

ENERGY AND NUTRIENT UTILIZATION BY THE CALF'S GUT

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Saskatoon, Saskatchewan

By

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ABSTRACT

Calf scours are caused by a variety of infectious agents. Oral rehydration therapy solutions are formulated with the objective of correcting dehydration and acidosis. Currently, oral rehydration therapy does not promote gut healing in diarrheic calves. However, investigators are examining the role of nutrition in promoting gut healing. Previous work has shown that the amino acid glutamine is important in nitrogen transport between tissues and is an indispensable nutrient for rapidly dividing cells such as lymphocytes, fibroblasts and enterocytes. Small intestinal epithelial cells depend mainly on glutamine, glucose, and ketone bodies for their energy under normal physiological conditions. The oxidative substrate preferred by large intestinal epithelial cells appears to be butyrate, followed by acetate, glutamine, and glucose. Research shows that glutamine supplementation increases intestinal protein synthesis. This may be one of the mechanisms by which glutamine exerts its protective effect on gut integrity and mucosal barrier function during critical illness. However, questions concerning the optimum dose and route by which glutamine is to be administered have yet to be addressed.

A surgical model was developed to chronically study the nutrient concentration differences across the portal-drained viscera (PDV) of preruminant calves. A bilateral subcostal approach was used to reach the portal area to provide access for proper placement of an ultrasonic transit time flow probe around the portal vein. The umbilical

vein was used as an entry point for the portal vein catheter. The femoral artery was also catheterized.

In the first experiment, gut metabolism (defined as the PDV) was investigated by intravenous infusion of nutrients in neonatal Holstein calves (n=4, 12.1 ± 2.2 days old). The experimental design consisted of a series of infusions conducted on four different study days in each calf. On the study days, 4 separate 1 h infusions of acetate, glucose, glutamine, saline (control) were administered intravenously via the jugular vein at 200 mmol/L/h in a different order. Venous and arterial blood were collected over the last 15 min of each 1h infusion. Blood flow was also measured. PDV uptake in $\mu\text{mol}/\text{kg}^{0.75}/\text{min}$ was 0.3 ± 1.1 for glutamine and 1.9 ± 3.1 for glucose during saline infusion. During acetate, glucose, and saline infusions, glucose was a greater source of energy than glutamine for the intestine. However, during glutamine infusion, PDV glutamine uptake ($29.9 \pm 11.2 \mu\text{mol}/\text{kg}^{0.75}/\text{min}$) increased significantly which was associated with a rise in ammonia production ($7.0 \pm 0.5 \mu\text{mol}/\text{kg}^{0.75}/\text{min}$). Therefore, glutamine can be used by the neonatal calf intestine and may have other important functions in the enterocytes.

A second experiment was designed to further quantify PDV glutamine utilization by neonatal calves, and to determine if glutamine uptake could be further stimulated either by longer term intravenous infusion or by chronic oral supplementation. PDV metabolism was investigated in four Holstein calves by measuring nutrient uptake during three intravenous infusions of glutamine over a 5 h period after an overnight fast. Prior to the first infusion, calves diet consisted of milk only. Diet was supplemented with oral

glutamine for the second and third infusions. Glutamine was administered via the jugular vein at a rate of 200 mmol/L/h. Venous and arterial blood was collected in duplicate every hour for 5 h. Blood flow was also measured. During glutamine infusion, there was an absolute increase in PDV uptake of glutamine associated with a significant production of ammonia. Feeding glutamine orally did not alter the PDV glutamine uptake.

Glutamine infusion did not increase the PDV uptake of essential amino acids. Neither chronic oral supplementation with glutamine, or infusion for periods longer than an hour, further increased PDV glutamine uptake. Arterial leucine concentration and PDV uptake declined during glutamine infusion suggesting that its supply became limiting. Thus glutamine supplementation may require the provision of a mixture of amino acids to be effective.

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PUBLISHED MATERIAL AND SUBMITTED

The first portion of the literature review (section 2.1) has been previously published in the *Compendium on Continuing Education for the Practicing Veterinarian*, volume 19, number 8, August 1997, S181-S190. This article reviews current oral rehydration therapy in diarrheic calves and gives recommendations for choosing optimal treatments. The second portion of the literature review (section 2.2) has been previously accepted in the *Journal of the American Veterinary Medical Association*. This paper describes PDV glutamine metabolism in healthy and disease states and summarizes the existing knowledge of glutamine supplementation in nutritional therapy. The surgical description of our chronic model for determination of the nutritional requirements of the portal-drained viscera of preruminant calves (section 4.0) has been recommended for publication in the *American Journal of Veterinary Research*. The data presented from the short-term intravenous infusions of glutamine and other nutrients (section 5.0) have been submitted to the *American Journal of Veterinary Research*. The data presented from the 5 h intravenous glutamine infusions (section 6.0) have also been submitted to the *American Journal of Veterinary Research*.

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Nappert G, Zello GA, Naylor JM: Determination of the glutamine requirements of the calves' gut. XIX World Buiatrics Congress, Edinburgh, July 1996, p. 110 in the proceedings of poster presentations.

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**LIST OF ABBREVIATIONS, SYMBOLS
AND ACRONYMS USED IN THIS THESIS**

Standard Abbreviations and Abbreviations and Symbols Used in Medicine

ATP:	adenosine 5'-triphosphate
cm:	centi ($\times 10^{-2}$) meter(s)
$^{\circ}\text{C}$:	degrees Celsius
EDTA:	ethylenediaminetetraacetic acid
et al.:	<i>et alia</i> , and others
g:	gram(s)
g/Kg:	gram(s) per kilogram
h:	hour(s)
i.e.:	<i>id est</i> ; that is
IU:	international unit(s)
IU/g:	international unit(s) per gram(s)
kcal/g:	kilo ($\times 10^3$) calories per gram(s)
kcal/mL:	kilo ($\times 10^3$) calories per milli ($\times 10^{-3}$) liter(s)
kcal/L:	kilo ($\times 10^3$) calories per liter(s)
kcal/Kg:	kilo ($\times 10^3$) calories per kilo ($\times 10^3$) gram(s)
kg:	kilo ($\times 10^3$) gram(s)
L:	liter(s)
mEq/L:	milli ($\times 10^{-3}$) equivalent per liter(s)
mg:	milli ($\times 10^{-3}$) gram(s)
min:	minute(s)
mL:	milli ($\times 10^{-3}$) liter(s)
mg/mL	milli ($\times 10^{-3}$) grams per milli ($\times 10^{-3}$) liter(s)
mm:	milli ($\times 10^{-3}$) meter(s)
mmol/L:	milli ($\times 10^{-3}$) mole(s) per liter
mOsm/L:	milli ($\times 10^{-3}$) osmole(s) per liter
No.:	number
p :	p -value or probability
r^2 :	correlation coefficient; a measure of the strength and direction of a linear association between two variables
SD:	standard deviation
SE:	standard error
SEM:	standard error of the mean
U:	units
$\mu\text{g/g}$:	micro ($\times 10^{-6}$) gram(s) per gram(s)
$\mu\text{g/mL}$:	micro ($\times 10^{-6}$) gram(s) per milli ($\times 10^{-3}$) liter(s)
$\mu\text{g}/100\text{ mL}$:	micro ($\times 10^{-6}$) gram(s) per 100 milli ($\times 10^{-3}$) liters

$\mu\text{mol}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$	micro ($\times 10^{-6}$) mole per kilo ($\times 10^3$) gram power 0.75 per minute
$\mu\text{mol/L}$:	micro ($\times 10^{-6}$) mole per liter(s)
x:	multiplied by
%:	percent or of each hundred
\pm :	plus and minus

Acronyms Used in this Thesis

ACD:	anticoagulant-citrate-dextrose
BW:	body weight
DDW:	double distilled water
DNA:	deoxyribonucleic acid
EGF	epidermal growth factor
Ig:	immunoglobulin
ORT:	oral rehydration therapy
PAH:	sodium para-aminohippurate
PDV:	portal-drained viscera
RP-HPLC	reverse-phase high performance liquid chromatography
TPN:	total parenteral nutrition
WCV:W	Western College of Veterinary Medicine

LIST OF COMMERCIAL PRODUCTS USED

<u>Product Description</u>	<u>Brand name, Manufacturer and Address</u>
Acetate	Sodium acetate, Sigma Chemical Co, St. Louis, MO.
Acetonitrile	PICO-TAG diluent, Waters Corporation, Milford, MA.
Amino acid column	Nova-Pak C ₁₈ , Waters, Millipore Corporation, Milford, MA.
Amino acid method	Pico-Tag Amino Acid method, Waters, Millipore Corporation, Milford, MA.
Ammonia method	A-GENT Abbot ammonia method, Abbot Laboratories, Abbot Park, IL.
Arterial blood pressure transducer	PB-240, Puritan-Bennett Corporation, Wilmington, MA.
Autoinjector	Waters 710 WISP, Waters, Millipore Corporation, Milford, MA.
Biopsy punch	Acu-punch®, Acuderm Inc., Ft. Lauderdale, FL.
Blunt needles	16 ga X 1 1/2 in, Sherwood medical, St. Louis, MO.
Catgut	Chromic gut, Davis & Geck, Cyanamid Canada Inc., Montreal, PQ.
Catheter introducer	Becton-Dickinson, Rutherford, NJ.
Chlorhexidine scrub	Hibitane, Ayerst Laboratories, Montreal, PQ.
Chromatography manager	Waters Millennium version 2.1, Waters Corporation, Milford, MA.
Computer software	Systat®, V. 6.1, SPSS inc., Chicago, IL.
Conform bandage	Kling X Plus, 15 cm X 4.5 cm, Johnson & Johnson Inc., Montreal, PQ.
Co-oxymeter	Ciba Corning Diagnostics Corp., Medfield, MA.
Disposable glass tubes	Aldrich Chemical, Milwaukee, WI.
Elastic adhesive bandage	Elastoplast®, 10 cm width, Smith + nephew, Lachine, PQ.
Filters	Scheicher & Schuell, Keene, NH.
Glucose	D-glucose, Sigma Chemical Co, St. Louis, MO.
Glutamine	L-glutamine, Sigma Chemical Co, St. Louis, MO.
Heparin	Hepalean, Wyeth-Ayerst Canada Inc., Organon Technica, Toronto.
High energy oral electrolytes	Revibe HE®, Langford Laboratories, Inc.
High energy oral electrolytes	Oralytes HE®, Rhône Mérieux Canada, Inc.
High energy oral electrolytes	V-Lytes HE®, Rhône Mérieux Canada, Inc.

High energy oral electrolytes	Enterolyte HE [®] , SmithKline Beecham Animal Health.
HPLC pump	Waters 600 pump, Waters Corporation, Milford, MA.
Injection caps	Intermittent infusion pluge, Sherwood Medical, St. Louis, MO.
Intravenous catheter	Angiochath, Becton Dickinson, Sandy, Utah.
Iron as ferric hydroxide	Dexafer, Vétoquinol Canada Inc., Joliette, PQ.
Microcentrifuge	Mirofuge 11 Centrifuge, Beckman, Instruments, Inc., Palo Alto, CA.
Oral electrolytes	Re-Sorb [®] , SmithKline Beecham Animal Health.
Oral electrolytes	Lectade [®] , SmithKline Beecham Animal Health.
Oral electrolytes	Lectade Plus [®] , SmithKline Beecham Animal Health.
Oral electrolytes	Revibe [®] , Langford Laboratories, Inc.
Oral electrolytes	Oralytes [®] , Rhône Mérieux Canada, Inc.
Oral electrolytes	V-Lytes [®] , Rhône Mérieux Canada, Inc.
Oral electrolytes	Hydra-Lyte [®] , Vet-A-Mix.
Organic acid reversed-phase column	Shodex KC-811, Showa Denko K.K., Tokyo, Japan.
PAH	Sodium para-aminohippurate, Sigma Chemical Co, St Louis, Mo.
Phenylisothiocyanate derivative	PITC, Pierce, Rockford, IL.
Polyglycolic acid sutures	Dexon, Davis & Geck, Cyanamid, Canada Inc., Montreal, PQ.
Pre-heparinized plastic syringe	Smooth-E [™] , Radiometer America Inc., Westlake, OH.
Silastic catheter	Specialties J.P. Arpin LTD, Anjou, PQ.
Silk ligatures	Silk, Ethicon, Somerville, NJ.
Skin sutures	Supramid-0, Serag-Wiessner, Naila, Germany.
Spectrophotometric autoanalyser	Abbott Spectrum system, North Chicago, IL.
Temperature control module	Waters Corporation, Milford, MA.
Wavelength absorbance detector	Waters 486, Waters Corporation, Milford, MA.
Ultrafiltration filter	Ultrafree-MC 10,000 NMWL, Millipore corporation, Milford, MA.
Ultrasonic perivascular flowprobe	8R series, Transonic Systems Inc., Ithaca, NY
Vacuum drier	PICO-TAG work station, Millipore corporation, Milford, MA.
Velour dacron	Meadox, Oakland, Rogar/STB NJ.
Vitamin E/selenium combination	Dystocel, Inc., Montreal, PQ.

1.0 GENERAL INTRODUCTION

Calf diarrhea (scours) is caused by a variety of infectious agents. Oral rehydration therapy (ORT) solutions are formulated with the objective of correcting dehydration and acidosis. Currently, oral rehydration therapy does not promote gut healing in diarrheic calves. However, investigators are examining the role of nutrition in promoting gut healing and it may become possible to speed intestinal healing by adding selected, limiting, nutrients to ORT solutions.

An understanding of the intestinal nutrient requirements is imperative for the proper treatment of severe enteritis, such as calf diarrhea caused by a variety of infectious agents. The amino acid glutamine is important in nitrogen transport between tissues and has been shown to be an indispensable nutrient for rapidly dividing cells such as lymphocytes, fibroblasts and enterocytes. Small intestinal epithelial cells depend mainly on glutamine, glucose, and ketone bodies for their energy under normal physiological conditions, with glutamine being the principal fuel. The oxidative substrate preferred by large intestinal epithelial cells appears to be butyrate, followed by acetate, glutamine, and glucose. Research shows that glutamine supplementation can increase intestinal protein synthesis. This may be one of the mechanisms by which glutamine exerts its protective effect on gut integrity and mucosal barrier function during critical illness. However, questions concerning the optimum dose and route by which glutamine is to be administered have yet to be addressed. The purpose of this thesis was to determine which

nutrients are used by the intestine of the calf and to determine if simple supplementation with these nutrients would promote intestinal metabolism.

