cholera did not cause increased nitrogen excretion in the study of Garlick et al. (1980) in subjects which were fed normally to preclude the anorectic response to infection. Infusion of interleukin-1 into fasted rats for 24 h also failed to increase urinary nitrogen excretion (Yang et al., 1983). Thus, it appears that short term fever or mild infection does not cause negative nitrogen balance regardless of the immediate nutritional status. Even though short-term infection models do not cause negative nitrogen balance, they do elicit more specific changes in nitrogen metabolism which are associated with infection.

D. Infection and Protein Turnover

The interpretation of whole body protein turnover measurements is plagued by the same problems as nitrogen balance measurements. The measurement of total protein synthesis and degradation in the body does not indicate which organs are involved, as the total rates observed are

a composite of the rates of these two processes in each organ. Infection generally causes increased whole body protein turnover. Increased protein synthesis and degradation have been shown to occur during short term infection following vaccination (Garlick et al., 1980) as well as during sepsis (Long et al., 1977). Whole body protein synthesis and degradation have also been shown to be increased following 4 h of endotoxin or interleukin-1 infusion in the guinea pig (Sobrado et al., 1983). These effects were not altered by the administration of ibuprofen, an inhibitor of prostaglandin synthesis. In contrast, Yang et al. (1983) reported no change in whole body protein synthesis in rats during a 24 h infusion of interleukin-1. The reason for this discrepancy could be a species difference or a difference in the time of exposure of the animal to the treatment. The fact that different labelled amino acids were used to measure protein turnover is also an important difference between these studies because there may be differential turnover rates of individual proteins containing substantial amounts of one of these amino acids.

Skeletal muscle is a likely site for the increase in protein degradation observed during fever as it contains the largest pool of protein in the body which is reasonably dispensable in the short-term. Lust (1966) examined net protein synthesis in a variety of tissues during both bacterial and viral infections in mice. The liver and

small intestine showed increased protein synthesis during bacterial infection and an initial decline followed by an increase during viral infection. More reliable methods have been developed since the time this study was conducted. For example, Fern et al. (1985) have also found decreased muscle protein synthesis and increased liver protein synthesis during malaria in rats. Although urinary N^{τ}methylhistidine excretion has been shown to be a poor quantitative indicator of skeletal muscle protein degradation, it is still a useful qualitative indicator. Long et al. (1981) found that N^{τ}methylhistidine excretion increased in septic humans indicating that myofibrillar protein degradation was increased in these individuals. Net muscle protein degradation has also been demonstrated to increase in incubated muscle from septic humans (Rosenblatt et al., 1983). Pedersen et al. (1986) measured protein metabolism in incubated liver slices from septic rats and reported increased rate of synthesis with no change in the rate of degradation.

Interleukin-1 has been shown to enhance the rate of protein degradation in muscle, in addition to its effect on body temperature (Baracos et al., 1983). The effect appears to be prostaglandin E_2 mediated. This observation does not fully agree with the results of Sobrado et al. (1983) regarding whole body protein degradation. Possibly the dose of ibuprofen administered to the animals in the study of Sobrado et al. (1983) may

not have been sufficient to affect muscle prostaglandin production and hence, the effect of interleukin-1 on muscle protein degradation would not be prevented in these animals.

E. Amino Acid Metabolism During Infection

There are alterations in the metabolism of individual amino acids during infection. Total plasma amino acid concentration is depressed during infection in humans (Clowes et al., 1980). The alterations in the levels of individual amino acids are somewhat different from those observed to arise directly due to fasting, and therefore, may be a direct consequence of the fever response. The plasma concentration of any individual amino acid is the net result of its rates of entry and exit from body tissues. The fate of the amino acids in the tissues varies with the type of tissue. Amino acids may be oxidized to carbon dioxide and ammonia, incorporated into tissue protein, converted to glucose or fatty acids, or converted to specialized nitrogen containing compounds such as, creatine and creatinine. Studies on amino acid metabolism of humans and animals in response to fever and infection have taken three forms: measurements of plasma and muscle concentrations (Askanazi et al., 1980); arterial-venous concentration difference measurements across various organs (Clowes et al., 1980; Lindberg and Clowes, 1981); and whole body turnover kinetics and oxidation of individual amino

acids (Long et al., 1976).

Most studies dealing with the response of amino acid metabolism to fever have dealt with septic or experimentally infected human patients. The plasma concentrations of many amino acids decrease during infection or sepsis. The branched chain amino acids, in particular leucine, have been implicated to have regulatory effects on protein metabolism in in vitro studies (Tischler et al., 1982). Thus, changes in the plasma levels of these amino acids may influence protein metabolism in the tissues of diseased animals. The results of studies vary, but in general the plasma concentrations of the branched chain amino acids are decreased (Wannemacher et al., 1972a; Wannemacher et al., 1972b; Wannemacher et al., 1971). Askanazi et al. (1980) however, found no change in plasma levels of branched chain amino acids in septic patients, although the free concentrations in skeletal muscle were increased. Similarily, Rosenblatt et al. (1983) found no difference in plasma branched chain amino acid concentrations between septic and fasted normal subjects.

The concentrations of non-branched chain amino acids have also been demonstrated to change during sepsis, but the results are not consistent between studies. Rosenblatt et al. (1983) only found increases in plasma concentrations of phenylalanine, methionine and glycine; while Wannemacher et al. (1971) found decreased concentrations of alanine, glutamine, threonine, and serine, among others. A study by

Wannemacher et al. (1972b) showed decreased plasma concentrations of all individual amino acids except lysine, histidine, and arginine within two days of exposure to a viral infection. By four days post-exposure however, the concentration of phenylalanine was significantly higher. The discrepancies between these studies may be due to the differences in the type and severity of infections studied and the type of subject used.

The plasma amino acid concentration response to endotoxin or interleukin-1 infusion is somewhat different than the response to sepsis. Sobrado et al. (1983) found no change in the plasma concentration of most individual amino acids except for increased tyrosine and phenylalanine and decreased aspartate concentrations in the guinea pig.

Amino acids are transfered from the peripheral tissues, such as skeletal muscle, to the liver during infection. Lindberg and Clowes (1981) measured hepatic uptake of amino acids in septic pigs and found increased uptake of all the individual amino acids except cysteine and hydroxyproline. The liver release of glutamate was significantly reduced during sepsis. The opposite pattern was apparent for arteriovenous concentration differences across peripheral tissues. Rosenblatt et al. (1983) determined that peripheral release rates of amino acids were increased to more than three times normal, while visceral fractional uptake rates were increased more than five times in septic patients.

Oxidative metabolism of individual amino acids has also been studied during sepsis. Long et al. (1976) observed no difference in whole body alanine oxidation between septic and normal patients. Sobrado et al. (1983) demonstrated that whole body leucine oxidation increased during endotoxin infusion. These effects were not changed by concommitant infusion of the prostaglandin synthesis inhibitor, ibuprofen. Oxidation of tyrosine and leucine has been shown not to differ in liver slices from septic and fasted rats by Rosenblatt et al. (1983).

F. Infection-Induced Changes In Insulin and Glucose Metabolism

Even though fasting would lower the circulating concentrations of glucose and amino acids, plasma insulin concentration is increased during peritonitis in fasted rats in comparison with fasted control rats (Ryan et al., 1974). Since insulin decreases muscle protein degradation in vitro (Jefferson et al., 1974), this rise in insulin concentration may have a regulatory effect on the extent of muscle wastage during disease. However, there is some evidence that muscle tissue is insensitive to insulin during infection (Ryan et al., 1974). Rat diaphragm preparations show no change in glucose oxidation or glycogen production during peritonitis, whereas, oxidation of glucose in adipose tissue from these animals was increased (Ryan et al., 1974). Whole body glucose

production from alanine is increased in septic patients even during glucose infusion (Long et al, 1978; Long et al., 1976). The subjects used in the study of Long et al. (1978) also showed increased blood insulin concentration.

G. Effects of Endotoxin in Ruminant Species

Very little work has been done on the response of ruminant species to endotoxin injection or experimental infection. Ruminant animals may exhibit different reactions to infection or endotoxin injection from those observed in monogastric species for three reasons. The large storage capacity of the rumen may ameliorate the effects of anorexia and reduced feed intake on metabolism. Since the ruminant does not normally absorb much glucose from the digestive tract, the relative importance of gluconeogenesis and the use of amino acids for this purpose may be increased during infection when compared to monogastric animals. There may also be differences between ruminant and monogastric animals in the metabolism of amino acids. Wijayasinghe et al. (1983) showed that there were apparent differences in leucine transamination and oxidation in response to fasting in liver, muscle, kidney, and adipose tissue in sheep compared with previous data from rats and humans. The studies presented in this thesis involved the use of sheep as the experimental animal because they are small ruminants which are easily handled and the literature is incomplete with respect to possible

unique responses of them to endotoxin and infection. Clinically, endotoxin injection into ruminants causes similar symptoms to those found in rats and humans such as; anorexia (Baile et al., 1981), depression (van Miert, 1973; Naylor et al., 1984), coughing and shivering (van Miert, 1973). Leukocyte number initially decreases after endotoxin (Cakala, 1965b; van Miert, 1973), and later becomes elevated (van Miert et al., 1983). Serum sodium concentration increases and serum potassium and inorganic phosphorus concentrations decrease following endotoxin injection (Cakala, 1965b).

The results of studies on plasma glucose concentrations following endotoxin injection in ruminants have been inconclusive. Allen et al. (1970) observed a small nonsignificant and transient increase in plasma glucose concentration during endotoxin induced fever in calves while Reece and Wahlstom (1973) reported an initial increase in plasma glucose concentration followed by a decrease in young calves injected with endotoxin. Endotoxin injection into sheep has also been shown to cause an initial increase in plasma glucose concentration, followed by a decrease (Cakala, 1965b; Yagi and Nakajima, 1983). Naylor et al. (1984) however, found that the concentration of glucose in plasma decreased following endotoxin administration without an initial increase.