2.0 REVIEW OF LITERATURE

2.1 Current practices in oral rehydration therapy for diarrheic calves

2.1.1 Introduction

When veterinary research yields new lifesaving therapies, they are often expensive and inaccessible to farmers. ORT is an exception to this rule and is the most effective therapy for mild or moderately affected diarrheic calves. ORT solutions are formulated to correct or prevent dehydration and acidosis. Such therapy is usually used when a calf is alert enough to drink. Calves with weak suck reflexes and those not adapted to feeding by hand can receive the ORT solution via tube feeding. Various oral rehydration solutions are available. Severely depressed calves that are unwilling to suck often have ileus and are best treated with intravenous fluids. ORT or intravenous fluids currently do not promote gut healing in diarrheic calves. As knowledge of the nutritional requirements of the calf's digestive tract becomes more precise, oral rehydration solutions are constantly being refined.

This chapter reviews current ORT in diarrheic calves and provides recommendations for choosing the best treatment. Differences between the gastrointestinal physiology of newborn calves and that of ruminant animals are discussed.

2.1.2 Physiology of digestion

Although the preruminant calf has a stomach with four compartments, the stomach functions differently from that of an adult. At birth the abomasum is the only functional part and is twice as large as the forestomachs (Leek 1993; Pond et al. 1995a). Milk and saliva are shunted past the reticulorumen and into the abomasum through a tube that is formed by closure of the esophageal groove. The groove consists of two lips that extend from the cardia to the reticulomasal orifice. The groove is closed by a reflex through the glossopharyngeal nerve; the reflex is stimulated by the intake of liquids. Solutions containing sodium ions are effective in closing the groove (Leek 1993).

Milk or electrolytes administered via stomach tube do not contact the pharynx; the reflex closure is not stimulated, and fluid are deposited in the forestomachs. In calves that are less than two weeks old, overflow normally occurs into the abomasum if more than 400 mL of fluid is present in the forestomachs (Chapman et al. 1986). Transition to ruminal digestion begins by approximately four weeks of age; at this time, the calf is capable of achieving an abomasal pH of approximately 2.0 and proteins that are not derived from milk are better digested. The reticulorumen is not fully functional until four months of age (Leek 1993); it does not completely develop dimensions proportional to those of an adult until nine to twelve months of age (Pond et al. 1995a).

Whole cow's milk contains 3% to 4% fat as micelles (triglycerides are the main component of milk fat), 3% to 4% protein (casein accounts for 80% of milk protein), and 4% to 5% lactose; the total dry matter content is 12% to 14% (Table 2.1) (Clive and

Table 2.1. Composition of midlactation whole milk

Parameter	Value
Specific gravity	1.032
Fat (%)	4.0
Total protein (%)	3.1
Casein (%)	2.5
Total immunoglobulin (Ig) (%)	0.09
IgG1 (mg/mL)	0.58
IgG2 (mg/mL)	0.06
IgM (mg/mL)	0.09
IgA (mg/mL)	0.08
Lactose (%)	5.0
Ash (%)	0.74
Calcium (%)	0.13
Magnesium (%)	0.01
Potassium (%)	0.15
Sodium (%)	0.04
Vitamin A ($\mu\text{g}/100\text{ mL}$)	34
Vitamin D (IU/g fat)	0.4
Vitamin E ($\mu\text{g}/\text{g fat}$)	15
Thiamin ($\mu\text{g}/\text{mL}$)	0.38
Riboflavin ($\mu\text{g}/\text{mL}$)	1.47
Vitamin B ₁₂ ($\mu\text{g}/100\text{ mL}$)	0.6
Folic acid ($\mu\text{g}/100\text{ mL}$)	0.2
Choline (mg/mL)	0.13

Adapted from Clive and Besser, 1991.

Besser, 1991; Etgen et al. 1987). Gross energy content is approximately 0.7 kcal/mL. Because digestibility is 95%, the digestible energy content is 0.67 kcal/mL (Naylor 1991). The enzyme that starts the process of milk digestion is salivary lipase, which begins fat hydrolysis (Tomkins and Jaster 1991). Shortly after the milk reaches the abomasum, casein coagulates under the influence of chymosin (or rennin) and pepsin. The fluid whey (which contains proteins, lactose, immunoglobulins, and minerals) is released and passes quickly into the duodenum. Digestion of the abomasal milk clot takes place over the next 12 hours under the influence of chymosin (optimum pH 3.5) with the assistance of pepsin hydrochloride (optimum pH 2.1) and muscular contractions.

In the lumen of the small intestine, the crypt cells secrete chloride ions. This inflow prompts a parallel flow of water and other ions (including sodium) from the blood to the gut. The fluid dilutes the nutrients, facilitating their breakdown by digestive enzymes (i.e., proteases, pancreatic lipases, and lactase) into molecules that are small enough to pass through the intestinal mucosa and into the blood. The mechanism of absorption into the intestinal epithelial cells of the villus and then into the blood depends on the nutrient (Kutchai 1993). The active cotransport system, which operates only when both sodium and glucose are present, is important in transporting most water-soluble nutrients. A preruminant calf has limited ability to digest carbohydrates, except for lactose, a disaccharide of glucose and galactose (Huber et al. 1961).

2.1.3 Energy, and macronutrient requirements

The net energy requirements of the neonatal calf consists of maintenance and growth requirements. Daily maintenance energy requirements in calves have been estimated at approximately at 50 kcal/kg (44.7 to 52.4 kcal/kg) of BW (Blaxter and Wood 1951; Brisson et al. 1975). Energy requirements for growth have been estimated at 3.0 kcal/g gain (2.68 to 3.07 kcal/g gain) (Pugh and Williams 1992; Brisson et al. 1975). Because whole milk contains approximately 0.7 kcal/mL, a 45 kg calf requires approximately 2250 kcal or 3.2 L (7.1% of BW) of milk for daily maintenance. In the first month of life, suckled beef calves drink about 12% of their BW daily as milk, which promotes growth (Boggs et al. 1980; Odde et al. 1985).

In practice, dairy calves are fed daily at 10% BW (0.3 kg/day of gain). Calf starters are also fed, from approximately four days of age until weaning, to provide additional calories for energy growth (Pond et al. 1995b). Most dairy calves are weaned at six to eleven weeks of age (Morrill 1991). Calves can tolerate 16% to 20% BW daily as fresh cow's milk without exhibiting diarrhea or maldigestion (Mylrea 1966a); however, less dry feed will be consumed and rumen development will be slower (Morrill 1991). To increase consumption of dry feed, calves should have access to clean water at all times (Kertz et al. 1984).

The maximum level of lactose tolerance in calves is unknown. Blaxter and Wood (1952) determined that feeding 125 g of lactose twice daily (equivalent to 5 L of milk/day)

caused diarrhea; Huber et al. (1984) found that lactose intake of 190 g twice daily caused no gastrointestinal problems. Variation in the sizes and ages of the calves, as well as the gradual increase in daily intake, might explain the differences.

The protein requirements of calves are influenced by the percentages of digested and absorbed protein retained in the body for maintenance and growth (or biologic value) (Brisson et al. 1975; Radostits and Bell 1970). As the biologic value decreases, digestible protein requirements increase proportionately (Brisson et al. 1975). The daily digestible protein requirement for maintenance is 0.5 g/kg of BW, and the amount required for growth is approximately 220 g/kg of BW gain (Jacobson 1969). Alternatively, the dietary protein requirement in calves is 22% crude protein (based on high digestibility) (Heinrich 1994). The protein requirement for sick calves is unknown.

The main role of fat in animal nutrition is as a concentrated energy source. Lipid has approximately twice the gross energy content of carbohydrates and protein. Providing energy is important because adipose tissue serves as a storage depot and becomes a primary energy source during periods of fasting (Leat and Cox 1980). Both brown and white adipose tissue may be present in neonatal animals (Schoonderwoerd et al. 1986). Most adipose tissue in newborn calves is brown, comprising 2% of the total BW (Alexander et al. 1975). Perirenal fat in calves also can be used as an energy source (Schoonderwoerd et al. 1986).

The unsaturated fatty acids linoleic acid and α -linolenic acid which are precursors to the eicosanoids, cannot be synthesized by mammals and thus are necessary part of the diet. A third fatty acid, arachidonic acid, can be synthesized from linoleic acid but may be required in the diet if levels of linoleic acid are marginal (Groff et al. 1995a). In calves, a level of 10% fat (on a dry-matter basis) is sufficient to supply the essential fatty acids; carry the fat-soluble vitamins A, D, E and K, and supply enough energy to achieve normal weight gain in thermoneutral conditions (Simons and Naylor 1991).

2.1.4 Pathogenesis of diarrhea

Diarrhea in calves that are younger than 30 days of age usually results from stimulation of secretion by intact intestinal epithelial cells or from structural damage to the absorptive intestinal surfaces (Argenzio 1985; Moon 1978). Infections with enterotoxigenic *Escherichia coli*, *Salmonella* species and possibly *Campylobacter* species cause secretory diarrhea in calves (Bywater 1977; Fromm et al. 1974). In enterotoxigenic *Escherichia coli* infection, the small intestinal cells are switched from net absorption of fluid to net secretion of chloride (or chloride and bicarbonate), sodium, and water into the lumen (Bywater and Logan 1974; Forsyth 1981; Tennant 1972). This additional fluid secretion overwhelms the absorptive capacity of the large intestine; diarrhea results. The glucose/glycine-sodium cotransport system (which brings sodium and glucose simultaneously into the cells from the intestinal lumen) remains mostly intact in calves with enterotoxigenic *Escherichia coli* diarrhea but is likely to be less functional in cases of viral

diarrhea in which there is destruction of the absorptive cells (Bywater 1977; Fromm 1974).

Rotavirus, coronavirus and cryptosporidial infections are associated with villous atrophy of the small intestine or colonic ridge atrophy of the large intestine. In human intestine, cryptosporidial infections are more likely to be associated with an enterotoxic than with a cytotoxic mechanism (Guarino et al. 1994). *Salmonella* species also damage the villi. Decreased lactase activity and nutrient malabsorption have been related to villous atrophy (Williams Smith 1962; Woode et al. 1978; Youanes and Herdt 1987). Undigested lactose may aggravate diarrhea by providing additional substrate for the development of intestinal bacterial overgrowth (Youanes and Herdt 1987) or by osmotically drawing fluids into the lumen after fermentation of undigested lactose in the large bowel (Roussel 1983). Diarrhea in calves with villous atrophy thus results from failure to absorb solute and water but can be exacerbated by the osmotic effects of undigested lactose, fat, and bacterial fermentation products (Argenzio 1985; Woode et al. 1978).

Diarrhea in calves results in a net loss of water, sodium, potassium, and chloride as well as nutrients (Argenzio et al. 1984; Bywater and Logan 1974; Forsyth et al. 1981). Water and electrolytes lost into the intestinal lumen come from plasma (Naylor 1987a; Phillips 1985; Phillips et al. 1971). Because milk is a poor source of electrolytes, whole-body depletion of electrolytes, especially sodium, occurs rapidly. Acidosis is most likely in calves that are older than 8 days (Naylor 1987b; Naylor 1989). The reasons are unknown but may be related to the level of absorption of water, electrolytes, and bicarbonate in

calves, which is believed to vary with age. Alternatively, older calves may produce more organic acids as a result of a greater capacity for bacterial fermentation of undigested nutrients.

The severity of acidosis is correlated with the degree of depression and the calf's loss of the ability to stand (Naylor 1989). Acidosis contributes to death resulting from heart failure by decreasing myocardial potassium and elevating extracellular potassium (Fisher 1965; Fisher and McEwan 1967; Phillips and Knox 1969).

2.1.5 Principles of oral rehydration therapy

Hyperkalemic dehydration and acidosis are the major causes of death in diarrheic calves (Phillips and Knox 1969). Hypothermia, hypoglycemia and septicemia are other factors that contribute to the death of affected calves (Phillips and Knox 1969). ORT solutions have been formulated to correct or prevent dehydration and acidosis. Standing beef calves with a suckle reflex are generally treated while still on the dam; if they are separated from the cow for ORT, it is usually for a short period. This maintains the cow's lactation and maternal instincts. Such calves can be coaxed to suck fluid from a bottle, but this is often difficult because they have been trained to suckle the dam. Gastric fill from ORT tends to reduce milk consumption.

Calves with a poor suckle reflex often benefit from ORT, but it may be necessary to administer the electrolytes by stomach tube (Naylor 1996). Concerns that ORT

solutions administered by intubation are not as efficacious as those nursed by calves have not been validated. Comparisons of the route of administration of rehydration solution to diarrheic calves (sucking or intubation) demonstrate slight differences in the rate of absorption of nutrients and electrolytes (Cleek and Phillips 1981). Furthermore, feeding by intubation may be more convenient, particularly in beef calves and depressed calves. Recumbent calves that lack a suckle reflex may have ileus and usually should be treated intravenously (Naylor 1996). Calves with rapidly progressing signs of dehydration also should be treated intravenously because fluids losses may be too rapid to be replaced orally (Naylor 1996). ORT solutions that pool in the forestomach may ferment and produce bloat (Naylor, 1990).

2.1.6 Components of oral rehydration therapy

Various commercial ORT preparations are available (Table 2.2). All are suitable for rehydration because they contain electrolytes and water to correct dehydration as well as glucose to facilitate sodium transport across the mucosa. Rehydration depends on sodium absorption. Sodium can enter mucosal epithelial cells independently, or it can be cotransported with glucose (Ferrante et al. 1988) or amino acids (Kutchai 1993). Water follows sodium; together they expand the extracellular fluid compartment. Amino acids are transported through the brush border by multiple carriers. Many have sodium-dependent mechanisms similar to those of the glucose carrier system; other carriers are independent of sodium (Groff et al. 1995b). Because different carriers are used, increased water and sodium absorption have been demonstrated when amino acids and glucose are used in combination (Armstrong 1987).

Table 2.2: Ingredients in some oral rehydration solutions for use in calves.

Products	Sodium mEq/L	Chloride mEq/L	Potassium mEq/L	Glucose mmol/L	Metabolized Energy Kcal/L	Glycine mmol/L	Citric acid mmol/L	Citrate mEq/L	Bicarbonate mEq/L	Acetate mEq/L	Phosphate mEq/L	Net alkalinizin constituent mEq/L
Entrolyte HE [®]	113	58	25	445	295	33	0	0	80	0	0	80
Re-Sorb [®]	78	78	17	120	90	44	4	1.6	0	0	16	-34
Lectade	73	73	16	112	0.6	41	4	1.4	0	0	15	-33
Revibe [®]	120	50	20	120	108	40	8	3	0	80	5	72
Oralytes V-Lytes												
Revibe HE [®]	120	50	20	422	301	40	8	3	0	80	5	72
Oralytes HE V-Lytes HE												
Hydra-Lyte	85	45	30	368	296	16	0	10	0	60	0	70
Lectade Plus	51	40	21	158	115	20	4	12	0	0	5	17

Maximal water and sodium absorption occur when 110 (Nalin et al. 1970) to 140 mmol/L (Modigliani and Bernier 1971) of glucose is included in the ORT preparation. Lower concentrations of glucose fail to maximize water absorption (Modigliani and Bernier 1971); however, if the concentration of glucose is 260 mmol/L, net water secretion occurs (Modigliani and Bernier 1971). There are conflicting data on the optimum sodium concentration. Sodium concentrations less than 90 mmol/L have been demonstrated to impair absorption (Spiller et al. 1987); in other studies, lower sodium concentrations (2.5 to 60 mmol/L) maximized water and sodium absorption (Lifshitz and Wapnir 1985; Saltzman et al. 1972). The optimum glucose-to-sodium ratio is apparently a 2:1 solution (Lifshitz and Wapnir 1985).

Glycine is the neutral amino acid that is most frequently used in ORT solutions (Naylor 1990). Alanine is also capable of maximizing intestinal water and sodium absorption (Wapnir et al. 1990) but can be harmful to growth and general health of rats with prolong use of large quantities (Chow et al. 1976). Glycine should be added so that the total of glucose and glycine is at least 150 mmol/L (Bywater 1980; Bywater and Wood 1980). Absorption decreases when the total amount of glucose and glycine exceeds 200 mmol/L (Hirschhorn 1982).

Solutions that contain large amounts of glucose are hyperosmolar and are absorbed more slowly than isotonic solutions, but the differences have no effect on hydration or small intestinal fluid composition in normal calves (Bywater et al. 1987).

Hypertonic solutions have been used to treat diarrheic calves (Jones et al. 1984); it has been argued that hypertonicity may be beneficial because it delays gastric emptying (Phillips 1985). However, glucose has been detected in the feces of calves fed high-energy rehydration solutions; this indicates that calves cannot always completely absorb high-glucose loads (Simmons et al. 1985).

In human medicine, cereal-based rehydration fluids have been proven to be superior to conventional glucose-electrolyte ORT in reducing the duration and severity of diarrhea (Thillainayagam et al. 1993). Starch, the dominant component in the cereals, is a large polymer of glucose that is digested into smaller polymers by intestinal amylase and then split by maltase into glucose at the intestinal brush border (Carpenter et al. 1988). Compared with the direct ingestion of an equivalent amount of glucose, this digestive process supplies more glucose molecules without increasing lumen osmolarity (Hirschhorn and Greenough 1991). In addition cereals contain protein, and other components, that may facilitate electrolyte absorption or help meet nutritional requirements.

The ionic composition of an ORT solution also affects absorption. Substituting acetate for part of the chloride doubles fluid and electrolyte absorption (Demigne et al. 1981). Substituting bicarbonate for some of the chloride aids absorption (Sladen and Dawson 1968). Adding citrate or citric acid to sodium chloride-based rehydration solutions (i.e., Re-Sorb[®], Lectade[®], and Lectade Plus[®]; Revibe[®] and Revibe HE[®]; Oralytes[®] and Oralytes HE[®]; and V-Lytes[®] and V-Lytes HE[®]) contributes to sodium and water absorption (Bywater 1977; Bywater et al. 1987; Atfield et al. 1972). Clinical signs

of potassium depletion include weakness and inability to concentrate urine and are known to occur in patients with diarrhea (Milne et al. 1957). Potassium thus is usually added during ORT at a concentration of 10 to 20 mmol/L.

The alkalizing ability is essential in counteracting acidosis during ORT, particularly in treating calves that are depressed or older than 8 days of age (Booth and Naylor 1987; Naylor 1987b; Naylor 1989). ORT should contain 50 to 80 mmol/L of alkalizing agent (Naylor 1992). Acetate, lactate, citrate, gluconate, and bicarbonate are used as alkalizing agents in ORT (Table 2.2). Bicarbonate has the advantage of combining directly with hydrogen ions; however, bicarbonate-rich rehydration solutions (i.e., Enterolyte HE[®]) interfere with milk clotting and reduce milk digestibility in diarrheic calves (Fettman et al. 1986; Heath et al. 1989). Subsequently, they promote bacterial fermentation in the lower intestinal tract (Naylor et al. 1990).

Metabolizable bases (i.e., D-gluconate, acetate, and citrate) are absorbed into the body and then metabolized to carbon dioxide and water, and hydrogen ions are consumed in the process. In healthy calves, bicarbonate and metabolizable bases are equally effective alkalizing agents (Naylor and Forsyth 1986). In calves that require intravenous fluid therapy, metabolizable bases are less effective alkalizing agents than bicarbonate (Kasari and Naylor 1986). Calves that receive ORT are usually in better metabolic condition than are calves that require intravenous therapy, and differences in alkalizing ability are likely to be much less noticeable. Acetate-based solutions (i.e., Revibe[®], Oralytes[®], and V-Lytes[®]) are the best choice for diarrheic calves that are still receiving milk. These solutions have

excellent alkalizing ability and do not interfere with milk clotting in the abomasum. In intravenous fluid therapy trials, acetate results in more rapid alkalization than does lactate (Kasari and Naylor 1986). Citrate (i.e., in Hydra-Lyte[®] and Lectade Plus[®]) is an effective alkalizing agent but is a strong inhibitor of milk clotting because it chelates calcium.

In one study, calves given a rehydration solution that contained an alkalizing agent demonstrated rapid correction of acidosis and 15% mortality rate in the recuperation period, compared with 40% mortality in calves given a rehydration solution without an alkalizing agent (Booth and Naylor 1987). Although oral rehydration solutions reduce mortality and improve fluid and electrolyte balance, as little as 60% of the fluid may be absorbed in patients with severe *Escherichia coli* diarrhea (Guard and Tennant 1986). An increased volume of diarrhea thus is expected in calves treated with oral rather than intravenous fluids.

2.1.7 Nutritional aspects

There is no consensus on the nutritional aspects of ORT for diarrheic calves. Physiologic studies have demonstrated that the ability of the calf's intestine to digest and absorb electrolytes and nutrients differs between healthy (Mylrea 1966a and b; Stiles et al. 1974) and diarrheic (Halpin and Caple 1976; Woode et al. 1978; Youanes and Herdt 1987) states. Healthy calves have considerable reserve capacity for milk digestion, and milk must be given at 20 to 25% of BW before the digestive capacity of the intestines is overloaded (Mylrea 1966b). Diarrheic calves perform better when fed cow's milk rather

than milk replacer (Dalton et al. 1960; Kertz 1977), and rehydration solutions that contain bicarbonate interfere with milk digestion (Heath et al 1989).

Studies with calves fed whole cow's milk demonstrate better weight gains when milk feeding at 10 to 15% of body weight is continued with an appropriate oral rehydration solutions despite the presence of severe diarrhea (Heath et al. 1989; McLean et al. 1972; Naylor et al. 1990). The digestibility of milk replacer is usually inferior to that of whole cow's milk (Naylor 1991). Diarrheic calves may perform poorly on milk replacer; this may be attributable to the lack of abomasal clotting related to the substitution of milk proteins with alternative protein sources (i.e., plant or animal proteins) (Khorasani et al. 1989).

Malabsorption can promote bacterial overgrowth and exacerbates diarrhea in calves that receive large carbohydrate loads (Blaxter and Wood 1953; Youanes and Herdt 1987). This has led to recommendations that diarrheic calves be deprived of milk for 48 to 96 h and fed a comparable volume of rehydration solution (Radostits et al. 1975). A documented problem with prolonged milk withdrawal is the development of cachexia (Schoonderwoerd et al. 1986). Furthermore, calves fed electrolyte solution may lose the ability to produce lactase (St. Jean et al. 1991). In one study of six healthy calves, however, withdrawal of milk and replacement with an oral rehydration solution for three days did not have a significant effect on jejunal mucosal lactase activity (St. Jean et al. 1991). Simple milk withdrawal thus does not result in lactase deficiency. Newly repopulated epithelial cells may not synthesize normal quantities of lactase if milk is

withdrawn from the diet. Our studies indicate that diarrheic calves have generalized malabsorption rather than specific lactose intolerance (Nappert et al. 1993). This might be expected in light of the fact that most enteric infections produce villous atrophy.

Replacing fluid and electrolyte losses must be the first priority in treating diarrheic calves (Tables 2.2 and 2.3). When this objective has been met, allowing the calf to voluntarily consume milk will help support bodily condition and, possibly, immune function and mucosal regeneration. Our studies support the concept that feeding small volumes of whole cow's milk and electrolytes free of bicarbonate (in separate feedings) to diarrheic calves combines the benefits of electrolyte feeding and nutritional support (Nappert et al. 1993). We recommend milk withdrawal while the calf is depressed and not interested in suckling. Bicarbonate-alkalizing ORT is acceptable if the calf is kept from milk and fed with a large amount of glucose (i.e., Enterolyte HE[®]). In most cases, ORT restores a calf's vigor within two days (Naylor 1996). Milk can then be reintroduced in small amounts (i.e., 1 L given two to four times a day).

Milk may be superior to high-energy ORT solutions because it contains protein and a broad range of other nutrients. However, high-energy products must be fed if milk deprivation is long-term. Assuming a 4 L daily intake and 100% digestibility of oral electrolyte nutrients, regular electrolyte solutions (i.e., Re-Sorb[®], Lectade[®], Revibe[®], Oralytes[®], and V-Lytes[®]) supply between approximately 15% and 25% of energy needs. If the calf is not interested in drinking or becomes depressed when reintroduced to milk, a

Table 2.3. Suggested ingredients and analysis for oral rehydration solutions for use in diarrheic calves.

Constituent	Analysis (mmol/L)
Sodium	105-120
Potassium	20
Chloride	50
Phosphate	5-15
Citrate	2-10
Alkalinizing agent	50-80*
Glycine	10-40
D-glucose	110-140
Energy (kcal/L)	90-110
Total Osmolarity (mOsm/L)	300-430

* May be provided as acetate, lactate, citrate, gluconate, or bicarbonate. Citrate and bicarbonate are less suitable if the calf is receiving milk.

high-energy ORT solution (i.e., Enterolyte HE[®], Revibe HE[®], Oralytes HE[®], or V-Lytes HE[®]) can be substituted to support bodily condition. These solutions provide approximately 50% of total daily energy needs if fed twice a day (total intake = 4 L) and approximately 75% if fed three times a day (total intake = approximately 6 L).

Rehydration solutions for ORT that contain mucilage are commercially available. Fettmann (1992) postulated that adding mucilage to an ORT might slow gastric emptying and give the small intestine a better chance to absorb. In addition to improving absorption, mucilage may be a factor in reducing the severity of diarrhea (Verschoor and Christensen 1990). Mucilage passes undigested through the intestine and gives a formed appearance to the feces. This improvement in fecal form is attributable to gelling of liquid and may be mistaken for a real improvement in the condition of the calf. One trial demonstrated no improvement in intestinal glucose absorption as a result of feeding mucilage-containing rehydration solution to diarrheic calves (Naylor and Liebel 1995).

For the future of ORT, the addition of glutamine to ORT will be of interest. The small intestine is usually the major site of glutamine utilization in mammals (Windmueller and Speath 1974 and 1975). Glutamine stimulates sodium uptake by the mucosal cells of the small intestine (Said et al. 1989). Investigations in laboratory animals have demonstrated that glutamine feeding improves growth and repair of the small intestinal mucosa and helps maintain intestinal immune function (Fox et al. 1988; Ardawi 1992).

Our research indicates that glucose and glutamine are important sources of energy for the calf's gut. This research has been performed in healthy calves, but may have implications in promoting gut healing in diarrheic calves (Nappert et al. 1996a). Glutamine has been categorized as a dispensable amino acid (Smith and Wilmore 1990). It may, however, be conditionally indispensable in pathophysiological conditions, such as diarrhea. In one trial, simply substituting glutamine for glycine in ORT solutions did not enhance the treatment of diarrheic calves or shorten the time of mucosal healing (Naylor et al. 1996). Further research thus is needed to determine the requirements for healing in diarrheic calves.

2.1.8 Conclusion

Treatment of diarrhea in calves is based primarily on correcting dehydration and acidosis via the use of oral and intravenous electrolyte-based solutions. The greatest differences among commercial products involve the ability to correct acidosis. Products that contain 50 to 80 mmol/L of alkalizing agent produce the highest recovery rates.

ORT is usually used when a calf is alert enough to drink the rehydration solution. However, calves with poor suck reflex often benefit from ORT (the electrolytes may have to be administered by stomach tube). Recumbent calves with no suckle reflex and/or rapidly progressing signs of dehydration are best treated via intravenous fluids that contain sodium bicarbonate; such calves may have ileus or may be losing fluid rapidly.

Milk withdrawal is most likely to be beneficial if a calf is depressed and has lost the suckle reflex. In such cases, ORT solutions should be administered at a rate of 4 to 6 L/day. As the calf becomes more alert, the amount of the solution can be decreased to 2 L/day and milk can be reintroduced as long as the calf has diarrhea. ORT solutions that contain bicarbonate can be used when the calf is kept from milk; acetate-based solutions which do not inhibit milk clotting, should be used in calves that are receiving milk.

2.2 Intestinal metabolism of glutamine and potential use as a therapeutic agent in diarrheic calves.

2.2.1 Introduction

In mammals, glutamine is the most abundant amino acid in plasma (concentrations of 600 to 900 $\mu\text{mol/L}$) (Windmueller and Spaeth 1974; Bulus et al. 1989; Smith and Wilmore 1990) and is found in high concentrations in cells (Bergstrom et al. 1974). Classically, glutamine was considered essential for cell proliferation in cell cultures (Eagle 1935; Eagle et al. 1956). Analysis of results of subsequent studies revealed that glutamine served as a major substrate for the renal ammoniogenesis pathways (Pitts 1964; Windmueller 1982; Welbourne 1987). It is also a precursor for the synthesis of other amino acids (Windmueller and Spaeth 1974; Kovacevic and Morris 1972; Frisell 1982; Jepson et al. 1988; Hammarqvist et al. 1989) and nucleotides such as ATP, purines, and pyrimidines. Glutamine is avidly consumed as a fuel by a number of rapidly dividing cells, such as malignant cells (Kovacevic and Morris 1972; Reitzer et al. 1979), fibroblasts (Donnelly and Scheffler 1976; Zielke et al 1978), renal tubular cells (Welbourne 1987), vascular endothelial cells (Leighton et al. 1987), reticulocytes (Newsholme et al. 1985a), macrophages (Newsholme and Newsholme 1989) and lymphocytes (Ardawi and Newsholme 1983; Newsholme et al. 1985b; Szondy and Newsholme 1990).