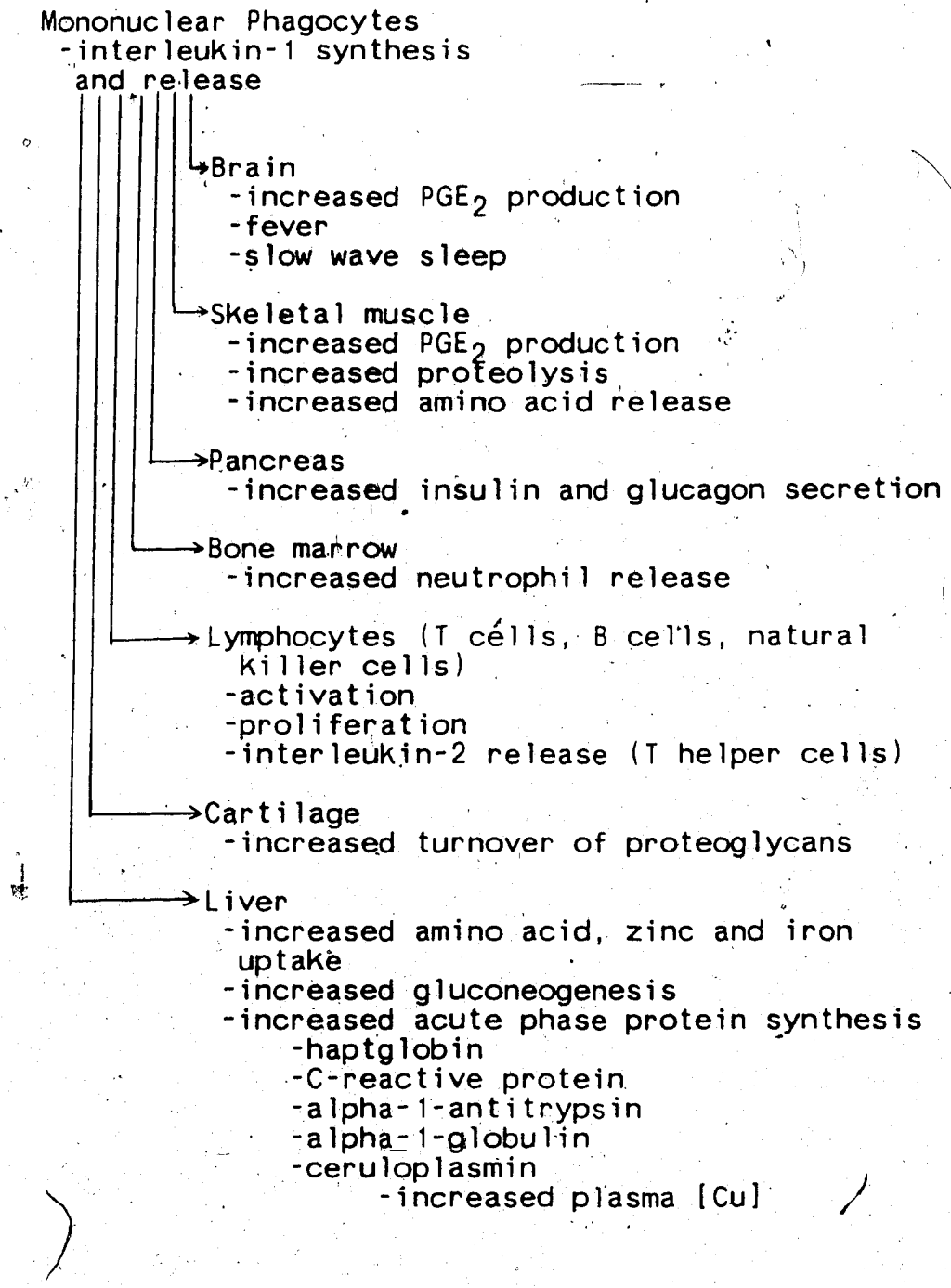
There are no reports in the literature dealing with amino acid metabolism in ruminants with respect to

infection and fever. The few studies conducted on the response of ruminants to fever have examined glucose concentration and gut motility. Since the consequences of the amino acid response could be important for animal growth and meat production as well as affecting results in other types of studies, the following experiments were conducted to elucidate the nature of the response in sheep. The objectives of the studies were:

1. to characterize a generalized response of sheep to endotoxin fever through measurement of the time course changes in plasma amino acid, glucose and insulin concentrations;
2. to determine whether endotoxin altered the fluxes of amino acids across various tissues;
3. to characterize the pattern of use of specific amino acids in hepatic tissue during endotoxin induced fever.

Difficulty was encountered in acheiving the second objective, but methodology and preliminary results are presented in Appendix 1.

Figure 1.1. Metabolic responses to infection mediated by interleukin-1.



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II. TIME COURSE CHANGES IN BLOOD METABOLITES DURING ENDOTOXIN FEVER IN SHEEP

A. Introduction

Rats, mice and humans respond to bacterial infection or endotoxin challenge by altering nitrogen metabolism. The changes observed include: alterations in individual plasma amino acid concentrations (Sobrado et al., 1983; Wannemacher, 1977), increased urinary nitrogen excretion (Beisel, 1966), and increased whole body protein turnover (Long et al., 1977a; Garlick et al., 1980). Increased skeletal muscle protein degradation has been observed in vitro in response to interleukin-1 (leukocytic pyrogen) (Baracos et al., 1983) and has been implied from in vivo studies of sepsis in rats (Ruff and Secrist, 1984), and from the measurement of urinary N^{τ}-methylhistidine excretion in infected humans (Long et al., 1977b). There appears to be a paucity of information in the literature regarding the response of ruminants to fever with respect to nitrogen metabolism. If the ruminant species commonly used for meat production exhibit this same type of catabolic nitrogen loss during fever, an understanding of this response would have important implications for the evaluation of the cost of diseases as well as recommendations for animal treatment to minimize

1. A version of this chapter has been accepted for publication: Southorn BG and Thompson JR. 1986. The Canadian Journal of Veterinary Research. in press

productivity losses during febrile periods.

During infection or sepsis, a chronic type of fever often develops and may last several days or weeks. Acute fevers also occur. These may be due to very mild illness, self-limiting viral disease, vaccination, or experimental endotoxin challenge. Acute fever models, such as that which is produced by endotoxin injection, provide repeatable, safe methods of studying the metabolic effects of endotoxin-induced fever. Since there is essentially no literature regarding the response of ruminant nitrogen metabolism to fever, the following study was conducted to characterize the time course changes in the concentrations of plasma amino acids and other metabolites, during and after acute endotoxin-induced fever in sheep.

B. Materials and Methods

Seven mature wethers, averaging 60.1 kg body weight, were individually housed in metabolism crates at an ambient temperature of 15-20°C with continuous lighting. Two of the animals had each been previously prepared with a rumen fistula and an externalized carotid artery. The animals were fed 2000-2500 g pelleted bromegrass hay containing 12 % crude protein, daily at 0800 h. Water and cobalt-iodized salt were available ad libitum.

Two trials were performed with each animal. During the first trial an acute febrile episode was induced in three wethers by a single intravenous injection of E. coli,

(serotype 055:B5) endotoxin (Sigma Chemical Company, St. Louis MO), while four wethers were injected with physiological saline and monitored as control animals. During the second trial seven days later, the treatments were reversed. The endotoxin solution was prepared prior to each trial, by dissolving endotoxin in sterile 0.9% NaCl to a concentration of approximately 0.20 mg/L. An endotoxin dose of 3 ug/kg BW was used, as preliminary trials indicated that a reproducible fever lasting approximately 6 h with a maximum temperature rise of 2.3°C could be obtained with such a dose. Rectal temperature was measured at 15 min intervals during the pre-injection period and during the first 8 h of each trial using a telethermometer probe inserted 12 cm into the rectum, (Yellow Springs Instuments Co., Yellow Springs OH).

A catheter was placed in a jugular vein one day prior to each trial for solution injection and blood sampling. The animals were fed routinely at 0800 h on the day of each trial. Feed consumption was monitored by weighing the residual feed and returning it to the animals every 2 h for 12 h post-feeding. Blood sampling (10 mL) commenced at 0900 h. Three pre-injection blood samples were obtained, at hourly intervals, the last one immediately prior to endotoxin or saline injection at 1100 h. After injection, blood samples were collected at 1.5,3,4.5,6,7.5,9,11, 13,19,31, and 55 h from both saline and endotoxin treated animals. An additional 12 blood samples were collected from

the endotoxin treated animals between 0.5 and 12 h post-injection, but were not used for analysis presented in this report.

A hematocrit value was determined on each blood sample using a micro hematocrit centrifuge. Plasma was separated from whole blood by centrifugation at 1000 g for 15 min. Plasma samples were divided into three equal aliquots and stored at -20°C until analysis.

Proteins were separated from plasma by the addition of acetonitrile according to the method of Jones and Gilligan (1983). Amino acids in the remaining plasma fraction were derivatized with o-phthaldialdehyde, prior to separation by high performance liquid chromatography using a Varian 5000 HPLC equipped with a Fluorochrom detector. Peak area integrations were performed by a Hewlett-Packard 2625A computer. It was not possible to separate threonine from citrulline and arginine from carnosine with the solvent gradient employed. Thus, these pairs of values are reported together.

Plasma insulin concentrations were assayed by a double antibody radioimmunoassay, using ovine standards (Sigma Chemical Company, St. Louis MO). Plasma glucose concentrations were determined by the neocuproine reaction using an automatic analysis system (Technicon Instruments, Tarrytown NY). Plasma creatinine values were obtained using the standard heat clot method and alkaline picrate reaction of London et al.(1967).

Analysis of variance was used as the statistical basis of comparison between trials; with each animal tested against itself as control, at a particular time in the trial.

Concentrations of the individual amino acids showed large variability between animals. To minimize the effect of this variation, the concentrations were expressed as a percentage of the pre-injection average for each animal. These values were also analyzed by analysis of variance.

A comparison of the relationship between glucose concentration changes and insulin concentration changes between trials was performed using linear regression and analysis of covariance. All statistical calculations were performed using the Statistical Package for the Social Sciences X.

C. Results

The rectal temperature of the wethers during the control trials remained relatively constant, while the rectal temperature of the endotoxin-treated animals increased almost immediately following injection; and was significantly ($P<0.05$) elevated compared with control animals from 0.75 to 6.75 h post-injection (Fig. II.1). The maximum rectal temperature attained was 2.3°C above control animals at 3.75 h post-injection.

The endotoxin-treated animals appeared lethargic during the course of the fever, recovering normal behavior

patterns once rectal temperatures had decreased to pre-injection values. Excessive salivation was observed in some of the animals during fever. The extent of anorexia was difficult to determine, because feeding occured prior to treatment. The animals consumed about two-thirds of their daily food allowance prior to treatment. During fever, the sheep tended not to eat, but finished their feed after defervescence. In general, all feed was consumed by 2000 h in both groups.

Hematocrit values became elevated ($P<0.05$) at 3 h post-injection and remained elevated until at least 6 h post-injection (Table II.1). Plasma creatinine concentrations were significantly ($P<0.05$) elevated (Table II.1) from 4.5 to 13.0 h post-injection. The concentrations reached a maximum value of 9 umol/L at 11 and 13 h post-injection, 4-6 h after defervescence.

Plasma glucose concentration decreased significantly ($P<0.05$) within 3 h of endotoxin injection and remained depressed through the trial until at least 55 h post-injection, as shown in Table II.1. Plasma insulin concentrations initially dropped ($P<0.05$) below control values at 1.5 h post-injection, and then increased ($P<0.05$) above control values from 4.5 to 13 h post-injection. The linear regressions of change in plasma glucose concentration on change in plasma insulin concentration showed no significant differences in slope ($P>0.05$) between control and endotoxin trials.

Total plasma amino acid concentrations were significantly decreased ($P<0.05$) in the endotoxin treated animals compared to the control animals by 4.5 h post-injection and remained depressed until at least 19 h post-injection, or 12 h after recovery of normal body temperature (Fig. II.2). The plasma concentrations of each of the individual amino acids decreased significantly ($P<0.05$) below control values at some point in the sampling period (Fig. II.2), with the exception of tryptophan and tyrosine. These two amino acids showed no significant alterations in their plasma concentrations throughout the experiment (data not shown).

The greatest change observed in the concentration of an individual plasma amino acid was for serine, which dropped almost immediately, reaching a low of 14% of pre-injection values at 9 h post-injection. At 4.5 h post-injection, shortly after fever peak, the concentrations of aspartate, arginine+carnosine, asparagine, isoleucine, leucine, methionine, serine, threonine+citrulline, and valine were all significantly ($P<0.05$) depressed in the endotoxin-treated animals when compared to the control animals. Significant ($P<0.05$) differences were not observed for a few amino acids until a number of hours after fever recovery. For example, glutamate and glutamine became significantly ($P<0.05$) depressed at 13 h post-injection in endotoxin treated sheep relative to control sheep. The concentrations of some amino acids,

such as arginine+carnosine, glutamate, histidine, and serine remained depressed 31 h post-injection ($P<0.05$). The plasma concentrations of all individual amino acids were not significantly different ($P<0.05$) from control values 55 h post-injection.

D. Discussion

In studying the effects of bacterial infections on nitrogen metabolism in ruminants it is of value to elucidate the actions of the bacterial endotoxins themselves. The intravenous administration of E. coli endotoxin to sheep permits the development of relatively reproducible biological responses in sheep as demonstrated by the consistency of time course changes measured in rectal temperature following endotoxin injection in this study (Fig. II.1) and in the study of Naylor et al. (1984). Studies of nitrogen metabolism involving the use of infected patients (Marchuk et al., 1977) or the use of experimental infection models (Wannemacher et al., 1971) are complicated by factors in addition to endotoxin which may lead to considerable difficulty in the interpretation of the signs developed.