Measurements of the arteriovenous difference across tissues drained by the nonhepatic splanchnic organs of dogs (Addae and Lotspeich 1968; Elwyn et al 1968; Weber and Veach 1979; Souba 1991), sheep (Wolff et al. 1972; Heitmann and Bergman

1978), rats (Windmueller and Spaeth 1974; Ishikawa et al. 1972; Matsutaka et al. 1973; Yamamoto et al. 1974; Hanson and Parsons 1977; Watford et al 1979; Windmueller and Spaeth 1980), and human beings (Felig et al. 1973a and b; Owen et al. 1981) indicate that the small intestine is the principal organ of glutamine consumption. Furthermore, glutamine serves as an important source for both enterocytes (Ashy et al. 1988) and colonocytes (Ardawi and Newsholme 1985; Ardawi 1986) and has a trophic effects on the intestinal mucosa (Souba et al. 1985a).

The small intestine has a central role in glutamine metabolism in normal and in pathophysiologic states. Glutamine supplementation increases intestinal protein synthesis, and this may be a mechanism by which glutamine exerts its protective effect on intestinal mucosa during critical illness (Higashiguchi 1993). Results of studies reveal that glutamine may be essential for maintenance of the structural and functional integrity of the small intestinal mucosa (Souba et al 1985a; Wilmore et al. 1988; Souba et al. 1990b; Fink 1991). Recently, glutamine has been the subject of considerable investigation, because its concentrations in blood and tissues decrease markedly during critical illness (Souba 1984; Souba et al. 1985a; Wilmore et al. 1988), and researchers suggest it may be a conditionally indispensable (i.e., dietary essential) amino acid (Souba 1991; Lacey and Wilmore 1990; Grimble 1993).

This review will describe the interorgan flow of glutamine in healthy and disease states and summarize our current knowledge of glutamine supplementation as part of nutritional treatment in neonatal calves.

2.2.2 Structure and principal metabolic pathways

Glutamine is classified as a neutral dispensable amino acid with a molecular weight of 147.1 (Souba et al. 1990a and b). Glutamine has a pivotal role as a vehicle for the transfer of nitrogen between tissues because of its two readily mobilizable nitrogen groups, an α -amino nitrogen and an amide nitrogen (Frisell 1982; Marliss et al. 1971; Souba 1987) (Figure 2.2.1). The α -amino nitrogen group is involved in carrying ammonia out of cells and in the formation of urea (Souba 1991). The amide nitrogen group is an essential requirement for nucleic acid biosynthesis (Frisell 1982; Martin 1987).

Ammonium ion (NH_4^+) formed from amino acid transamination or deamination can combine with glutamate to form glutamine (Groff et al. 1995b) (Figure 2.2.2). Glutamine is synthesized in all tissues rich in glutamine synthetase, especially muscle and the lungs (Souba 1991). Because glutamine is important in ammonia removal from muscles, its concentration in skeletal muscle, the principal organ of glutamine synthesis and storage, is 30 times greater than the circulating plasma concentration (Bergstrom et al. 1974; Muhlbacher et al. 1984). Studies in dog and humans have also established the liver to be an important site for glutamine production, especially during fasting (Miller et al. 1983; Cersosimo et al. 1987), and periods of acidosis where glutamine serves as a temporary storage for toxic ammonia (Cersosimo et al. 1987; Fine 1982). In liver, kidney, and intestine, glutamine is catabolized by glutaminase, which removes the amide nitrogen to yield glutamate and ammonia (Groff et al. 1995b) (Figure 2.2.3).

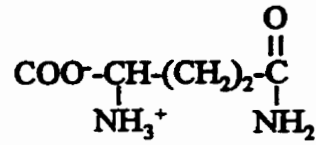


Figure 2.2.1 Structure of glutamine at physiologic pH. Glutamine contains an amide (NH₂) and α-amino (NH₃⁺) nitrogens.

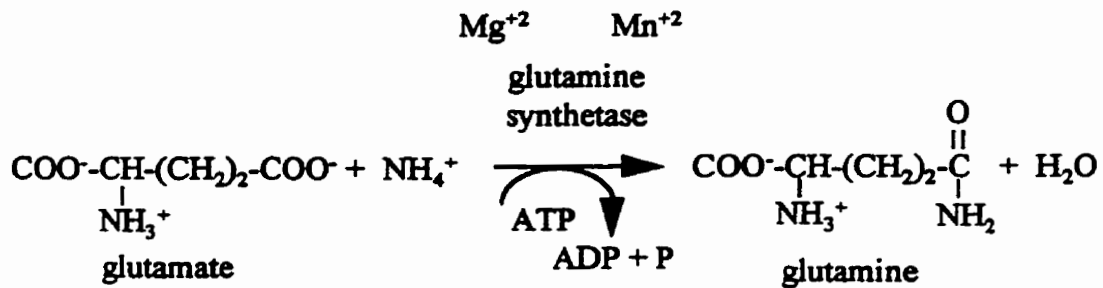


Figure 2.2.2 Synthesis of glutamine from glutamate and ammonium ion. The reaction is catalyzed by glutamine synthetase and requires ATP and magnesium (Mg⁺²) or manganese (Mn⁺²).

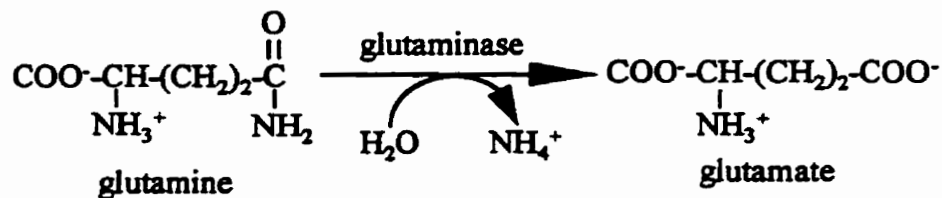


Figure 2.2.3 Catabolism of glutamine.

Gastrointestinal metabolism of glutamine is associated with the production of ammonia by intestine (Weber et al. 1988). The intestine is well suited to metabolize glutamine, because the ammonia produced when glutamine is hydrolyzed by mitochondrial glutaminase is released into the portal blood and extracted by the liver before it reaches the systemic circulation (McFarlane-Anderson et al. 1976). In the small intestine, glutaminase activity is greater than glutamine synthetase activity in mature villous tip cells, as well as in the rapidly dividing mucosal crypt cells (Pinkus and Windmueller 1977). High activity of glutaminase (3 to 6 $\mu\text{mol/h/mg}$ protein) has been demonstrated in duodenal, jejunal, and ileal mucosa of rat (Windmueller and Spaeth 1974; Pinkus and Windmueller 1977; Herzfeld and Raper 1976), dog, cat, hamster, rabbit, and monkey (Windmueller 1982). These glutaminase rich tissues would be expected to be major glutamine users. Glutamine intestinal utilization is entirely from the plasma fraction of the blood, since the glutamine concentration of erythrocytes circulating through the intestine does not change (Windmueller and Spaeth 1975).

In all species studied during high-protein meals, uptake of glutamine by the skeletal muscles (Groff et al. 1995b) and the liver is considerable with net release occurring in the postprandial period (Matsutaka et al. 1973; Yamamoto et al. 1974; Cersosimo 1987). Glutamine and alanine transport two thirds of the circulating amino acid nitrogen content and comprise more than half of the amino acids released from skeletal muscle (Askanazi et al. 1980; Ruderman 1975; Felig 1975). Glutamine leaves skeletal muscle, and is taken up by liver, kidney, intestine, and organs such as the pancreas (Souba 1991) (Figure 2.2.4). Intestinal glutamine metabolism supplies precursors and nitrogenous end products for

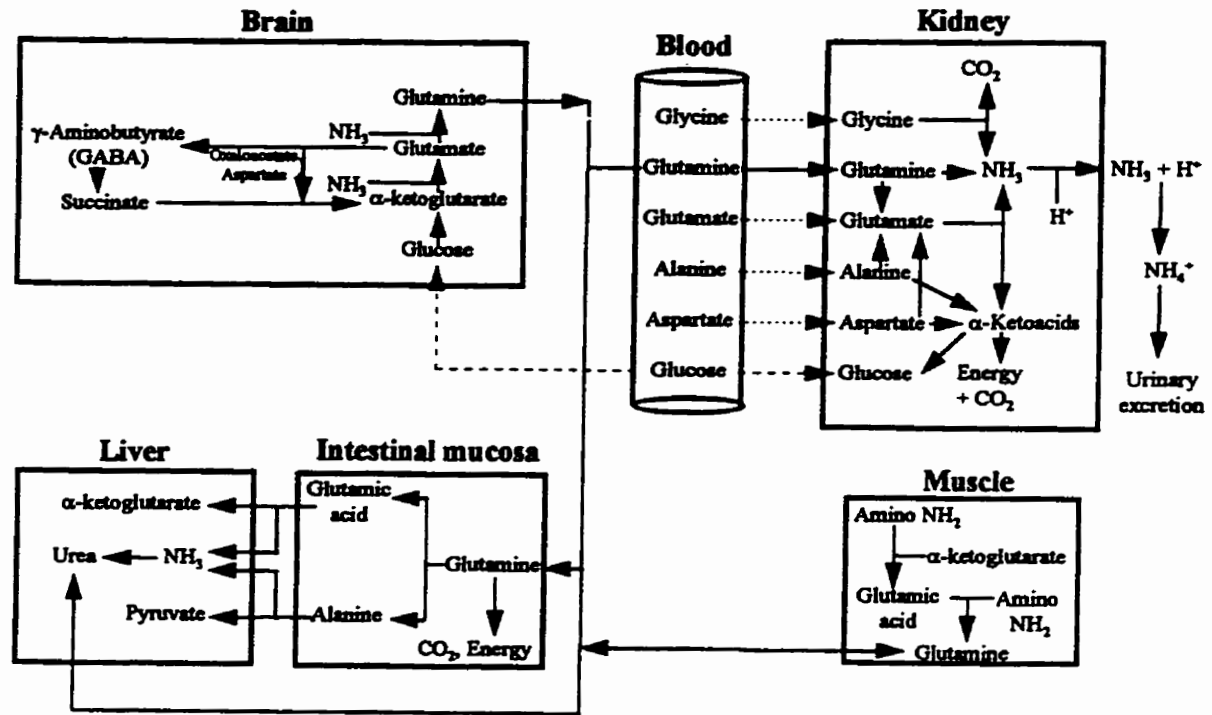


Figure 2.2.4 Glutamine metabolism in selected organs.

hepatic gluconeogenesis and for urea synthesis, respectively (Addae and Lotspeich 1968; Meijer et al. 1985; Sies and Häussinger 1984; Windmueller 1984). Its splanchnic handling differs markedly when compared with alanine. Alanine is produced by the gastrointestinal tract to a small extent, and the major organ of uptake is the liver (Souba and Wilmore 1983; Masola et al. 1985). While alanine is taken up by the liver and serves as a principal gluconeogenic amino acid, glutamine uptake occurs predominantly in the gut. Analysis of results of *in vitro* studies suggest that approximately a fourth of the glutamine extracted by the intestines is converted to alanine, and a major portion of the glutamine is oxidized by enterocytes as a preferred respiratory fuel (Windmueller 1982). Renal handling of glutamine and alanine differs: glutamine (Van Slyke et al. 1943) and other amino acids, particularly glycine (Pitts 1971), are extracted by the kidneys and donate an amino group for renal production of ammonia, whereas alanine (Cahill and Aoki 1975) and serine (Schröck and Goldstein 1981) are released by the kidneys.

When blood glucose reaches the brain, it can be converted to glutamate via reductive amination (Groff et al. 1995b). Whenever there is excessive ammonia in the brain, glutamine is formed through the action of glutamine synthetase. Glutamate must be synthesized in the brain because this amino acid penetrates the neural cell membrane poorly (Groff et al. 1995b). Glutamine, on the other hand, is freely diffusible and can move easily into the blood or cerebrospinal fluid, thereby allowing removal of toxic ammonia from the brain (Groff et al. 1995b).

Glutamine metabolism is complex. Therefore, it is important to keep in mind that the amino acid glutamine is a major nitrogen carrier between peripheral and splanchnic tissue (Windmueller and Spaeth 1980). It is the most abundant amino acid in blood and has been shown to be an essential nutrient for rapidly dividing cells such as lymphocytes, fibroblasts and enterocytes (Newsholme et al. 1985). The intestine is a major organ of glutamine utilization (Windmueller and Spaeth 1974) and skeletal muscle is the main source (Groff et al. 1995b).

2.2.3 Intestinal glutamine uptake

Glutamine use by enterocytes is via the brush border (from the intestinal lumen) or the basolateral membrane (from the plasma fraction of the blood) (Davies et al. 1987; Mircheff et al. 1980). Glutamine uptake across the brush border membrane and basolateral membrane vesicles from the jejunum of human beings (Said et al. 1989; Ghishan et al. 1990; Souba and Copeland 1992), rats (Ardawi 1986; Mircheff et al. 1980; Ghishan et al. 1990; Souba and Copeland 1992; Bradford and McGivan 1982; Kovacevic and McGivan 1984; Ghishan et al. 1989; Taylor et al. 1989; Van Voorhis et al. 1989), dogs (Bulus et al. 1989a and b), horses (Salloum et al. 1993b), and baby pigs (Rhoads et al. 1990) is principally via a sodium-dependent system specific to the transport of neutral amino acids (system B), and, to a less extent, via a sodium-independent pathway similar to system L. Lynch and McGivan (1987) have described a similar system in bovine kidney brush border membrane responsible for the transport of glutamine from the luminal space

to the intracellular space. The relative importance of each of these systems to the overall metabolism of glutamine remains to be determined.

2.2.4 Intestinal energy sources

In rats, small intestinal epithelial cells depend mainly on glutamine, glucose, and ketone bodies for their energy supply under normal physiological conditions (Hanson and Parsons 1977; Windmueller 1984; Kimura 1987), with glutamine being the principal fuel in the fed state (Windmueller 1982; Windmueller and Spaeth 1980; Souba et al. 1985) and ketone bodies in the starved state (Hanson and Parsons 1978; Windmueller and Spaeth 1978). Windmueller and Speath (1978) reported that the jejunum took up glucose from arteries and released lactate, but in a later article they (1980) found that very little lactate was produced from glucose after absorption from the lumen of the jejunum. Whale et al (1971 and 1972) studied glycolysis in the small intestine of sheep and found that glucose was consumed by the small intestinal mucosa and that lactate was one of the products of the glycolytic metabolism. Wolff et al (1972) reported utilization of arterial glutamine by the portal drained viscera of sheep fed alfalfa. Reynolds and Huntington (1988 a and b) reported uptake of butyrate and glutamine by mesenteric-drained viscera of steers fed alfalfa, suggesting that these tissues use ketones and glutamine for energy. Our work shows that glucose and glutamine are important sources of energy for the calf's gut (Nappert et al. 1996a).