Body fluid distribution changes are commonly associated with endotoxin injection, but are relatively short-lived as shown by van Miert et al. (1983) following administration of E. coli endotoxin to dwarf goats. In our experiment the injection of endotoxin resulted in a slight increase in

hematocrit values during the febrile period, suggesting that only at this time was there possibly a significant shift in body fluid distribution.

Various reports in the literature have indicated that sepsis and endotoxin-induced fever alter plasma insulin concentrations and/or carbohydrate metabolism. Ryan et al. (1974) reported increased plasma insulin concentration in rats suffering from peritonitis while there was no change in plasma glucose concentrations. These authors also reported that the responses of skeletal muscle pyruvate dehydrogenase and glucose oxidation to insulin were impaired during peritonitis while their responses in adipose tissue remained normal. These results suggest that skeletal muscle in monogastrics becomes refractory to elevated insulin concentration during sepsis.

There is limited information concerning the effect of bacterial endotoxins on insulin and carbohydrate metabolism in ruminants. Intravenous administration of E coli 011:B4 endotoxin to cattle has been reported to result in nonsignificant changes in plasma glucose concentration (Allen et al., 1970). In wethers, Naylor et al. (1984) demonstrated that plasma glucose concentration was depressed from four until at least twenty-four hours after intravenous injection of E. coli (serotype 055:B5) endotoxin in the presence or absence of the anti-pyretic drug dipyrone. Plasma insulin concentration was not reported in either of these studies. In the present study,

intravenous injection of E. coli serotype 055:B5 endotoxin simultaneously reduced plasma glucose concentrations and increased plasma insulin concentrations. Possibly sheep respond to bacterial endotoxin by increasing peripheral glucose uptake due to increased circulating concentration of insulin, and this response is independent of elevated body temperature.

In monogastric animals infection is characterized by a negative nitrogen balance (Powanda et al., 1972) due largely to an increased rate of breakdown of skeletal muscle which is reflected in an increased excretion of urinary creatinine (Garlick et al., 1980; Long et al., 1977b; Powanda et al., 1972). The elevated plasma concentrations of creatinine measured in our study may also indicate an increased rate of skeletal muscle breakdown.

Amino acids from the skeletal musculature provide substrates for increased rates of gluconeogenesis and protein synthesis in the liver (Rosenblatt et al., 1983). Intravenous administration of endotoxin has been shown to increase the rates of whole body amino acid appearance, oxidation and incorporation into protein in the guinea pig (Sobrado et al., 1983). Very little information is available to characterize the time course change in amino acid metabolism during the development and onset of sepsis or endotoxin-induced fever. Wannemacher et al. (1972) studied human volunteers infected with sandfly fever virus and found that by 47 h after inoculation, before the onset

of fever or other clincial indications of infection, the concentration of most individual plasma amino acids was significantly depressed below pre-inoculation values and remained depressed for as long as six days. Sobrado et al. (1983) reported that only the concentration of plasma aspartic acid was depressed while phenylalanine and tyrosine concentrations were increased four hours after infusion of Salmonella enteriditis endotoxin into guinea pigs. There is no information concerning the response of ruminant nitrogen metabolism to sepsis or endotoxin administration.

The decrease in plasma amino acid concentrations observed in this experiment following intravenous administration of endotoxin may initially be due, in part, to decreased absorption from the intestine, resulting from rumen stasis and altered intestinal-fluid dynamics. van Miert et al. (1983) showed that the frequency and amplitude of rumen contractions dropped during endotoxin fever, but returned to normal as temperature decreased. Thus, it is unlikely that the prolonged effects on amino acid concentrations observed were simply due to decreased rumen motility. Since very little glucose is absorbed from the digestive tract of the ruminant, a decrease in rumen motility due to endotoxin injection would also not be expected to directly affect plasma glucose concentration. Previous trials in our laboratory, with higher doses of endotoxin, have shown prolonged, severe diarrhea suggesting

fluid shifts in the intestinal tract (Southorn, B.G. and Thompson, J.R., unpublished observations). The lack of diarrhea in this experiment suggests that absorptive function may have been relatively normal.

The extent to which the plasma concentrations of individual amino acids decreased following endotoxin administration was variable. Plasma serine concentration decreased most rapidly and to the greatest extent of any amino acid in the present experiment. Plasma glycine followed the same pattern, but showed a slightly lesser drop. Previous studies of infection with rats and humans have shown either no change (Askanazi et al., 1980) or very little change (Wannemacher et al., 1971; Wannemacher et al., 1972) in plasma serine concentration. Endotoxin infusion in the guinea pig also caused no change in plasma serine or glycine concentration (Sobrado et al., 1983). The reason for this discrepancy remains unclear, but future studies using flux measurements across tissues may provide evidence for the fate of serine in the febrile ruminant.

Alanine may be an important amino acid in the host response to infection because it is a major substrate for gluconeogenesis. Some authors have found no change in alanine concentrations during infection (Marchuk et al., 1977; Askanazi et al., 1980), while others report decreases in alanine concentration starting before infection induced fever appears (Wannemacher et al., 1972). During four hours of endotoxin infusion in guinea pigs, the plasma

alanine concentration did not change (Sobrado et al., 1983). Alanine concentrations in the present study remained unchanged until after fever had subsided. As is the case with all individual amino acids, the flux of alanine through the circulatory system may change greatly during fever and infection with little or no change in absolute concentration.

Decreased branched chain amino acid concentrations appear to occur in monogastric species during infectious fever. In experimental infections, the branched chain amino acid concentrations decrease before fever appears (Wannemacher et al., 1971; Wannemacher et al., 1972). In contrast to the present experiment, Wannemacher et al. (1972) found that the branched chain amino acids showed a more prolonged concentration drop than the other amino acids. Marchuk et al.(1977) found a decreased release of these amino acids from skeletal muscle during sepsis, suggesting their increased use as energy substrates this tissue. In the guinea pig, no change in the concentrations of the branched chain amino acids occured during a four hour endotoxin infusion (Sobrado et al., 1983). In the ruminant however, the oxidation of branched chain amino acids in muscle is limited compared to that in the monogastric (Wijayasinghe et al., 1983). The difference in the normal metabolism of leucine suggests that the alterations in branched-chain amino acid metabolism due to fever and infection may not be the same between these two

types of animals. This hypothesis is confirmed by the plasma branched-chain amino acid concentration data of the present experiment.

The plasma concentration of phenylalanine did not change significantly ($P>0.05$) during fever and failed to show the evening increase above preinjection concentration seen in the control trials. Endotoxin administration in the guinea pig causes increased plasma phenylalanine concentration (Sobrado et al., 1983). In the studies of others, plasma phenylalanine concentration during infection has been reported to show an initial drop (Wannemacher et al., 1972) followed by a large rise (Marchuk et al., 1977; Wannemacher et al., 1971; Wannemacher et al., 1972). Askanazi et al. (1980) found no change in plasma phenylalanine concentration during sepsis but the muscle concentration was elevated.

The changes in plasma creatinine and amino acid concentrations reported here suggest that rapid and extensive changes are also occurring in the patterns of tissue protein metabolism in the ruminant in response to endotoxin administration. It is possible that these changes may contribute to economic losses incurred by livestock producers during infectious disease outbreaks. A more complete understanding of the mechanisms of catabolic nitrogen loss may help to alleviate this problem. The results of the present experiment also suggest that some differences may exist between ruminant and monogastric

species, with respect to amino acid metabolism during infection. In order to elucidate the origin of the observed changes in plasma amino acid concentrations, studies are currently in progress to measure the flux of these amino acids across representative tissues during endotoxin induced fever.

Figure II.1. Time course change in rectal temperature in control (——) and endotoxin-treated (-----) sheep.
*, Temperatures were significantly different between treatments from 0.75 to 6.25 h ($P<0.05$) n= 4-7 animals

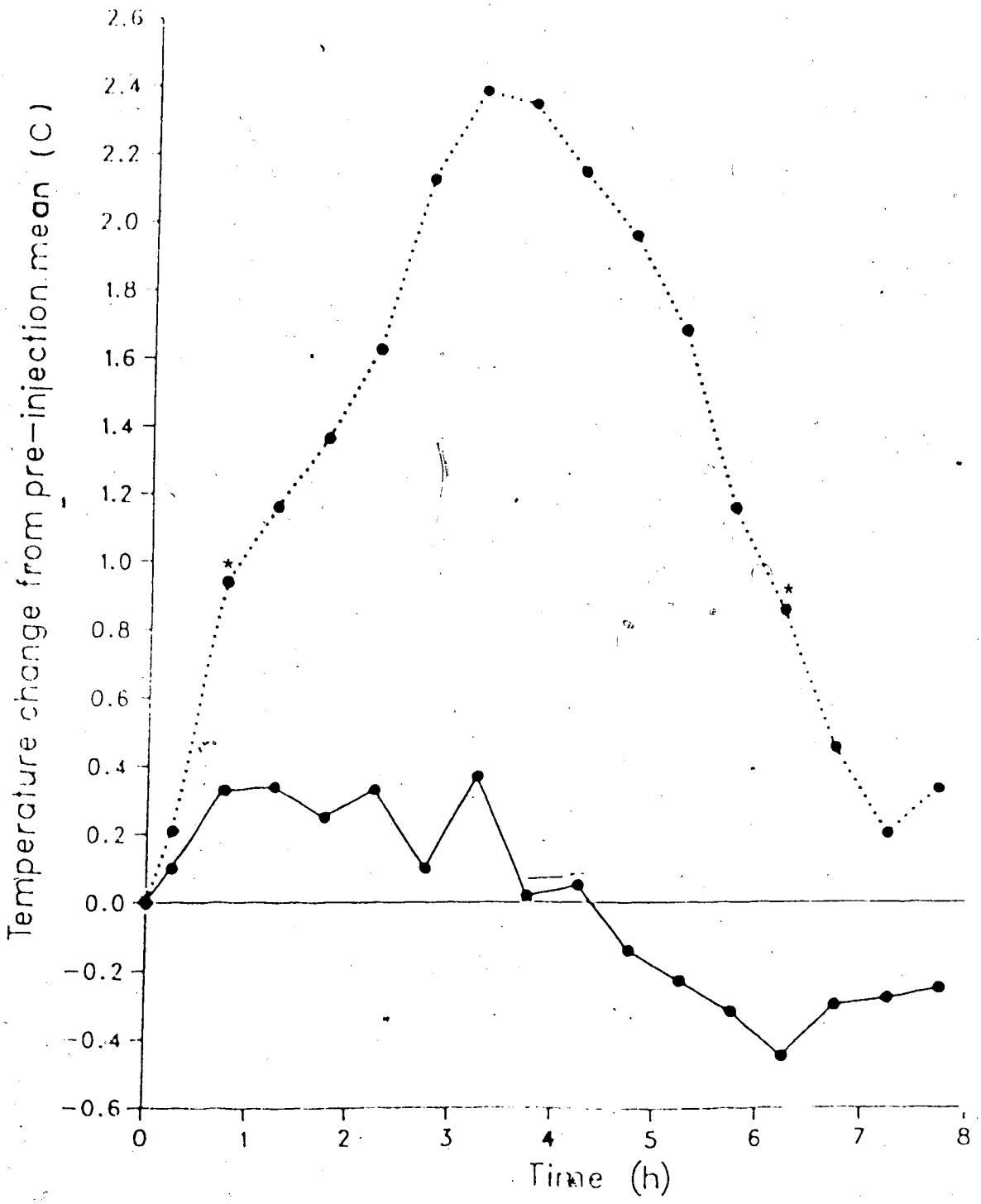
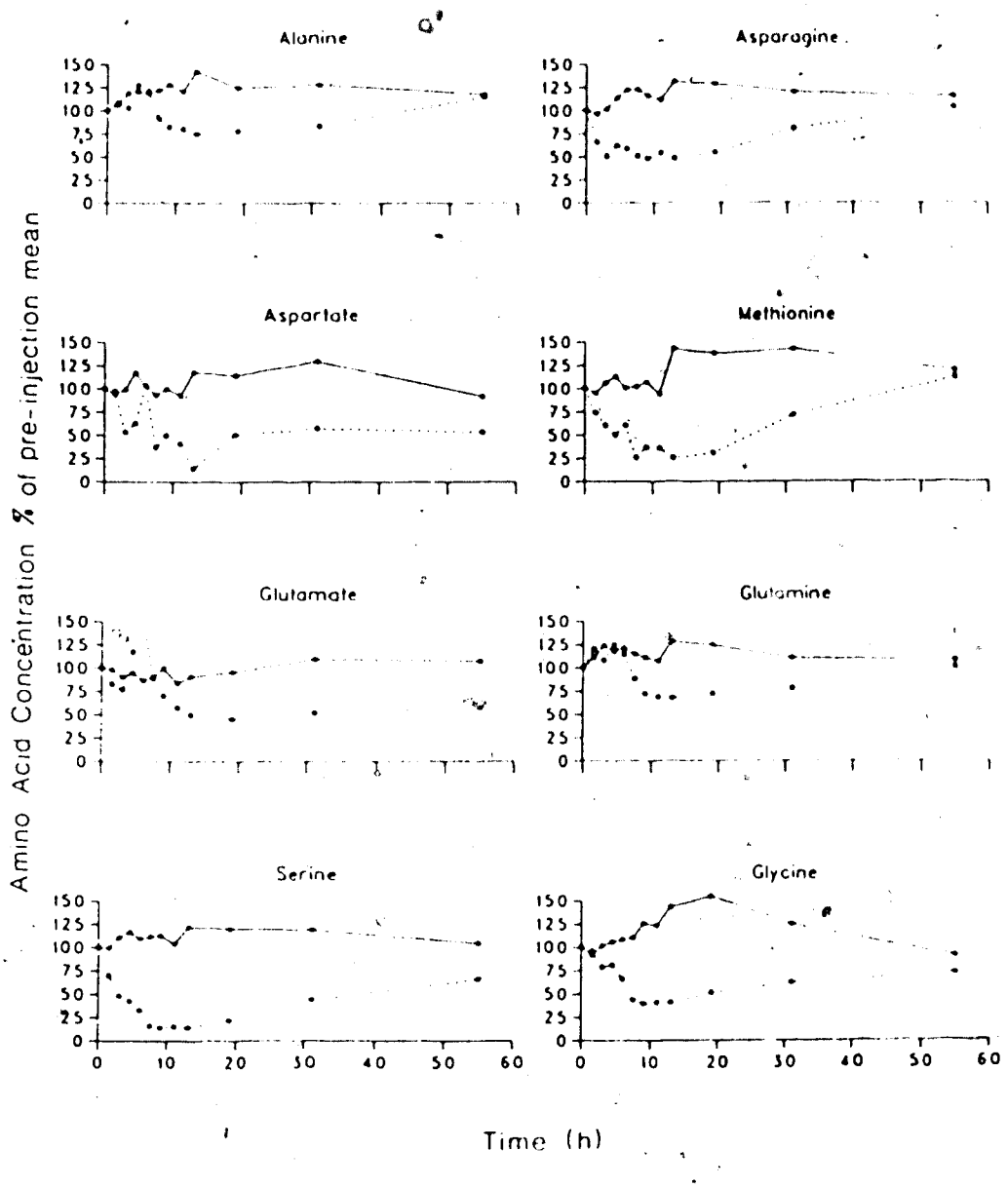