In the large intestine, microbial fermentation of dietary constituents, including soluble dietary fibers and unabsorbed starch, leads to the production of short-chain fatty

acids (Wolin 1981). Acetate, propionate and butyrate are the major short-chain fatty acids produced in the large intestine of monogastric mammals (Kass et al. 1980; Cummings 1981; Kennelly 1981; Ehle et al. 1982; Storer et al. 1983; Fleming et al. 1991). Butyrate is the major fuel source for colonic epithelium in human (Roediger 1980; Chapman et al. 1993), in rats (Roediger 1982), and in pigs (Darcy-Vrillon et al. 1993), followed by acetate, propionate, glutamine and glucose (Ardawi and Newsholme 1985; Fleming et al. 1991; Roediger 1982; Darcy-Vrillon et al. 1993). Butler et al. (1990), using isolated colonocytes from rats, reported that butyrate independently decreased pyruvate dehydrogenase activity and reduced pyruvate oxidation through the tricarboxylic acid cycle. These reports (Fleming et al. 1991; Butler et al. 1990; Britton and Krehbiel 1993) suggest that, although large intestine epithelial tissue can metabolize a variety of substrates, preference is shown for butyrate. Absorption of short-chain fatty acids from the large bowel seems to occur via passive diffusion because a linear relationship between the rate of absorption and luminal short-chain fatty acids concentration has been demonstrated in the human colon (McNeil et al. 1978), human rectum (Ruppin et al. 1980), and rat cecum (Yeo 1988).

2.2.5 Intestinal glutamine use in healthy animals

Methods incorporating radiolabeled substrates and quantification of labeled carbon dioxide ($^{14}\text{CO}_2$) as the end product of respiration have been used to assess respiratory fuels of isolated cells. In the small intestine of rats, glutamine carbon may be metabolized intramitochondrially via two principal routes, forming Δ^1 -pyrroline-5-carboxylate or α -ketoglutarate (Figure 2.2.5) (Herzfeld and Raper 1976; Wakabayashi and Jones 1983).

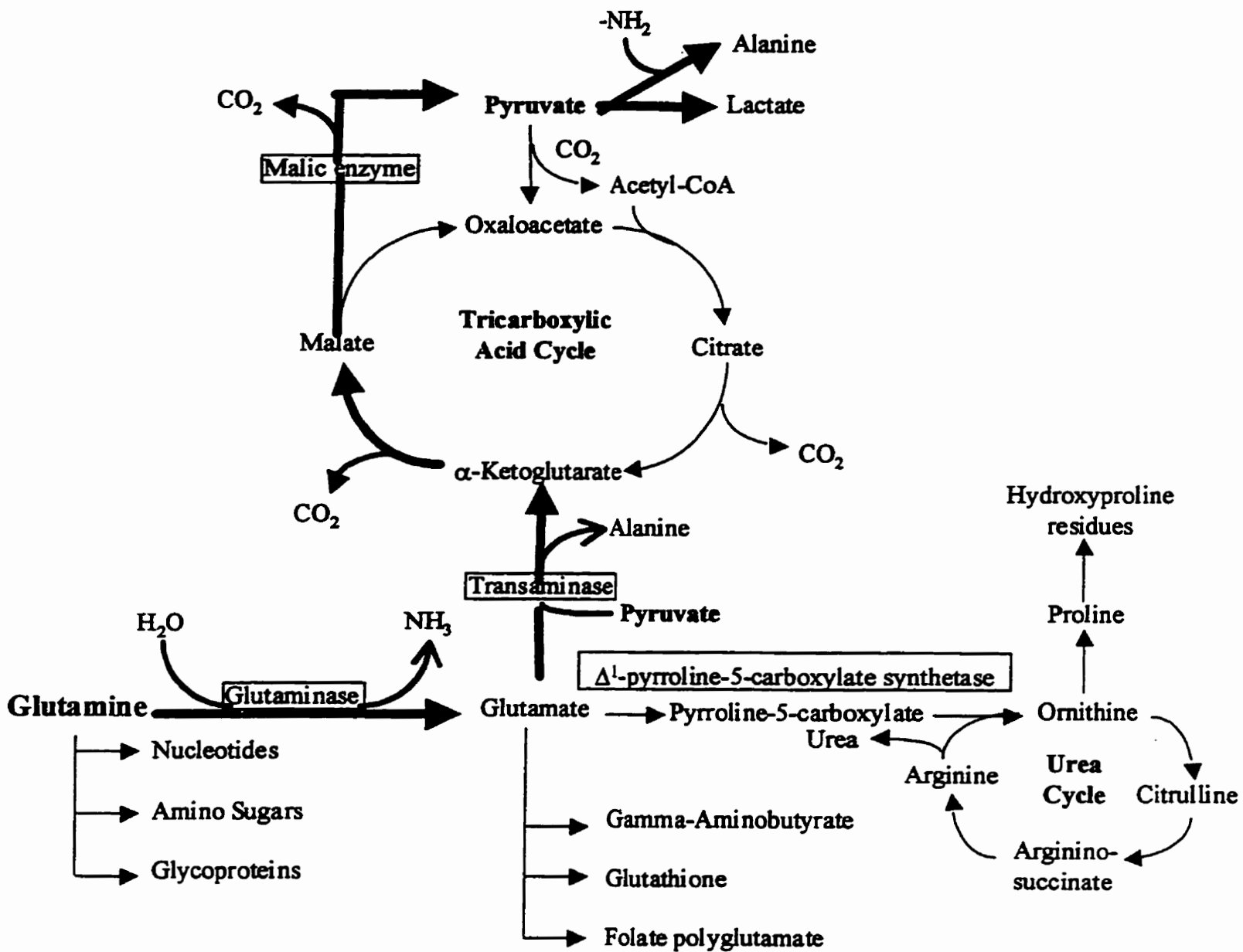


Figure 2.2.5 Intestinal glutamine catabolism. Bold lines indicate pathway of glutaminolysis.

The former pathway leads to formation of proline, ornithine, and citrulline (Windmueller and Spaeth 1981) which are released from small intestine and account for a tenth of the glutamine carbon used (Windmueller and Spaeth 1974). Another 10% to 15% of glutamine carbon is incorporated into tissue protein (Windmueller and Spaeth 1974). α -Ketoglutarate, a citric acid cycle intermediate, accounts for the major proportion of glutamine use (Windmueller and Spaeth 1974; Pinkus and Windmueller 1977; Windmueller 1984). Glutamine provides carbon for alanine synthesis (Watford et al. 1979), and the remaining glutamine carbon is recovered as citrate, lactate, other organic acids, and glucose (Windmueller and Spaeth 1974, 1975, and 1978). Glutamine nitrogen taken up by intestine appears in citrulline (34%), alanine (33%), ammonia (23%), and proline (10%) (Windmueller and Spaeth 1974, and 1980; Windmueller 1982).

Glutamine is similarly metabolized whether it enters the mucosal cells across the brush border from the lumen or across the basolateral membrane from arterial blood (Windmueller 1982; Windmueller and Spaeth 1975). The same product distribution of the glutamine carbons has been detected in various rat intestine preparations by other groups (Matsutaka et al. 1973; Watford et al. 1979) as well as *in vivo* in dogs (Elwyn et al. 1968), sheep (Wolff et al. 1972), and human beings (Felig et al. 1973 a and b). In tissue culture medium containing glucose and glutamine, the contribution of glutamine oxidation to the energy requirement ranges between 30 to 50% (Zielke et al. 1984). Reasons for intestinal epithelial cells requiring glutamine for energy remain unclear, but characteristic of their metabolism is that only a small portion of the glutamine consumed is actually fully oxidized (Mallet et al. 1986). Newsholme et al. (1985a) suggest that, in rapidly dividing

cells, high rates of glycolysis and glutaminolysis are required to permit high rates of cell division and mucosal cell renewal. Glycolysis and glutaminolysis generate energy and also provide precursors for macromolecular synthesis to specific regulators to permit high rates of proliferation when required (Newsholme et al. 1985a).

2.2.6 Intestinal glutamine use in the catabolic state

In dogs, starvation for periods of < 4 days is associated with substantial adaptations in glutamine metabolism by the intestines, despite a lack of substantial changes in arterial glutamine concentration (Miller et al. 1983). Glutamine use by the intestinal tract increases, while the liver switches from uptake to release such that the overall effect is of glutamine balance across the splanchnic viscera. Circulating concentrations of glutamine are diminished after surgical stress despite augmented release from skeletal muscle, which may be depleted of more than half of its glutamine content (Souba et al. 1985b and c; Ruderman 1975; Felig 1975; Souba and Wilmore 1983; Aulick and Wilmore 1979). Intestinal glutamine consumption is increased by three fourths in dogs after a standard laparotomy (Souba and Wilmore 1983) and appears to be mediated, in part, by glucocorticoid hormones (Souba et al. 1985b). After glucocorticoid treatment, intestinal glutamine use increases more than twofold, and the intestine switches from an organ of glucose uptake to one of glucose release (Souba et al. 1985b).

During sepsis and endotoxemia, intestinal glutamine use decreases slightly (Souba et al. 1990a and c; Salloum et al. 1991), but intestinal lymphocytes and macrophages continue to use large amounts of glutamine (Ardawi and Newsholme 1983; Newsholme

and Parry-Billings 1990; Newsholme and Carrié 1994). Glutamine is essential for lymphocyte proliferation in response to antigenic challenge, as a precursor for nucleotide biosynthesis, and as a major energy source (Newsholme and Parry-Billings 1990). Analysis of results of cell culture studies revealed that failure to supplement culture media with glutamine impairs the ability of lymphocytes to respond to mitogenic stimulation (Taudou et al. 1983). The reduction in intestinal glutamine use appears to be cytokine mediated (e.g., interleukin-1) and associated with the decrease in mucosal glutaminase activity, and increased passage of bacteria and microbial products from the intestine to other organs (Salloum et al. 1991; Austgen et al. 1992). Therefore, during sepsis, bacteria and endotoxins can more readily translocate the mucosa and stimulate macrophages to release monokines (tumor necrosis factor and interleukin-1) that activate the pituitary-adrenal axis (Souba et al. 1990a and c). Release of cortisol is believed to accelerate muscle glutamine release and enhance intestinal lymphocytes use of glutamine as well as supporting renal ammoniogenesis (Souba et al. 1990a and c). If a catabolic state is prolonged, tissue consumption of glutamine typically exceeds skeletal muscle production, and plasma and intracellular concentrations of this amino acid decrease. In this situation, the lungs play an important role by releasing additional glutamine (Souba et al. 1990a; Plumley et al. 1990).

Evidence exists showing that epidermal growth factor (EGF) is required for development and maintenance of the intestinal epithelium, and exogenous administration of EGF enhances cell replication and enteropeptidase activity (Ulshen et al. 1986; Hiramatsu et al. 1985). EGF is also implicated in the regulation of amino acid transport

activity in jejunal brush border membrane vesicles (Salloum et al. 1993a). This hormone is resistant to gastrointestinal enzymatic digestion (Taylor et al. 1974), is secreted directly into the duodenum by Brunner's glands and, also, gains access to the gastrointestinal tract through the salivary and biliary routes (Ulshen et al. 1986). The most global effect of EGF on the intestinal mucosa is a generalized stimulation of DNA synthesis (Yeh et al. 1981). Studies suggest that the effect of glutamine and EGF on the small intestine and colonic mucosa are additive (Jacobs et al. 1988; Swaniker et al. 1995).

Controversies exist regarding the contribution of small intestine to glutamine metabolism in acidotic conditions. There are reports of increased (Addae and Lotspeich 1968; Heitmann and Bergman 1978), decreased (Lund and Watford 1976), and unaltered (McFarlane-Anderson et al. 1976; Shröck and Goldstein 1981; Hanson and Parsons 1988; Welbourne et al. 1986a; Tizianello et al. 1978) intestinal glutamine metabolism. Baverel and Lund (1979) suggested the use of glutamine for the urea cycle diminished in an acidotic state and that the liver released glutamine for transport to, and uptake by, the kidneys. Enhanced glutamine efflux from skeletal muscle also is evident during periods of acidosis (Phromphetcharat et al. 1981). In clinically normal dogs, urinary ammonium is produced at a rate nearly equivalent to that of glutamine plasma extraction, whereas urinary ammonium production is almost two-fold greater than the rate of glutamine plasma extraction during acidosis (Halperin and Ching-Chen 1987). Therefore, the rate of glutamine metabolism in the kidneys of clinically normal dogs and dogs with chronic metabolic acidosis was influenced by the plasma glutamine concentration (Halperin and Ching-Chen 1987). Results of studies indicated an increase in renal glutamine uptake and

urinary ammonia production during acidosis for dogs (Cersosimo et al. 1987; Vinay et al. 1989), sheep (Heitmann and Bergman 1978), and rats (Welbourne et al. 1986b; Krebs et al. 1980). However, results are conflicting for human beings (Waterlow et al. 1994).

2.2.7 Glutamine supplementation and therapeutic considerations

Glutamine is included in manufactured diets only at the amounts characteristic of its concentration in plant and animal protein (about 3 to 10% of total amino acids) (Lacey and Wilmore 1990; Swails et al. 1992), because it has been categorized as a dispensable amino acid (Lacey and Wilmore 1990; Souba et al. 1990; Meister 1980). However, for the maintenance of intestinal mucosal metabolism, structure, and function, glutamine is believed to be a conditionally indispensable amino acid during illness associated with inflammation and injury (Windmueller and Spaeth 1974; Windmueller 1982; Souba 1991; Souba et al. 1985a; Lacey and Wilmore 1990; Ziegler et al. 1992).