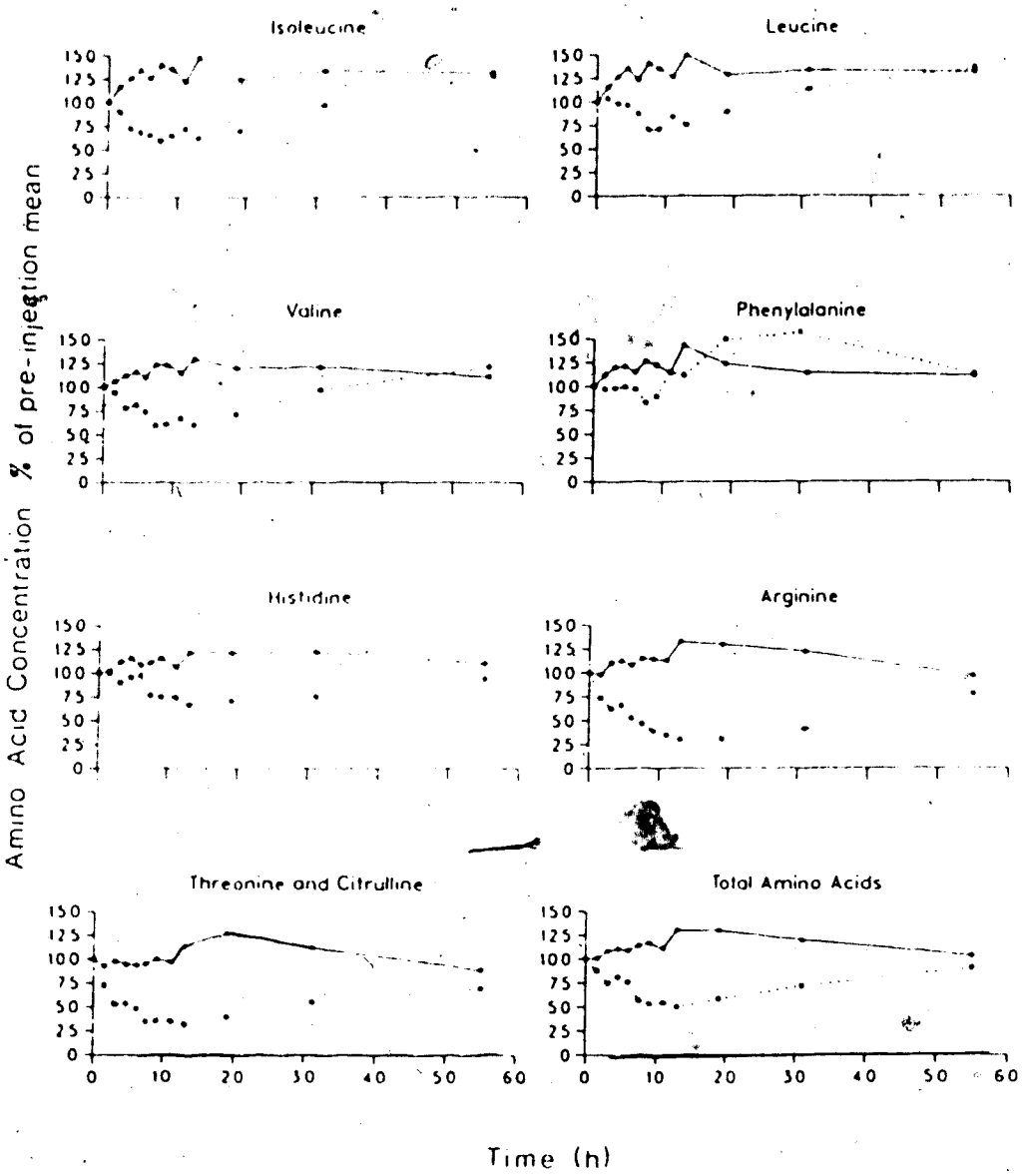
Table II. Time course changes in concentration of blood constituents [a]

		Time Post-injection (h) 0	1.5	3.0	4.5	6.0	7.5	9.0	11.0	13.0	19.0	31.0	55.
Hematocrit	C	0.27 (0.00)b	0.25 (0.01)	0.26 (0.01)	0.26 (0.01)	0.26 (0.01)	0.26 (0.01)	0.26 (0.01)	0.26 (0.01)	0.27 (0.01)	0.26 (0.01)	0.26 (0.00)	[illegible]
	F	0.29 (0.01)	0.30 (0.02)	0.32A (0.02)	0.34A (0.01)	0.32A (0.02)	0.30 (0.02)	0.28 (0.02)	0.29 (0.02)	0.27 (0.02)	0.27 (0.02)	0.27 (0.01)	[illegible]
Creatinine (μmol/L)	C	61 (3)	61 (4)	61 (2)	55 (3)	60 (4)	53 (4)	64 (5)	62 (2)	57 (2)	60 (4)	66 (4)	[illegible]
	F	59 (0)	61 (9)	74 (6)	78A (7)	80A (8)	88A (8)	89A (5)	91A (6)	91A (7)	82 (7)	76 (6)	[illegible]
Glucose (mmol/L)	C	2.9 (0.1)	3.1 (0.1)	3.3 (0.1)	3.2 (0.1)	3.2 (0.1)	3.3 (0.2)	3.5 0.1)	3.4 (0.0)	3.4 (0.1)	3.3 (0.2)	3.3 (0.1)	[illegible]
	F	2.9 (0.0)	2.8 (0.2)	3.1A (0.2)	2.5A (0.2)	1.8A (0.2)	1.7A (0.1)	1.7A (0.1)	1.8A (0.1)	1.9A (0.1)	1.9A (0.1)	2.1A (0.1)	[illegible]
Insulin pmol/L	C	291 (15)	288 (38)	238 (15)	289 (24)	253 (17)	272 (15)	265 (22)	243 (24)	238 (21)	231 (19)	244 (21)	[illegible]
	F	284 (17)	186A (40)	720 (141)	578A (101)	508A (67)	565A (93)	523A (72)	442A (60)	487A (52)	360 (62)	358 (33)	[illegible]

a - Mean values from 5 - 7 animals
b - SEM
C - Control trials
F - fever trials
A - significantly different ($P<0.05$) from control value at the time indicated.

Figure II.2. Time course changes in individual and total amino acid concentrations in control (——) and endotoxin-treated (- - -) sheep. Values are expressed as percentage of the pre-injection mean for each amino acid. The preinjection plasma concentrations were: alanine 208, asparagine 48, aspartate 7, methionine 22, glutamate 128, glutamine 302, serine 116, glycine 580, isoleucine 117, leucine 163, valine 327, phenylalanine 65, histidine 64, arginine+carnosine 220, threonine+citrulline 526, and total amino acids 3174 μM. mean values from 5-7 sheep.


Alanine
Asparagine
Aspartate
Methionine
Glutamate
Glutamine
Serine
Glycine
150
125
100
75
50
25
0
0
10
20
30
40
50
60
Amino Acid Concentration % of pre-injection mean
Time (h)


Isoleucine
Leucine
Valine
Phenylalanine
Histidine
Arginine
Threonine and Citrulline
Total Amino Acids
Amino Acid Concentration % of pre-injection mean
Time (h)

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III. THE EFFECT OF ENDOTOXIN INDUCED FEVER ON HEPATIC SERINE AND ALANINE METABOLISM

A. Introduction

Infection and fever are known to induce marked changes in nitrogen metabolism in humans (Beisel,1966). These changes include onset of negative nitrogen balance (Beisel, 1966); altered rates of protein synthesis and degradation both in the whole body (Garlick et al., 1980) and in individual tissues (Fern et al., 1985; Pedersen et al., 1986); and changes in amino acid oxidation and distribution (Rosenblatt et al., 1983). It has been shown that the release of amino acids from peripheral tissues such as muscle and the uptake of amino acids by visceral tissues such as the liver are increased during sepsis (Rosenblatt et al., 1983; Clowes et al., 1980). The amino acids which are extracted from the blood by the liver have a variety of fates. They may be deaminated and oxidized, incorporated into hepatic protein or converted to glucose and fatty acids. Long et al. (1976) showed that during sepsis, the rate of alanine oxidation was unchanged but the rate of gluconeogenesis from alanine was elevated. Sobrado et al. (1983) have shown that endotoxin infusion alone is effective in altering leucine metabolism in the guinea pig. They demonstrated increases in both whole body protein synthesis and degradation, as well as increased leucine oxidation.