Some investigations in laboratory animals have indicated that glutamine supplementation in feed improves growth and repair of the small intestinal mucosa, and helps maintain intestinal immune function (Ulshen et al. 1986; Burke et al. 1989; Alverdy 1990). Oral administration of glutamine to rats revealed that glutamine helps maintain the integrity of the intestinal mucosa, reduces the prevalence of bacterial translocation (Karatzas et al. 1991; Gianotti et al. 1995), and decreases morbidity and mortality after abdominal irradiation (Karatzas et al. 1991; Klimberg et al. 1990a). After partial exsanguination, glutamine infusion restores intestinal blood flow (Flynn et al. 1992). Glutamine also promotes repair of injured intestine (Fox et al. 1988), possibly through its

stimulation of proliferative events including enhanced sodium-hydrogen ion exchange and ornithine decarboxylase activity, which has been shown in several cell lines (Chen and Canellakis 1977; McCormack et al. 1990; Rhoads et al. 1994). Chemotherapy and radiotherapy for treatment of animals with cancer cause devastating injury to the intestinal mucosa (Neu et al. 1996). Enterocolitis associated with abdominal radiation in rats was markedly diminished with a glutamine-enriched diet given before radiotherapy (Klimberg et al. 1990b). Glutamine supplementation may be beneficial, therefore, in the treatment of severe enteritis.

A glutamine-enriched diet increases renal arginine production (Houdijk et al. 1994; Welbourne 1995), another amino acid required for an adequate host response to injury (Barbul 1986; Barbul et al. 1990; Prior and Gross 1995). Arginine is generated in the proximal convoluted tubule of the kidneys by the conversion of circulating citrulline (Featherston et al. 1973; Dhanakoti et al. 1990). The most important source for circulating citrulline is the intestine (Windmueller and Spaeth 1981; Wu et al. 1994), where the production of circulating citrulline is dependent upon the continual metabolism of circulating or luminal glutamine (Windmueller and Spaeth 1975 and 1978). Little of the circulating citrulline released into portal blood is taken up by the liver; instead, it becomes the sole precursor for renal arginine synthesis (Windmueller and Spaeth 1981). Glutamine and arginine promote the release of pituitary growth hormone (Welbourne 1995) and nitric oxide, a free radical molecule with several servoregulatory and cytotoxic functions (Hibbs 1991; Moncada 1992; Nathan 1992). It has been speculated that growth hormone and

glutamine or arginine may cause anabolic synergy when given to human beings with human immunodeficiency virus infection (Neu et al. 1996).

In rats, glutamine-enriched total parenteral nutrition (TPN) solutions improved intestinal immune function (Burke et al. 1989; Alverdy 1990; Alverdy et al. 1992), substantially decreased villous atrophy associated with use of regular TPN solutions (Hwang et al 1987; Tamada et al. 1992; Platell et al. 1993; Kaibara et al. 1994), and improved nitrogen retention in tumor-bearing rats during chemotherapy without enhancing tumor growth (Grant and Snyder 1988). Simultaneously, glutamine-enriched TPN solutions stimulated intestinal glutaminase activity and glutamine consumption (Klimberg et al. 1989). However, in unstressed rats, the supplementation of TPN solutions with glutamine did not seem to improve intestinal barrier function or mucosal immunity (Spaeth et al. 1993). Glutamine supplementation improved survival rates in septic mice and prevented sepsis-induced multiple system organ failure (Neu et al. 1996). In baby pigs, glutamine supplementation of TPN solutions did not completely prevent TPN-associated intestinal mucosal atrophy (Burrin et al. 1994). In human patients receiving bone marrow transplants, glutamine-enriched TPN solutions decreased the incidence of clinical infection and microbial colonization (Ziegler et al. 1992). In addition, the mean duration of hospitalization was less than that of the control group.

Studies have failed to reveal toxicoses associated with glutamine-supplemented TPN solutions. Such studies have been carried out in human volunteers (Lowe et al. 1990; Ziegler et al. 1990), dogs (Souba 1984; Souba et al. 1985), rats (Salloum et al.

1993a), and calves (Nappert et al. 1996a). However, in baby pigs, glutamine supplementation to TPN solutions promotes sodium reabsorption in renal tubules and leads to disturbances in water balance caused by extracellular fluid expansion (House et al. 1994). Concerns exist about intravenous infusions of glutamine because of the production of ammonia during its metabolism, but increased circulating concentrations of ammonia have not been reported. Administration of glutamine to animals with hepatic shunt might be contraindicated (Dimski 1994).

General supplementation of TPN solutions with glutamine does not seem necessary but a biochemical rationale for glutamine supplementation in parenterally administered solutions exists, specially in critically ill patients. However, its unfavorable chemical properties, such as instability (Meister 1956; Herskowitz et al. 1990) especially during heat sterilization and storage, and limited solubility (3 g/100 mL at 20°C) hamper its use as parenteral nutrition substrate in routine clinical settings (Fürst et al. 1990; Khan and Elia; Khan et al. 1991). The drawback of glutamine instability can be overcome by the use of alanine-glutamine or glycine-glutamine dipeptides (Fürst et al. 1990; Stehle et al. 1989; Jiang et al. 1993). Glutamine dipeptides are highly soluble in commercially available amino acid solutions, have a long shelf-life, and are stable after sterilization at 121°C (Stehle et al. 1984). Research results also indicate that crystalline glutamine solutions can be a safe, effective means of providing glutamine parenterally when cost is not a factor, provided the solutions are not stored for long periods and are sterilized by cold filtering (Lowe et al. 1990; Stehle et al. 1989).

2.2.8 Glutamine use in diarrheic calves

In diarrheic calves, oral rehydration therapy (ORT) is formulated with the objective of correcting dehydration and acidosis (Nappert et al. 1997a). Standard oral rehydration solutions generally contain glucose and glycine to replace fluid deficits in diarrheic calves by stimulating absorption across separate Na^+ -coupled carriers in the apical membrane of the intestine (Nappert et al. 1997a). Currently, ORT does not aim to promote intestinal healing in diarrheic calves. Glutamine has been considered as a promising component of ORT, because it stimulates sodium uptake by the mucosal cells of the small intestine (Said et al. 1989) and may aid in intestinal tissue repair (Souba et al. 1990b). Some studies with baby pig jejunum indicated that glutamine stimulated both sodium ion and neutral sodium chloride absorption in control and rotavirus-infected baby pigs (Rhoads et al. 1991). However, high concentrations are required to enhance absorption; therefore, improvement of oral rehydration solution with glutamine supplementation may be limited (Rhoads et al. 1991).

Analysis of results of our studies confirm that glutamine has a role in intestinal mucosal function of neonatal calves (Nappert et al. 1996a). Infusing glutamine (200 mmol/L/h) intravenously increases the intestinal uptake of glutamine. This result would indicate that glutamine may stimulate cell growth and may be important in promoting intestinal healing. Diarrheic calves often have bacteremia (Fecteau et al. 1997). The high frequency of septicemia in calves greater than one week of age may be explained by injury to their gastrointestinal tract, which predisposes to translocation of opportunistic bacteria in the intestine. The countercurrent blood circulation in the intestinal villi make them

extremely susceptible to damage from lack of oxygen when blood flow is low (Phillips 1985). In our study, most of the intestinal oxygen consumption was used to metabolize nutrients from the intestinal lumen. Therefore, nutrient metabolism from the intestinal lumen was more important than nutrient metabolism from the vascular system. This finding suggests that ORT solutions are more important than intravenous administration of fluid for providing nutritional support to diarrheic calves. Our research primarily focused on the nutritional uptake of the intestine of neonatal calves and in calves receiving milk proteins from whole-milk of cows, because these contain particularly high quantities of glutamate and glutamine (20% of the total amino acids content) (Davis et al. 1994). A better understanding of the nutrients used by intestinal tissues may allow us to support the concept that feeding small volumes of whole milk and electrolytes free of bicarbonate (in separate feedings) to diarrheic calves combined the benefits of electrolyte feeding and nutritional support (Nappert et al. 1993). Results of one study indicated that substituting glutamine (40 mmol/L) for glycine in ORT solutions did not cause improvement of diarrheic calves or decrease the time needed for mucosal healing (Naylor et al. 1997). However, in another study, high-glucose (378 mmol/L) oral rehydration solutions containing glutamine (30 mmol/L) were more effective in correcting plasma, extracellular fluid, and blood volume in diarrheic calves than high-glucose amino acid-free solutions (Brooks et al. 1997). Various nutrients combined with glutamine might be more successful, because glutamine supplementation increases the use of other amino acids (Nappert et al. 1996a). Therefore, additional research is needed to determine the requirements for intestinal healing in diarrheic calves. In future studies, the choice of the appropriate control will also have to be given careful thought. In human medicine, use of

cereal-based rehydration fluids causes dramatic improvements, compared with conventional glucose electrolyte oral rehydration treatment, by reducing the duration and severity of diarrhea (Thillainayagam et al. 1993).

2.2.9 Conclusion

Glutamine has a role in intestinal mucosal function and metabolism. Results of several studies indicated that endogenous glutamine may not be sufficient to meet metabolic needs during critical illness. Glutamine supplementation could provide a new approach to promote intestinal healing when treating animals with enteritis. However, additional studies must be performed before there is sufficient information to properly recommend that glutamine supplementation is safe and effective in clinical treatments.

3.0 RATIONALE AND OBJECTIVES

Diarrhea is a major problem for calf producers. Treatment of diarrheic calves mainly relies on electrolyte solutions to replace the fluid lost during the diarrhea and on correcting acidosis through the use of oral and intravenous electrolyte based solutions. Antibiotics are also used, but they only work against a few of the many causes of diarrhea. At the present time, these treatments do not promote gut healing in diarrheic calves. Instead, they keep the calf alive so that the body can slowly heal itself. We have shown that calves with diarrhea have damage to the lining of the gut and reduced absorption of nutrients. We know that holding animals off feed results in atrophy of the gut. If we knew which nutrients the gut uses, we might be able speed healing by adding the required nutrients to an electrolyte mix. As our knowledge of the nutritional requirements of the calf's gut becomes more precise, oral rehydration solutions could be refined. The purpose of our study was to determine the requirements for glutamine, glucose, and acetate by the neonatal calf's gut using an arteriovenous difference technique.

3.1 Objective of the surgical model

The relative inaccessibility of the portal vein in preruminant calves has made studies of gut utilization difficult. The purpose of our study was to develop a surgical model to study the nutrient concentration difference across the portal-drained viscera of preruminant calves over a two-week period. The bilateral subcostal approach has never been described to reach the portal area in calves. Surgical techniques for measuring blood flow with an ultrasonic flow probe have not been reported in preruminant calves. The

surgical model was developed and used in nine healthy preruminant male Holstein calves. These results may have implications in the development of treatment to promote gut healing in diarrheic calves.

3.2 Objective of the short-term intravenous study

The role of glutamine as an energy source and a precursor for amino acid synthesis in the gut of neonates, such as the preruminant calf, is not clear. The objective of this study was to quantify PDV glutamine utilization by neonatal calves and to assess the relative importance of glutamine, glucose, and acetate as fuels for the enterocytes.

3.3 Objective of the long-term intravenous study

In the previous study, we showed that a short-term intravenous infusion of 200 mmol/L of glutamine over a period of an hour resulted in increased portal-drained viscera (PDV) glutamine uptake in association with increased PDV ammonia production and a tendency for oxygen consumption to increase. The objective of this study was to quantify further portal-drained viscera glutamine utilization by neonatal calves, and to determine if glutamine uptake could be further stimulated either by longer term intravenous infusion or by chronic oral supplementation.

4.0 Description of a chronic surgical preparation to determine the nutritional requirements of the portal-drained viscera of preruminant calves.

4.1 Introduction

Study of the nutritional requirements of the gut *in vivo* often requires catheterization of the major blood vessels that supply and drain the gut. PDV blood flow, measured with an ultrasonic flow probe, and arteriovenous concentration differences across the gut can be used to calculate net uptake of nutrients. The technique can be combined with tracer infusions to determine the end products of PDV substrate utilization. These studies depend on successful placement of catheters and flow probes. Prevention of inflammation associated with peritonitis and/or sinus tract infection is extremely important to keep the experimental model relevant. There is little information in the literature as to the type, dimension, and best location for chronic intestinal catheterization and flow probe placement in the preruminant calf. Catheterization of the portal vein has been reported in calves via the twelfth right rib resection (Conrad et al. 1958; McGilliard 1971; McGilliard and Thorp 1971) and via the umbilical vein (McGilliard 1968). Portal blood flow rates have been measured in calves by isotope or dye dilution (Conrad et al. 1958; McGilliard et al. 1971; Fries and Conner 1961), thermodilution (Bensadoun and Reid 1965), and the doppler shift principle (Carr and Jacobson 1968). Surgical techniques for measuring blood flow with an ultrasonic flow probe have not been reported in preruminant calves. The purpose of our study was to develop a surgical model to study the nutrition of the calf's gut over a two-week period. The preparation of specialized blood sampling catheters and

blood flow probes, surgical preparation, pre- and postoperative care, catheter maintenance, and possible complications are described. This model has been developed successfully on healthy calves, but the study may have implications for the study of diarrheic calves.

4.2 Materials and methods

4.2.1 Implant Preparation

Two silastic catheters, 100 cm in length (diameters in mm: portal vein, 1.57 inside diameter X 3.17 outside diameter; femoral artery, 1.01 inside diameter X 2.16 outside diameter) and one 8 mm ultrasonic transit time flow probe for the portal vein, were cleaned with chlorhexidine scrub and rinsed with distilled water. Two-layer double velour dacron cuffs were attached to the skin ends of the catheter (Figure 4.1) and to the Konigsberg button of the flow probe (Figure 4.2) as previously described (O'Brien et al. 1991). The skin grows into the dacron from above and below sealing off the exit site wound. This device has minimized the incidence of infections developing at the skin interface with subsequent sinus tract infection and possibly systemic infection in dogs (O'Brien et al. 1991).

4.2.2 Calves

Nine male Holstein-Friesian calves were obtained at 4.5 ± 3.5 (mean \pm SD) days old and fed 10% of their body weight daily of whole cows milk in two feedings. Body weight was recorded weekly. A day before the surgery, a physical examination and a complete blood cell count were performed. The calves received vitamin E (3 IU/kg) and

Figure 4.1. Construction of a two-layer double velour dacron flange adhered together and to the catheter with medical grade silastic adhesive to prevent sinus tract infections.

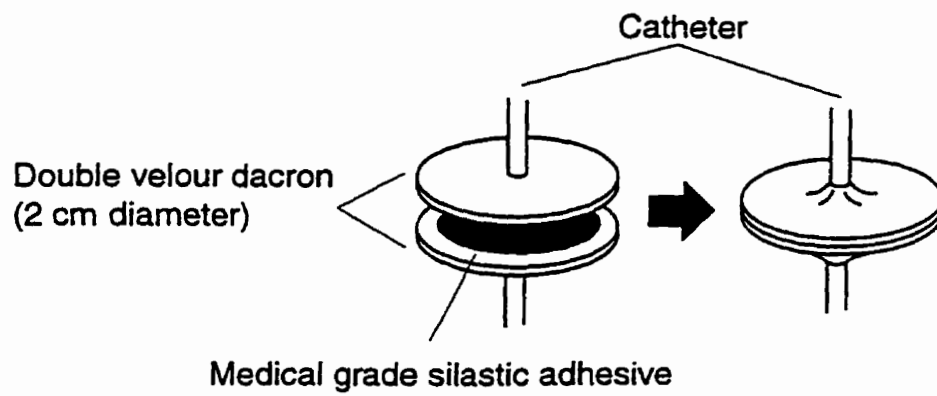
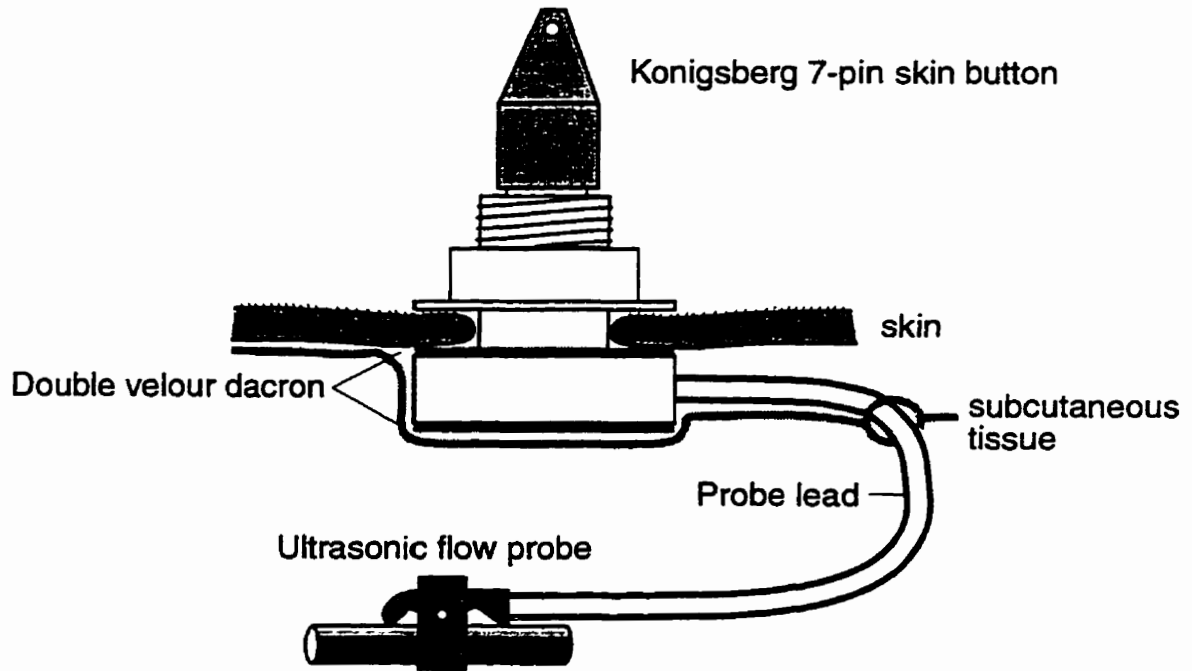


Figure 4.2. Construction of a two-layer double velour dacron flange adhered to the Konigsberg skin button of the probe lead with medical grade silastic adhesive.



selenium (0.07 mg/kg) combination, iron (4 mg/kg) as ferric hydroxide, and ceftiofur (1 mg/kg) intramuscularly a day before the surgery. Pre-operative analgesia consisted of intramuscular injections of flunixin meglumine (1.1 mg/kg/day) at 24 h and at 2 h before the surgery.

After an overnight fast, a mixture of diazepam (0.5 mg/kg) and ketamine (5 mg/kg) was administered through a 14-gauge jugular catheter. When the animal was sedated, the calf was intubated orotracheally and maintained at 2% halothane in 100% O₂ using intermittent positive-pressure ventilation. The medial aspect of the right thigh, the abdomen and the right paralumbar fossa were clipped and scrubbed with 10% povidone iodine topical solution and isopropyl alcohol. The calves were placed in left lateral recumbency for the subcutaneous placement of the catheters and the flow probe, and then moved to dorsal recumbency for catheterization of the femoral artery and portal vein, and placement of the flow probe around the portal vein. The calves were kept on a circulating hot water blanket to maintain their body temperature during anesthesia. Direct arterial blood pressure was monitored with a transducer.

4.2.3 Surgical technique

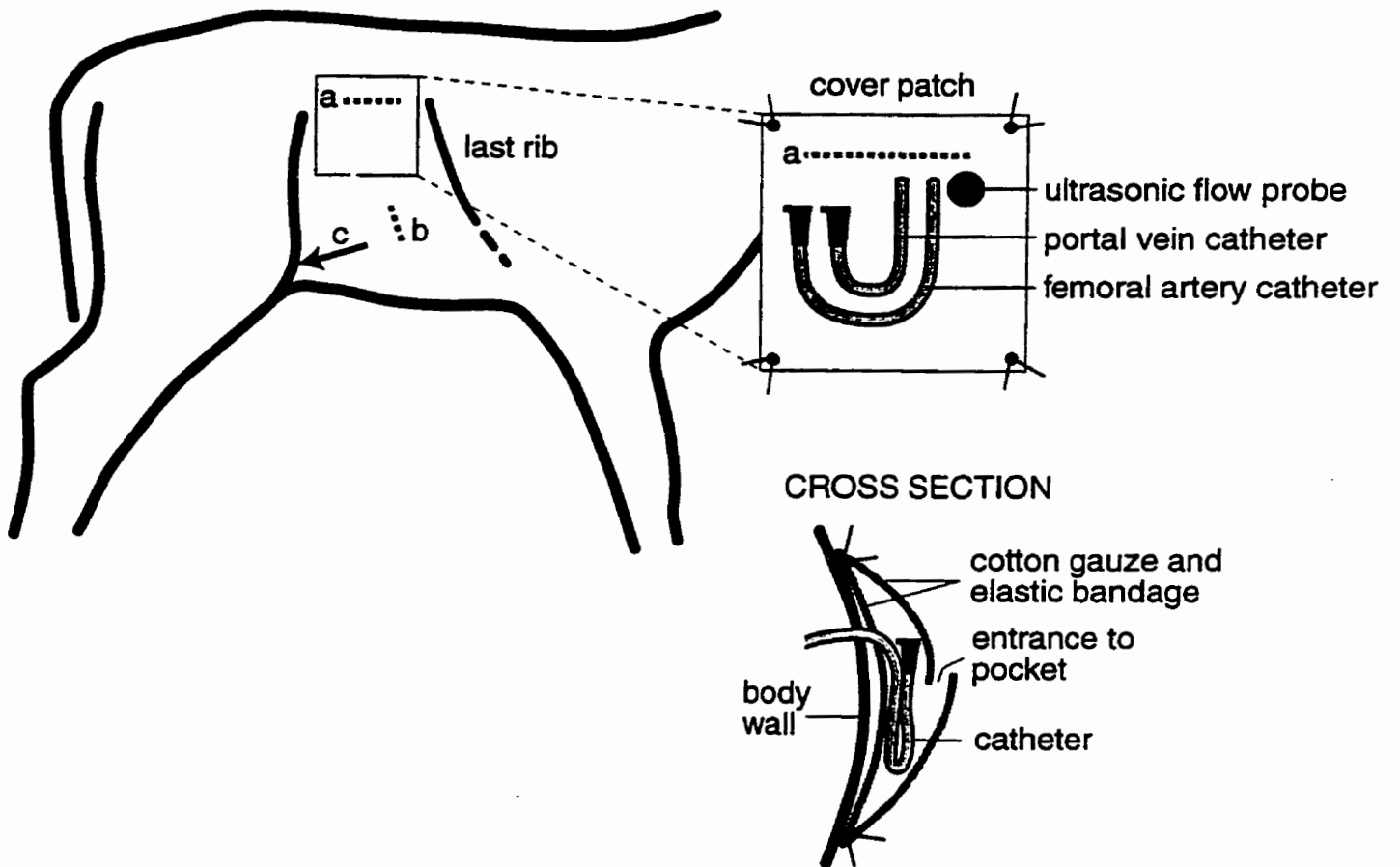
A horizontal 15 cm skin incision was made caudal to the last right thoracic rib and parallel to the transverse processes of the lumbar vertebrae to access a row of 3 subcutaneous pockets made by blunt dissection (Figure 4.3; incision a). Another 5 cm skin incision was made along the right flank caudal and parallel to the right paracostal arch (Figure 4.3; incision b). A trocar was tunneled subcutaneously from the flank incision into

the cranial-most pocket of the paralumbar incision. The ultrasonic transducer of the flow probe was tied to the end of the trocar and was pulled through the tunnel and positioned to allow the Konigsberg skin button to lay in the dorsal pocket. A 4 mm hole was made with a biopsy punch at the skin exit points to accommodate the pin connectors. The trocar was tunneled again subcutaneously toward the second pocket of the paracostal incision. The vessel end of the portal vein catheter was then tied to the trocar and pulled through into the second pocket until the dacron flange lay deep within the dorsal pocket. A 2 mm stab incision was made in the middle of the second pocket about 3 cm from the midline incision using a No. 11 scalpel blade and the external end of the catheter was pulled through using mosquito hemostatic forceps, until the flange lay against the subcutis. The femoral artery catheter was introduced through the third pocket in the paralumbar incision with the same technique used for the portal vein catheter. From the flank incision (Figure 4.3; incision b), a blunt incision was made with Metzenbaum scissors through the abdominal wall. The vessel end of the portal vein catheter and the transducer of the flow probe were introduced into the abdomen.

The abdominal wall was closed with a single layer of 1 chromic catgut. The right flank incision (Figure 4.3; incision b) was closed with one subcutaneous layer of simple continuous 2-0 braided polyglycolic acid sutures, and the skin was closed with simple interrupted non absorbable skin sutures. A 2 cm skin incision was made at the cranial aspect of the thigh (Figure 4.3; incision c). The vessel end of the femoral artery catheter was tunneled subcutaneously to the cranial incision of the thigh using Ochsner hemostatic forceps. The vessel end of the femoral artery catheter was coiled and placed in

**Figure 4.3. A calf placed in lateral recumbancy showing the incision sites.
The body bandage placed on the calf postoperatively is also illustrated.**

- a) paralumbar incision
- b) flank incision
- c) incision at cranial aspect of thigh



a sterile plastic bag. The bag was clamped to the thigh with dressing forceps. The first suture layer of the paracostal incision (Figure 4.3; incision a) was 2-0 dextron, placed close to the velour flanges to eliminate dead space. A second simple continuous subcutaneous layer of 2-0 braided polyglycolic acid sutures was followed by continuous everting mattress non absorbable skin sutures. Blunt needles and injection caps were placed on the free end of the catheter, secured with a silk tie, and the catheter was flushed with heparin 1000 U/mL. The drapes were then removed and the calf turned in dorsal recumbency, prepared, and draped for catheterization of the vessels, and placement of the flow probe.

The femoral artery was exposed as previously described (Naylor et al. 1985). The femoral artery catheter was tunneled subcutaneously from the cranial incision of the thigh toward the medial incision with a hemostat. The skin incision of the cranial aspect of the thigh was closed in routine manner. A segment of the femoral artery was exposed. Loose 2-0 silk ligatures were placed on each side of the catheterization point and used to elevate the vessel to prevent backflow from the artery. A catheter introducer facilitated placement of the catheter through a small incision in the vessel. The catheter was advanced for 8 cm towards the aorta and fixed in place using preplaced silk ligatures. The catheter was replaced in a subcutaneous position to prevent kinking of the catheter and the muscles were apposed with simple continuous 2-0 braided polyglycolic acid sutures. The skin incision was closed in routine manner.

A bilateral subcostal incision was made parallel to the paracostal arch and the xiphoid (Figure 4.4). Bleeders were ligated with 0 chromic catgut. The duodenum was

Figure 4.4. An anesthetized calf in dorsal recumbancy showing the bilateral subcostal and femoral incision sites.