Although there is an absence of literature regarding the response of ruminant animals to endotoxin-induced fever with respect to nitrogen metabolism, it has recently been demonstrated that sheep exhibit decreased concentrations of plasma individual amino acids and glucose when subjected to this treatment (Chapter II.). The mechanisms responsible for these plasma concentration changes have not been elucidated. The principal objective of this study, therefore, was to characterize the hepatic metabolism of two amino acids, serine and alanine, during endotoxin induced fever in sheep. Serine was chosen since it is largely metabolized in the liver and its concentration in plasma was reduced to the greatest extent during endotoxin-induced fever in sheep. Alanine is an important glucogenic amino acid and its role in gluconeogenesis has been previously demonstrated in septic humans (Long et al., 1976).

B. Materials and Methods

[U-^{14}C] serine and [U-^{14}C] alanine were obtained from ICN Radiochemicals, Montreal, PQ. Radiochemical purity was confirmed to be greater than 99% by paper chromatography employing a solvent system of tertiary butanol, methylethylketone, water, and ammonium hydroxide (4:3:2:1). Endotoxin from E. coli serotype 055:B5 was obtained from Sigma Chemical Company, St. Louis, MO.

Six adult wether sheep weighing 40-57 kg were housed in

metabolism cages at an ambient temperature of 20°C. They were shorn prior to the experiment and three had been previously fitted with rumen cannulae. The sheep were fed 1000 g of mixed concentrate/alfalfa pellets once daily, at 0800 h. Cobalt-iodized salt and water were available ad libitum throughout the experiment.

Each sheep was used in two trials; control (injection of pyrogen free saline) and endotoxin (injection of 4 µg/kg body weight E. coli serotype 055:B5 endotoxin dissolved in pyrogen free saline). The endotoxin solution was prepared in one batch and stored frozen in single dose syringes. The trials were separated by at least two weeks.

A single polyvinylchloride catheter was inserted into the jugular vein one day prior to each trial for solution administration and blood sampling. On the morning of a trial, the animals were not fed. Body temperature was monitored and recorded at one minute intervals by a microcomputer connected to a thermocouple inserted 10 cm into the rectum of the sheep. Blood samples were obtained at -2,-1,0,1.5,3.0,4.5,6.0,7.5,9.0, and 24 h postinjection. Packed cell volume was determined using a micro-hematocrit centrifuge. Plasma was separated by centrifugation at 1000g and stored at -20°C until analysis.

At approximately 6 h postinjection liver biopsies were obtained according to the method of Pearson and Craig (1980) using a Tru-Cut mammary biopsy needle (Travenol

Laboratories Inc., Deerfield, Ill.). Ten biopsies of approximately 10-15 mg wet weight and 2 mm in diameter, were placed in buffer (see below) until incubation.

When possible five replicate incubations were performed for each treatment and amino acid. In addition, incubations were performed without tissue to correct for background. The biopsies were weighed and preincubated at 39.0°C for 30 min in 2 mL of media saturated with 95%:5% O_2:CO_2. They were then transfered to 2 mL of fresh media in 25 mL incubation bottles fitted with center wells for subsequent collection of respired CO_2, and incubated for a further 2 h. The incubation medium was Krebs-Ringer bicarbonate buffer (Umbreit et al., 1972) with 10mM HEPES, 10 mM glucose, 5mM acetate and sheep plasma concentrations of amino acids as previously determined in our laboratory. The HEPES was added as an additional buffer. The glucose, acetate and amino acids were added to provide substrates for the maintainance of normal metabolism by the tissue. In addition, each flask contained either 0.5 μCi[U-^{14}C]-serine or 0.1 μCi[U-^{14}C]-alanine. The initial medium specific activity was 3098 dpm/nmol for serine and 598 dpm/nmol for alanine.

The metabolism of the tissue was terminated after 2 h by the addition of 0.15 mL 70% $HClO_4$ to the incubation medium. Protosol (0.20mL) (New England Nuclear, Lachine PQ) was added to the center well in each bottle to trap CO_2. The incubations were continued for a further 45 min

to ensure complete collection of CO_2. The center wells were removed and placed in scintillation vials. Fifteen mL of scintillation cocktail (toluene 500mL, methylcellusolve 500mL, PPO 5g, POPOP 0.2g) were added and the radioactivity in CO_2 was determined using a MarkI liquid scintillation counter (Nuclear-Chicago) and sample channels ratio for quench correction. The tissue and incubation media were stored at -20°C until analysis.

The tissue and incubation media were homogenized using a glass homogenizer. The homogenizer was washed twice with 1 mL of 0.6 N $HClO_4$ containing either 0.1N alanine or 0.1N serine. After centrifugation (1000g, 15 min) the supernatant was decanted and saved for glycogen analysis. The protein pellet was washed a further 3 times with 0.6 N $HClO_4$ containing 0.1 N alanine or 0.1 N serine. The protein was resuspended in 0.25 mL of water and solubilized in 0.75 mL Soluene (Packard Instrument Company Inc., Downer's Grove, Ill) overnight at 20°C. The samples were counted after addition of cocktail (Scintiverse Bio-HP, Fisher Scientific Company, Fair Lawn, NJ). Correction for contamination from free serine and alanine adhered to protein was done using media from flasks incubated without tissue and the same procedure as the samples. Frozen liver tissue which was not incubated with isotope was added as a source of protein.

The supernatant from the initial protein precipitation was mixed with 10 mg glycogen. Three volumes of 75%

ethanol were added and the samples allowed to stand overnight at 4°C to precipitate glycogen. After centrifugation (1000 g, 15 min) and decanting of the supernatant, the glycogen pellet was redissolved in 0.5 mL 0.6 N $HClO_4$ containing 0.1 N serine or 0.1 N alanine and reprecipitated with ethanol three more times. The final precipitate was dissolved in 0.5 mL water and counted. Correction for residual radioactivity associated with glycogen was performed by repeating the same procedure using media containing ^{14}C-alanine and ^{14}C-serine from flasks incubated without tissue.

Plasma samples were deproteinized with trichloroacetic acid prior to derivatization of amino acids with o-phthaldialdehyde according to the method of Jones and Gilligan (1983). Amino acid concentrations were determined using high performance liquid chromatography (Varian 5000 liquid chromatograph equipped with a Fluorichrom detector) with peak integrations performed by computer (Hewlett Packard 2625A). Plasma glucose was determined using a reagent kit (Sigma Chemical Company, St. Louis, MO).

Calculations of the amount of carbon from each amino acid partitioned to the three fractions studied were as follows. The amount of label appearing in any one fraction (CO_2, protein, or glycogen) was calculated on a dpm/mg wet tissue basis. This value was divided by the specific activity of the incubation medium for serine or alanine, the result was multiplied by 3 to correct for the number of

carbon atoms in each amino acid and expressed as μmole substrate C per mg tissue wet weight.

Data were analyzed statistically using the Statistical Package for the Social Sciences X. The basis of comparison between control and endotoxin incubations was analysis of variance and f-test. To minimize the effects of variation between animals in the absolute plasma concentrations of amino acids, the valueswere expressed as percentages of preinjection mean concentrations. Time course changes in plasma amino acid and glucose concentrations and rectal temperatures were assessed using paired t-tests.

C. Results

The time course change in rectal temperature for control and endotoxin treated animals is shown in Figure III.1. Rectal temperature of endotoxin injected sheep was elevated ($P<0.05$) above that of the controls from 4.25 h to 9.0 h postinjection. The maximal temperature elevation above preinjection values was 2.43°C at 5.5 h postinjection. The rectal temperature of the control sheep also increased ($P<0.05$) transiently after injection of saline.

The symptomatic reaction of the sheep to endotoxin treatment was quite variable. Most animals exhibited some shivering and excessive salivation shortly after injection. Only one animal developed a severe reaction to the endotoxin, characterized by diarrhea and shock. Two of

the endotoxin treated animals, including the sheep which exhibited the severe reaction, died of shock after the liver biopsy procedure, but this problem was not encountered in any of the control animals.

By 3 h postinjection the hematocrit values of the endotoxin treated sheep had risen significantly ($P<0.05$) above those of the control animals as shown in Table III.1. They remained elevated until at least six hours postinjection. Plasma glucose concentration had decreased ($P<0.05$) by 3.0 h postinjection, reaching a low of 1.55 mM 24 h postinjection (Table III.1).

Plasma total amino acid concentration became significantly ($P<0.05$) depressed by 9.0 h postinjection and remained so until at least 24 h postinjection, as shown in Figure III.2a. Plasma serine concentration decreased significantly ($P<0.05$) by 3.0 h postinjection reaching a minimum of 26 percent of the preinjection concentration at 9.0 h postinjection (Figure III.2b.). The serine concentration of the control sheep also decreased but not to the same extent. Plasma alanine concentration showed a nonsignificant ($P>0.05$) increase during the initial part of the trial in the endotoxin treated sheep, as shown in Figure III.2c. At 24 h postinjection, the plasma concentrations of both serine and alanine were reduced ($P<0.05$) in the endotoxin injected sheep in comparison with controls. Details of the plasma concentrations of other amino acids may be found in Appendix 2.

Endotoxin injection caused a 62% increase ($P<0.05$) in hepatic oxidation of ^{14}C-serine (Table III.2). Although there was no significant ($P>0.05$) change in $^{14}CO_2$ release from ^{14}C-alanine due to endotoxin treatment, there was a trend towards an increase. Significantly ($P<0.05$) more alanine than serine was oxidized in both the endotoxin and saline injected animals (Table III.2). The amount of serine and alanine carbon appearing in hepatic protein was increased ($P<0.05$) by 72% in biopsies from endotoxin treated sheep as shown in Table III.3. Incorporation of serine and alanine carbon into hepatic glycogen (Table III.4) was also increased ($P<0.05$) by endotoxin treatment. Although the increase was substantial (275% for serine and 325% for alanine) the total amount of activity in glycogen after 2 h of incubation was 100-400 times less that that found in CO_2.

D. Discussion

The objective of this study was to partially characterize the hepatic metabolism of serine and alanine during endotoxin-induced fever in sheep. It was determined that the overall hepatic metabolism of both serine and alanine increased due to this treatment. This finding provided a partial explanation for the large decrease in plasma serine concentration observed due to this treatment in a previous experiment in our laboratory (Chapter II).

The rise in body temperature due to endotoxin was not

as rapid in the animals studied in this experiment as compared to the animals studied in the previous experiment (Chapter II), however, the rectal temperature was elevated for a longer period of time in this experiment. The animals in this experiment did not show the characteristic biphasic temperature rise which is often observed in studies following endotoxin injection into sheep (van Miert et al., 1983; Chapter II). The rectal temperature of the sheep during the control trials became elevated during the first few hours following the injection of saline. The reason for this temperature rise is unclear.

The decrease in plasma glucose concentration observed due to endotoxin injection was similar to that observed in the previous experiment (Chapter II). Likewise, the rise in hematocrit recorded in this experiment reflected the data of the previous experiment as well as that of van Miert et al. (1983).

Ruminant animals differ from monogastric animals in areas other than the digestive tract. They are more dependent on gluconeogenesis than monogastric animals because very little glucose is absorbed from the gastrointestinal tract (Wolff and Bergman, 1972). Ruminants may also differ with respect to the effects of fasting and the relative importance of various tissues in amino acid oxidation (Wijayasinghe et al., 1983). The literature is incomplete with respect to the metabolic responses of ruminant animals to endotoxin induced fever.

A few reports have dealt with the reduction in rumen motility associated with endotoxins (van Miert et al., 1983; van Miert, 1973). Other authors have reported changes in plasma glucose concentration in endotoxin treated ruminants (Naylor et al., 1984; Cakala, 1965); but they have not investigated the metabolic basis of these observed changes. Van Miert et al. (1983) reported increased plasma urea concentration in goats 24 h following endotoxin injection. There have been no other reports of any change in the metabolism or concentration of nitrogen containing compounds in ruminants during endotoxin induced fever except for that in Chapter II.

Sobrado et al. (1983) studied guinea pigs during endotoxin infusion and found no change in the concentrations of selected amino acids, except for a decrease in plasma aspartate and an increase in plasma tyrosine and phenylalanine concentrations following 4 h of infusion. Infusion of interleukin-1 had the same effect as infusion of endotoxin on plasma amino acid concentrations in their study. These results are consistent with the values obtained in this study except that plasma serine was dramatically decreased in the sheep used in this experiment but was not affected in the guinea pigs studied by Sobrado et al. (1983).

The conversion of serine and alanine to the products measured in this experiment represents the actual appearance of carbon from these two amino acids in the

fractions studied. These are valid measurements of the rate of oxidation, net incorporation into protein and glycogen if it is assumed that the intracellular specific activity of the precursor amino acid is equivalent to the medium specific activity, an assumption normally made in this type of study (eg. Mitch and Clark, 1984). The intracellular specific activity was not measured, but since the volume of medium greatly exceeded the volume of the tissue incubated, this was probably a valid assumption. The values calculated would be underestimates, however, because the intracellular specific activity would never exceed the medium specific activtiy. The effect of this difference would be slight as shown by Fornander et al. (1985). The estimation of the contribution of serine or alanine to oxidative metabolism in the liver tissue is also underestimated by the method used in this experiment because some of the label from the amino acids would be lost through equilibration with unlabelled carbon atoms in the TCA cycle (Brosnan, 1982).

Serine oxidation during infection or fever has not been previously reported. The rate of whole body oxidation of alanine has been previously shown to be unaffected in septic patients (Long et al., 1976). Liver tyrosine oxidation increases during sepsis in humans but not in rats (Rosenblatt et al., 1983). The oxidation of tyrosine increases in rats during interleukin-1 infusion (Yang et al., 1983), however, illustrating possible differences

between reactions to infection and induced fevers. Similarily, endotoxin infusion in the guinea pig has been demonstrated to increase whole body leucine oxidation (Sobrado et al., 1983). In general, it appears that oxidation of amino acids increases during fever or sepsis. This supports the hypothesis that the role of amino acids as energy sources during this type of metabolic stress is increased.

Short-term fever following vaccination has previously been demonstrated to increase whole body protein synthesis (Garlick et al.,1980). Numerous studies in the past have reported increases in hepatic protein synthesis during fever. This has been demonstrated both in chronic infections (Lust, 1966; Fern et al., 1985; Rosenblatt et al., 1983) and in short term fevers induced by endotoxin or interleukin-1 (Sobrado et al., 1983; Loda et al., 1984; Yang et al., 1983). In the present experiment hepatic protein synthesis was not directly measured, but the increased net incorporation of serine and alanine carbon into hepatic protein suggests that net protein synthesis was increased in the liver of the sheep in response to endotoxin administration. Protein synthesis of both the secretory and non-secretory fractions of hepatic protein is increased by interleukin-1 infusion into the guinea pig (Sobrado et al., 1983). Four hours of endotoxin infusion did not increase hepatic protein synthesis in their experiment. However, in the study of Loda et al. (1984)

the increase in *in vitro* hepatic protein synthesis in response to endotoxin injection was [illegible]ed until 12 h postinjection; a time when the increase due to interleukin-1 had attenuated. The results of the present experiment suggest that the time course change in hepatic protein synthesis is shorter in the sheep when compared with the rats in the study of Loda et al. (1984).

In monogastric species the production of glucose by glycogenolysis is increased until the liver glycogen pool is exhausted, following endotoxin injection (McCallum and Berry, 1973). In this experiment, liver glycogen content was not measured, but examination of the incorporation of serine and alanine carbon into glycogen suggests that in sheep liver, the rate of glycogen synthesis may have increased in response to this treatment. If the turnover rate of liver glycogen was extremely elevated, it would also be possible to have increased labelling of the glycogen even if the stores were depleted. Alternatively, the excess glucose present in the incubation media may have stimulated glycogen synthesis. Without data on the glycogen content of the liver biopsy samples and the rate of glucose production from glycogen it is impossible to distinguish between these two possibilities.

This experiment clearly illustrates that endotoxin induced fever increases the hepatic use of alanine and serine in sheep. The increased hepatic use, particularly of serine, may partially explain the decreased plasma

concentration of this amino acid due to endotoxin treatment observed in this study and the previous one (Chapter II). Further experiments on the fate of the carbon skeletons of other amino acids in this situation would provide valuable information on the reaction to endotoxin induced fever in sheep.

Figure III.1. Time course change in rectal temperature in control and endotoxin treated sheep - Experiment 2. mean values from 4-6 sheep.

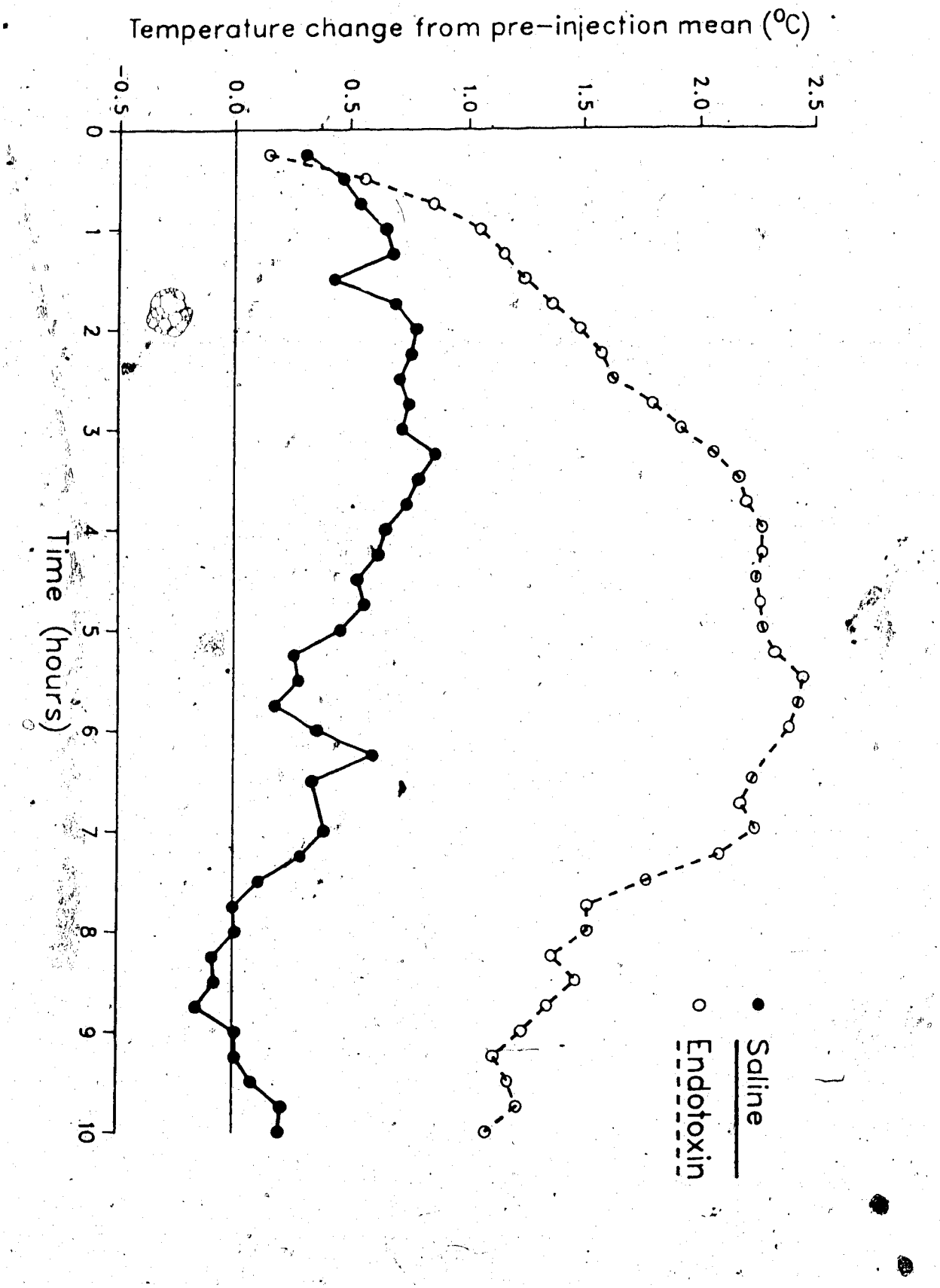
Temperature change from pre-injection mean (°C)
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
Time (hours)
0
1
2
3
4
5
6
7
8
9
10
Saline
Endotoxin

Table III.1. Time course change in plasma glucose concentration and hematocrit. [a]

		Time (h)							
		0.0	1.5	3.0	4.5	6.0	7.5	9.0	24.0
Hematocrit	C	.25	.25	.26	.27	.27	.24	.24	.24
		(.01)	(.01)	(.01)	(.01)	(.01)	(.01)	(.02)	(.01)
	F	.26	.32	.33A	.32A	.32A	.24	.24	.24
		(.01)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.03)
Glucose (mmol/L)	C	3.11	3.25	3.33	2.99	2.87	3.23	3.27	2.92
		(.12)	(.33)	(.38)	(.19)	(.10)	(.23)	(.15)	(.10)
	F	2.84	3.24	2.48A	2.18A	2.13A	1.65A	1.83A	1.54A
		(.15)	(.65)	(.28)	(.21)	(.28)	(.51)	(.28)	(.14)

[a] mean values from 4-6 sheep
( ) standard error of the mean
C - saline trial
F - endotoxin trial
A - significantly different from saline trial at time point indicated ($P<0.05$)

Figure III.2. Time course changes in plasma amino acid concentrations following endotoxin injection into sheep. mean values from 4-6 sheep.

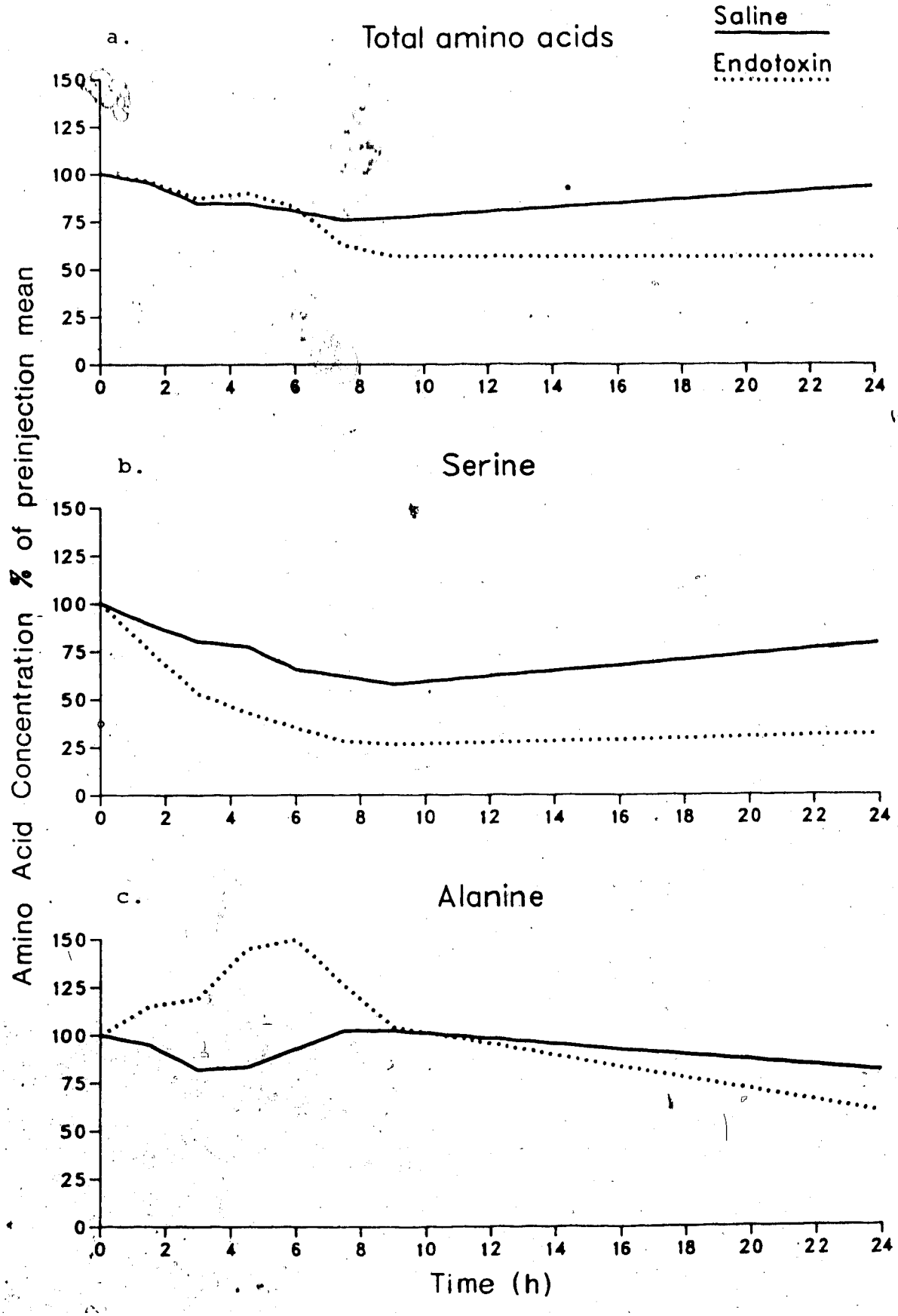
a.
Total amino acids
Saline
Endotoxin
b.
Serine
c.
Alanine
Amino Acid Concentration % of preinjection mean
Time (h)
150
125
100
75
50
25
0
0
2
4
6
8
10
12
14
16
18
20
22
24

Table III.2. Hepatic oxidation of serine and alanine.

Treatment	Substrate: Serine(S) (umole C)(mg liver wet weight)$^{-1}2h^{-1}$	Alanine(A) (umole C)(mg liver wet weight)$^{-1}2h^{-1}$	(A/S)100%	Signifcance
Saline(Sa)	633(123)+	1329(228)	210%	*
Endotoxin(E)	1026(99)	1638(123)	160%	*
(E/Sa)100%	162%	123%		
Significance	*			

n=6
+ mean-+ SEM
* significantly different ($P<0.05$)

Table III.3. Appearance of labelled carbon in hepatic protein.

Treatment	Substrate			
	Serine(S)	Alanine(A)	(A/S)100%	Significance
	(umole C)(mg liver wet weight)$^{-1}$2h^{-1}			
Saline(Sa)	354(33)+	288(54)	81%	
Endotoxin(E)	612(81)	495(84)	81%	*
(E/Sa)100%	173% *	172%		
Significance	*	*		

n=6
+ mean ± SEM
* significantly different ($P<0.05$)

Table III.4. Conversion of serine and alanine to glycogen.

Treatment	Substrate: Serine(S) (umole C)	Alanine(A) (mg liver wet weight)$^{-1}$2h^{-1}	(A/S)100%	Significance
Saline(Sa)	3.24(.57)+	3.30(1.29)	102%	
Endotoxin(E)	8.91(1.35)	10.71(2.88)	120%	
(E/Sa)100%	275%	325%		
Significance	*	*		

n=6
+ mean ± SEM
* significantly different ($P<0.05$)

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IV. GENERAL DISCUSSION AND CONCLUSIONS

There is considerable interest in the effects of infection and fever on protein and amino acid metabolism in human patients and monogastric animal models (eg. Sobrado et al., 1983; Powanda et al., 1972; Clowes et al., 1980). The overt result of these changes in amino acid and protein metabolism is muscle tissue wasting which may be substantial, especially as a result of prolonged illness. There are no published scientific studies of the extent of this problem in livestock species and it is conceivable that the effect of infectious disease is to drastically influence the efficiency of animal production. Because ruminant animals differ from monogastric animals in a number of ways, it is unreasonable to directly extrapolate from the published studies on mongastric animals and apply the results to estimate the economic loss due to disease in ruminant animals. Therefore, the experiments presented in this thesis were conducted to provide new information on amino acid metabolism of ruminants during fever.

Two experiments were conducted. In the first experiment (Chapter II) the objective was to determine whether endotoxin influences plasma amino acid concentrations and to characterize any time course changes in plasma amino acid concentrations following the injection of endotoxin. The objective of the second experiment (Chapter III) was to examine hepatic metabolism of serine

and alanine in response to endotoxin challenge.

In experiment one it was demonstrated that the plasma concentrations of most individual amino acids and glucose were decreased by endotoxin injection. The fever produced by the endotoxin injection lasted approximately 6 h but the concentration of many of the plasma amino acids remained decreased for more than 24 h. This experiment indicated that there were likely substantial changes in amino acid metabolism in ruminant animals in response to endotoxin injection, but the nature of these changes could not be determined simply from alterations in plasma concentrations. The observed changes in plasma concentrations of amino acids were not entirely consistent with data from monogastric animals subjected to similar treatments (Sobrado et al., 1983). For example, plasma serine concentrations decreased to 14 % of the preinjection mean early in the time course measured in the sheep, but did not change in the guinea pig.

The results from experiment two showed that hepatic oxidation of serine was increased by endotoxin treatment, while hepatic oxidation of alanine was not significantly affected. The incorporation of carbon from both serine and alanine into protein and glycogen was also increased in endotoxin treated animals. It has been demonstrated that septic patients release amino acids from peripheral tissues such as the leg (Clowes et al., 1980). Amino acid uptake into visceral tissues, especially the liver, increases in

septic patients as demonstrated by Rosenblatt et al., (1983). These authors also demonstrated increased incorporation into hepatic protein and oxidation of tyrosine, corroborating the results of experiment two.

The changes in plasma amino acid concentrations in sheep in response to endotoxin injection from the second experiment of this series (Chapter III and Appendix 2) did not agree completely with the changes observed in the first experiment (Chapter II). The plasma concentration of many of the individual amino acids did not decrease to the same extent in the second experiment as they did in the first experiment. The possible effect of feed intake on data of this type is clearly evident. In the first experiment, the sheep were fed at 0800 h on the morning of the trials whereas in the second experiment, they were not fed the morning of the trial and any feed remaining from the previous day was removed. This possibly contibuted to the marked reductions in plasma concentrations of most individual amino acids during the saline trials in experiment two as compared to experiment one.

The rectal temperature responses were also different between the two experiments. In the first experiment, the sheep developed a fever lasting 6 h, while in the second experiment the sheep developed a longer fever lasting until 9 h after injection of a similar amount of endotoxin. Also, in the second experiment the saline injected control animals showed increased rectal temperature during the

initial part of the recording period. There were three differences between the two experiments in the treatment of the sheep. The first difference between the sheep in the two experiments was the feed intake prior to treatment and the type of feed. The animals in the second experiment were fed a concentrate as part of their ration, while the animals in the first experiment were fed pelleted bromegrass hay. Secondly, the surgical stress of the biopsy procedure may have prolonged the fever in the endotoxin treated animals in experiment two. Thirdly, the sheep used in experiment one were handled daily by the experimenter for two months prior to and during the experiment. In contrast, the sheep used in the second experiment were fed and cleaned by departmental staff and therefore were not accustomed to handling by the experimenter except during the actual trials. Psychological stress due to the lack of conditioning to the experimenter could provide a partial explanation for the temperature rise observed in the sheep following saline injection and the prolonged fever following endotoxin injection observed in the second experiment. The variation in rectal temperature response may also be partially due to differences among the animals in prior exposure to endotoxin through natural infections and consequent tolerance due to antibody formation.

An objective of this research was to determine if sheep react to fever through alterations in amino acid metabolism

which could impair production of meat. Clearly, the sheep does exhibit modified amino acid metabolism during endotoxin induced fever as shown in Chapters II and III. Although there were some differences in the data between the two experiments, qualitatively the data showed the same response in both. The increase in hepatic oxidation of serine demonstrated in Chapter III provides a partial explanation for the large decrease in its plasma concentration observed in both experiments. Alanine oxidation may occur in many tissues other than liver, so the data presented in Chapter III concerning hepatic oxidation may not as accurately represent the response of alanine metabolism to endotoxin challenge in the sheep. There are still many aspects of the responses of amino acid and protein metabolism to endotoxin-induced fever in sheep which require further thought and investigation.

The experiment attempted to determine the effects of endotoxin on amino acid fluxes between organs in the sheep, which is summarized in Appendix 1., would provide valuable information. A combination of this type of experiment and experiments involving isotope infusion of specific amino acids would permit an accurate estimation of quantitative inter-organ exchange of individual amino acids coupled with information on their metabolic fates in the organs studied. Measurement of fractional protein synthesis and degradation rates in various tissues of the sheep, especially skeletal muscle, during fever could also be

directly related to estimating the economic costs of disease in meat production. A comparative study of endotoxin and infection induced fevers would provide valuable insight into the relative importance of endotoxin in the infectious process in sheep. And finally, in the interest of attempting to control the loss of body protein during fever, it would also be valuable to [illegible] effect of commonly used veterinary antipyretic drugs on protein turnover under disease situations.

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APPENDIX 1

SURGICAL PROCEDURE AND PRELIMINARY DATA FOR AMINO ACID FLUX MEASUREMENTS ACROSS ORGANS OF SHEEP

In order to further investigate the changes in plasma amino acid concentrations observed in experiment one, measurement of inter-organ exchange of amino acids was attempted. To acheive this goal, catheters were implanted into six blood vessels: the hepatic vein, the portal vein, a mesenteric vein, the femoral vein, one femoral artery and either the other femoral artery or a mesenteric artery. The purpose of this appendix is to detail the surgical procedures and report some of the preliminary data obtained before the study was abandoned because of a number of technical difficulties.

Surgery Procedure

Three types of catheters were used during this study. Catheter type one was polyvinylchloride (PVC) tubing (0.066 in OD) which had silastic tubing stretched over it using xylene. The tip of the catheter had approximately two mm of silastic protruding over the end of the PVC. Catheter type two was a commercial silastic catheter (Broviac) in two sizes. Catheter type three was large size (0.062 in ID x 0.125 in OD) stiff silastic tubing. Infusion catheters for this phase of the study were made of thin silastic

tubing (0.058 in ID x 0.077 in OD) stretched over PVC tubing using xylene. All catheters except the Broviac type had cut off 16 and 18 gauge needles inserted in the distal end as adapters. All catheters were capped with Luer lock rubber septa.

Surgery with catheter types one and two was done according to the method of Katz and Bergman (1969a). The animal was anesthetized with Halothane (5% initally; 2% for maintenance) and placed on the table on its left side. An incision was made along the lower edge of the ribs using a scalpel. A section of intestine was extracted from the incision following blunt dissection through the subcutaneous muscle layers and peritoneum. After isolation of a large mesenteric vein, ligatures of 2-0 Tichron were placed loosely around it. The ligatures were pulled away from each other in order to occlude blood flow and a small incision was made in the vessel using scissors. The catheter was inserted into the vein and guided to the the portal vein via the mesenteric vein. Once placement was confirmed, the catheter was secured in place by tightening the ligatures on the vein and placing one more ligature around the catheter and mesentery.

A second catheter was placed into another mesenteric vein branch in an analogous fashion. It was not extended as far into the vessel as it was to be used for infusion. The hepatic vein catheter was placed in the left lobe of the liver. The ribs were retracted with a large abdominal

hand held retracter. The liver was grasped and twisted up into the incision. The hepatic vein was located by palpation of the fissure in underside of this lobe. A scalpel blade (No. 11) was used to cut into the vein. The catheter was inserted into the incision in the vein and threaded through the vein to the junction with the inferior vena cava. The catheter was secured into place with a purse-string suture and two or three other sutures around the catheter and liver capsule. The catheters were exteriorized through a stab wound in the right side. The catheters were pulled through a trocar to acheive this end. The catheters were checked to ensure hemostasis and sterile saline was poured into the cavity to minimize adhesion formation. The incision was closed in three layers with 2-0 Dexon or chromic gut. The catheter ends were placed in a plastic bag which was attached to the animal's back using tag cement.

The animal was moved to expose the inner surface of the hindlimbs, which were shaved and scrubbed with Betadine. The leg surface was palpated to locate the saphenous artery and vein. An incision was made distal to these vessels, along the fissure in the muscle groups. Blunt dissection between the muscles revealed the branches of the femoral artery and vein. The skin and muscles were held open with a Weitlaner self-retaining retracter. Both vessels were cannulated in a manner analogous to the gut vessels. The catheters were inserted towards the vena cava and aorta in

order to collect blood from and infuse the entire hindlimb area.

The catheters were either exteriorized to the flank using a trocar as a guide or through a stab wound on the back of the leg. The incision was closed in one layer using 2-0 Dexon. The other leg was incised, and a catheter was inserted into the artery for sampling.

In the animals where the leg catheters were exteriorized on the leg, they were guided to the holding bag using wide tape held in place with t[redacted]nt.

Catheter type three was implanted using the method of Naylor et al. (1985). This is a modification of the original method which facilitates the installation of the hepatic vein catheter.

The sheep was placed on its back after anesthetizing with halothane. A 20 cm incision was made across the midline and up the right side along the edge of the ribs. Blunt dissection with forceps was performed through the subcutaneous muscle and fat layers. The position of the oblique muscle layers necessitated cutting through fibers, but this was minimized. The catheters were inserted and secured using the same method as before, except that a small plastic catheter introducer was used to hold the vessel open during insertion of the catheters.

In order to facilitate insertion of the hepatic catheter the rumen was deflated using a 16 gauge needle surrounded by gauze. The ribs and liver were each lifted

by assistants to allow the surgeon free access to the hepatic vein. An arterial sampling catheter was inserted into a mesenteric artery using a similar procedure to that for the mesenteric vein in some of the animals. The catheters were exteriorized through (three) four separate stab wounds on the upper right side of the animal. They were pulled through the wound using long hemostats. The order of the catheters on the outside of the body was always the same to aid in identification of the vessel sampled. The peritoneum and muscle layers were closed with 2-0 Dexon sutures and the skin was closed with 2-0 silk.

Catheters were installed in the right leg in both the artery and vein using the same procedure as with the other types of catheter. A small catheter introducer was used to facilitate insertion of the tubing in the vessel. The catheters were exteriorized through separate stab wounds on the rear of the leg. If no arterial catheter was installed in a mesenteric artery then one was placed in the left leg.

Tape "butterflies" were wrapped around all of the catheters at the site of exit from the body and stitched to the body with 2-0 silk. The leg catheters had additional butterflies attached and stitched to the skin with 2-0 silk as they were brought up the back of the legs. A catheter bag of rip-stop nylon was stitched to the back of the animal. All of the catheters were placed in the bag and it was tied closed. Eye wound powder was applied to all of the incision sites and the animal was administered 4 mL of

Terramycin and 500 mg naproxen. Terramycin therapy was continued for four days post surgery. The sheep were normally allowed ten days for recovery from the effects of surgery.

The patency of the catheters was maintained by twice daily flushing with 5 mL of heparinized saline (30 IU/mL). For experiments, the sheep were infused with para aminohippuric acid (PAH) to estimate blood flow. In order to estimate blood flow in the hindlimb and viscera simultaneously, two solutions of PAH were infused. A 1% solution was infused into the hindlimb arterial infusion catheter and a 2% solution was infused into the mesenteric vein catheter, both at a rate of 60 mL/h. A priming dose of 10 mL 2% PAH solution was injected prior to infusion to shorten the time required to reach plateau background concentration. PAH concentration in whole blood samples was analyzed by a colorimetric assay (Katz and Bergman, 1969b).

Results

Initially, catheters were placed into four cattle of varying age. These animals developed infections in the subcutaneous catheter tracks probably due to the use of the trocar to exteriorize the catheters. The trocar was long and difficult to maintain sterile throughout the procedure. Trials using endotoxin were conducted on two of these animals (data not shown).

Sheep were implanted with catheter types two and three. Various problems were encountered with these animals, including chewing of catheters, post-surgical systemic infection in one sheep, strangulated bowel in two sheep, inexplicable death in two sheep, and plugging or loss of catheters.

In the few sheep that survived surgery and the recovery period with intact, patent catheters, experiments were conducted to measure the effect of endotoxin induced fever on amino acid flux across the hindlimb, portal drained viscera, and liver. During each experiment, blood was collected at four time points: prior to injection, and at 3,6 and 24 h postinjection. At each sampling, three sets of samples were obtained to minimize variation in the blood flow estimation.

Blood flow measurements were still variable. One sheep had extremely variable blood flow, especially in the hindlimb, as shown in Table A1.1. This appeared to be due to the animal moving during sampling, ie. muscular contraction inhibiting blood flow.

A comparison of preinjection amino acid exchange data for the same sheep in four consecutive experiments separated by one week showed that the sheep were not consistent in the direction or magnitude of flux over the period (Table A1.2). Due to many technical problems and large variability encountered with these preparations, this approach was abandoned.

Table A1.1. Blood Flow measurements from two trials, one animal

Sample	10 d		17 d	
	Femoral Vein+	Portal Vein	Femoral Vein	Portal Vein
1	7299	1844	668	1793
2	2492	4836	409	2391
3	-	-	1451	1618
4	-	1529	-	2004
5	786	3376	-	2084
6	491	1789	-	2505
7	-	2294	3887	1315
8	406	2033	21768	1739
9	439	1570	12093	2338
10	-	994	54420	10523
11	1161	1028	-	-
12	7299	2294	-	-

+ mL/min

- apparently negative flow, not calculated

Table A1.2. Plasma exchange of selected amino acids across the hindlimb and portal-drained viscera at various intervals post-surgery, data from one animal

	Time Post-surgery (d)							
	10		17		24		31	
Amino Acid	HL+	PDV*	HL	PDV	HL	PDV	HL	PDV
Alanine	0.33	1.72	-0.68	MS	MS	3.39	MS	-1.81
Serine	0.28	-0.67	0.28	MS	MS	0.48	MS	-1.94
Glutamine	1.02	7.08	-0.11	MS	MS	11.50	MS	2.18
Glycine	1.98	8.61	1.40	MS	MS	14.40	MS	-4.13
Leucine	0.80	0.15	0.29	MS	MS	6.07	MS	-0.02
Valine	1.01	1.62	0.28	MS	MS	14.62	MS	0.49
Isoleucine	0.54	0.12	0.14	MS	MS	4.13	MS	-0.51

+ hindlimb exchange mmole/h
* portal drained viscera exchange mmole/h
MS missed sample

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APPENDIX 2

TIME COURSE CHANGES IN PLASMA AMINO ACID CONCENTRATIONS DURING ENDOTOXIN INDUCED FEVER

EXPERIMENT 2

Table 2.1. Time course changes in plasma amino acid concentrations - Experiment 2

		Time (h) 0.0	1.5	3.0	4.5	6.0	7.5	9.0	24.0
		µM							
Aspartate	C	4.0	59.2%	52.5%	48.9%	133.4%	61.6%	36.3%	53.0%
	F	4.2	98.6	100.9	115.6	155.7	61.9	53.3	61.7
Glutamate	C	71.2	95.9	87.8	86.5	94.7	89.3	86.1	98.2
	F	66.2	128.8	135.6	154.2A	167.6	100.0	102.9	68.6
Asparagine	C	42.7	84.8	72.6	73.0	62.4	49.5	50.9	76.7
	F	42.6	70.3	55.0	59.2	53.2	32.9	29.2	39.6A
Serine	C	136.4	89.1	80.2	77.4	65.4	61.8	57.7	79.1
	F	114.7	75.5	52.9A	42.9A	34.9A	27.9A	26.4A	31.6A
Glutamine	C	296.9	94.5	84.5	86.0	79.1	77.3	72.9	97.2
	F	304.4	88.0	97.8	121.1	119.3	103.1	95.4	74.9
Histidine	C	49.2	79.1	70.5	73.6	71.3	68.9	72.6	79.4
	F	47.3	128.0	87.3	107.8	88.6	79.2	84.8	73.9
Glycine	C	841.2	98.7	91.1	92.3	89.4	84.8	85.8	122.6
	F	759.6	99.7	87.6	88.6	79.5	59.8A	56.3A	49.3A
Threonine	C	164.2	89.8	79.2	74.6	65.1	57.6	56.2	79.7
	F	177.3	75.5	77.4	63.2	52.1	74.1	67.7	78.6
Citrulline	C	203.3	90.0	84.2	84.4	76.5	65.2	63.4	79.5
	F	187.4	77.1	57.4	58.6	55.5	37.1A	35.0	35.5A
Arginine	C	142.9	87.8	80.1	73.6	64.6	64.1	59.4	100.9
	F	139.1	86.2	62.7	59.3	52.6	34.8	26.6	46.4A

Table A2.1. cont.

		0.0	1.5	3.0	4.5	6.0	7.5	9.0	24.0
Taurine	C	24.4	110.4	78.7	83.6	99.8	94.9	89.1	99.2
	F	19.2	172.9	143.1A	145.6A	146.2	78.7	85.6	123.1A
Alanine	C	173.4	94.8	81.8	83.3	92.8	102.2	102.1	80.9
	F	169.6	115.1	119.0	144.8	149.6	125.7	104.0	59.3A
Tyrosine	C	62.8	93.4	81.1	78.1	76.8	72.2	74.2	81.2
	F	54.4	92.5	85.5	87.6	82.2	57.2	57.7	71.8
Tryptophan	C	44.0	94.3	81.4	72.5	65.7	57.7	56.1	68.5
	F	44.7	87.7	97.2	100.4A	71.8	48.7	30.2	31.3A
Methionine	C	17.7	96.3	80.7	80.8	81.1	74.4	78.8	101.0
	F	16.7	93.1	79.8	74.1	63.6	44.7	38.2	40.2A
Valine	C	210.3	95.1	84.0	81.1	77.1	70.7	75.7	73.1
	F	163.1	107.9	93.8	89.6	76.1	54.1A	45.9A	59.4
Phenylalanine	C	43.8	98.9	84.9	80.0	83.1	81.0	84.1	94.8
	F	40.7	100.0	101.6	104.8A	93.8A	73.6	71.3	145.3
Isoleucine	C	84.7	91.2	80.0	78.0	73.3	67.1	76.8	80.0
	F	72.7	94.2	66.4	57.4	45.6	36.8A	33.2A	45.9A
Leucine	C	116.5	100.6	75.0	84.6	85.8	80.6	94.5	85.7
	F	95.8	125.8	105.7	98.6	81.1	65.6	56.9A	94.5
Lysine	C	103.3	95.2	82.2	80.4	73.4	60.2	70.9	102.0
	F	102.5	94.8	82.6	69.8	51.0	30.7	32.4	45.1

C - Saline trial
F - Endotoxin trial
A - Significantly different from saline trial ($P<0.05$)