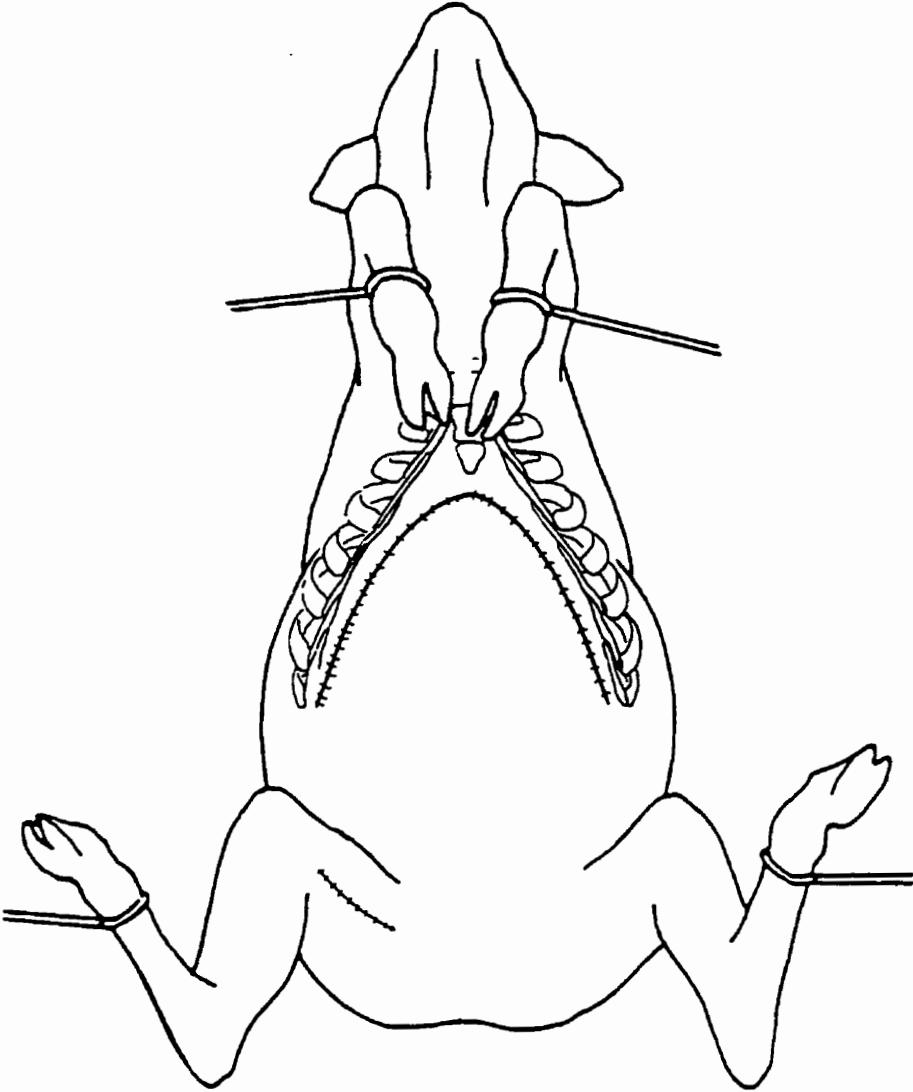
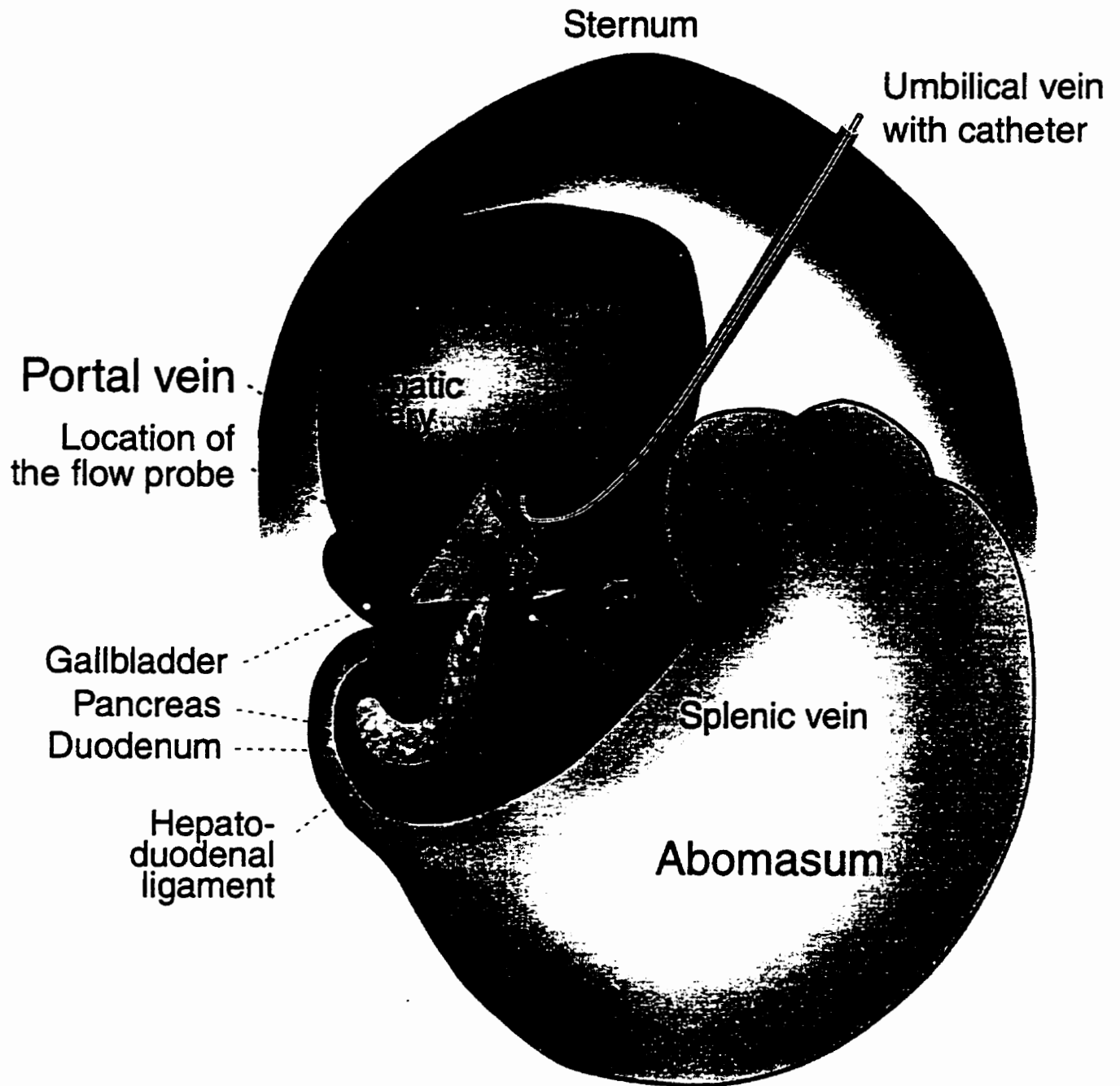


Figure 4.5. View from the bilateral subcostal approach where the portal vein is lying under the hepato-duodenal ligament.



retracted to expose the pancreas and the portal vein area. The small intestine was packed off using a moist laparotomy sponge. The entrance of the splenic vein into the portal vein was located. A 2 cm section of the portal vein was freed from the pancreas and mesentery by gentle blunt dissection downstream to the splenic vein (Figure 4.5). The freed section of portal vein was elevated using a cardiovascular surgical instrument (gemini forceps) and the transducer was placed around the vessel and anchored to the surrounding tissue with 2-0 silk sutures.

The umbilical vein, which is immediately evident on the caudal border of the falciform ligament, was utilized as an entry point to the portal vein catheter. The portal vein catheter was inserted into the umbilical vein and passed cranially until it could be palpated within the common trunk of the portal vein. It was fixed in place with 2-0 silk ligatures in the portal vein. To prevent strangulation of the small intestine, slack catheters and flow probe leads were sutured in place in coils along the right body wall. The peritoneum and abdominal musculature were closed with a single layer of 1 chromic catgut, using a simple continuous pattern. A simple continuous subcutaneous layer of 2-0 braided polyglycolic acid sutures was followed by continuous everting mattress non absorbable skin sutures.

4.2.4 After care

A body bandage was placed on the right flank over the external end of the catheters to keep them clean. The inner layer of the pack was made from conform

bandage, the outer layer from elastic adhesive bandage. A 2 cm incision was made in the middle of the pocket on the side facing the incision site and the external end of the catheters were pulled through. Each corner of the bandage was attached to the skin with a single non absorbable skin sutures. Immediately after surgery, 0.1 mg/kg of butorphanol was given intramuscularly. The injection was repeated 8 h later. Ceftiofur (1 mg/kg) was given intramuscularly once daily for four more days.

4.2.5 Catheter care

The catheters were always flushed every three days by cleaning the injection caps with isopropyl alcohol, then flushing with heparin 1000 U/mL to fill the catheter dead space. After a week of healing, the heparin strength was suddenly increased from 1000 U/mL to 10,000 U/mL. The injection caps were changed every three days.

4.3 Results

The day of the surgery, the calves were 7.1 ± 2.2 days old and weighed 52 ± 1 kg. The calves were under general anesthesia for 210 ± 30 min and the surgery lasted for about 150 ± 30 min. Of the nine calves on which the procedure was performed, seven recovered without complications. An intestinal hernia from the flank incision was noticed in one calf within 24 h of surgery. The hernia started when the calf was hold only by the legs to be transferred from the surgery table to its pen. An exploratory laparotomy was performed under general anesthesia. A non strangulating intestinal hernia was found and repaired. The calf recovered without further complications. One calf was euthanatized a week after the surgery because it developed septicemia secondary to catheter-related

infections. On necropsy, the infection spread via the subcutaneous portal vein catheter tunnel. A septic peritonitis was found.

Within four days post surgery, seven calves drank 10% of their body weight daily as whole cows milk, divided into three feeds, daily. Two weeks after the surgery, the calves weighted 54 ± 0.7 kg. The technique allowed blood to be collected and portal vein blood flow to be measured over a two week interval beginning five days after placement of instrumentation (Nappert et al. 1996b). The calves' livers were examined at necropsy, and the portal veins were opened to reveal the catheters. The catheters and vessel walls did not reveal any abnormalities in eight out of nine calves, but intestinal fibrinous adhesions were noticed around the portal area in all the calves.

4.4 Discussion

Research on intestinal nutrient metabolism in the preruminant calf has been limited in comparison with dogs (Weber et al. 1982; Souba and Wilmore 1983; Karner and Roth, 1989), pigs (Wu et al. 1994; Prior and Gross 1995), rats (Windmueller and Spaeth 1975, 1978, and 1980), and sheep (Burrin et al. 1989 and 1990; Wahle et al. 1971 and 1972; Wolff et al. 1972; Heitmann and Bergman 1981). The relative inaccessibility of the portal vein in preruminant calves has made studies of gut utilization difficult. The bilateral subcostal approach has not been described to reach the portal area in calves and was helpful in our study. Nevertheless, several blood vessels crossed the abdominal wall and ligation of these blood vessels was required with this approach. Cardiovascular surgical

instruments were also required for proper placement of the ultrasonic flow probe around the portal vein.

Measurement of PDV blood flow by use of a flow probe is difficult, because of the dissection required to isolate the portal vein. However, estimation of PDV blood flow using an ultrasonic probe is much easier than constant infusion of 3% PAH into a mesenteric vein (Naylor et al. 1985). The application of an ultrasonic flow probe also has significant advantages over other blood flow meters (i.e., electromagnetic, doppler) for chronic application. These advantages include direct measurement of volume rate of flow regardless of vessel dimension, nonconstrictive fit of the probe on the vessel, and accuracy over the period of long-term implant (O'Brien et al. 1991; Burton and Gorewit 1984).

Another factor contributing to the success of our model has been the use of a catheter inserted into the portal vein via the umbilical vein. Catheterization of the portal vein via the umbilical vein in calves older than two weeks of age may not be possible, however, if the umbilical vein has degenerated (McGilliard 1968). Furthermore, catheterization of the portal vein will not be recommended via the umbilical vein on animals with omphalitis. The combination of chronic blood flow and arterio-venous nutrient measurements allowed us to measure nutrient flux across the portal-drained viscera (abomasum, small and large intestine, mesenteric fat, pancreas, and spleen). This surgical model has already been successfully employed to measure transintestinal fluxes of amino and organic acids in calves (Nappert et al 1996a).

Catheter-related infections pose a hazard to the calves. The development of an implantable flange for application at the skin exit sites of catheters and flow probe connectors was considered useful to prevent infections via the subcutaneous catheter tunnel. Although other types of catheter flanges utilizing other materials have been reported (Schmidt et al. 1988), expense limited our acquisition of prefabricated devices and prompted us to construct our own implantable catheters that have proven to be successful in dogs (O'Brien et al. 1991). In prevention of post surgical infections, keeping our calves on straw in a clean area combined with the use of systemic antibiotic was successful in almost all cases. We did not measure the serum immunoglobulin levels of the calves prior to the surgery, but we measured the serum protein levels. In the absence of dehydration, we considered serum protein levels over 5 g/dL associated with a successful passive transfer. All the calves were fed 10% of their body weight as colostrum in the first 24 hours of life and had a serum protein levels over 5.4 g/dL a day before surgery. One calf was euthanatized because of catheter-related infection. The application of dilute antibiotic spray around the skin interfaces twice a day as previously recommended (O'Brien et al. 1991) might have helped prevent catheter-related infection in this calf. Flushing the catheter lumens every three days with a concentrated solution of chymotrypsin and gentamicin (Palm et al. 1991) or an anticoagulant-citrate-dextrose solution with formaldehyde might also have been beneficial (O'Brien et al. 1991). When not in use, it was essential to have the catheter filled with an anticoagulant solution. Another problem was occasional loss of the injection caps at the external end of the catheter. A pinch-clamp placed on the external end of the catheters helps eliminate the

possibility of blood flowing back into the catheter in the event of loss of the injection caps (Conner and Fries 1960).

Significant problems with skin healing using a continuous everting pattern in this research project were not observed. The continuous nature of the pattern allowed rapid closure which reduced the anesthetic time of the calf. Wound healing in the calf with this suture pattern has proven to be very good in both healthy and debilitated calves in hundreds of cases in our clinic. Other suture patterns would also be very effective but with the exception of skin staples, would not be as rapidly applied.

In spite of normal food intake within four days post surgery, the calves only gained 2 kg over the two-week period. None of the calves lost weight a week after the surgery. Estimated daily maintenance energy requirements in calves have been determined approximately at 50 kcal/kg of body weight (BW) (Naylor 1991). Energy requirements for growth have been estimated at 3.0 kcal/g BW gain (Naylor 1991). Since whole milk contains about 0.7 kcal/mL, a 45-kg calf requires approximately 2250 kcal or 3.2 L (7.1% of BW) of milk for daily maintenance. In practice, dairy calves are fed daily at 10% BW (0.3 kg/day of gain) (Naylor 1991). There was no evidence of an infectious inflammatory process to explain this lack of weight gain. It is possible that with the dietary intake and the surgical procedure including fasting that the gain measured would be real close to that expected. Abdominal surgery is known to increase nutrient demands (Ralston and Naylor 1991). This is why it is important to allow a recovery phase. The calves may have grown if they were fed above average nutrient demands and the study had been performed over a

longer period of time. Since this experimental set-up was only maintained for two weeks, the potential for longer experimental periods needs to be investigated.

With the use of a catheter inserted into the portal vein via the umbilical vein, we have developed an *in vivo* flux model in calves to quantitate the net exchange of nutrients across the portal-drained viscera (small and large intestine, pancreas, and spleen) by measuring blood flow and by sampling the concentration of the nutrients in the femoral artery and portal vein (Nappert et al. 1996b). However, some limitations may be associated with this chronic *in vivo* model. The flux rates of nutrients are crude in that they do not provide information about the relative contributions of the individual cell populations in the tissue to the net overall exchange rate (Souba 1993). Although arteriovenous concentration difference studies do not provide detailed information about transport and metabolism in individual cells, they do provide important information about how organ handling of specific nutrients changes during feeding, starvation, and disease states. Glutamine is similarly metabolized whether it enters the mucosal cells across the brush border from the lumen or across the basolateral membrane from arterial blood in rats, dogs and sheep (Nappert et al, 1997). In fed piglets, glutamate is the preferred substrate for mucosal glutathione synthesis if it enters the intestinal cells from the lumen in comparison with the bloodstream (Reeds et al, 1997). Additional research is needed in calves to determine the differential utilization of nutrients depending on whether they are presented to the intestinal cells intraluminally or from the bloodstream. Therefore, combined with data obtained from other *in vitro* models (i.e., regional perfusion model,

cultured intestinal cells), this model will help us to understand the metabolism of the intestine of the neonatal calf.

5.0 Portal-drained viscera metabolism in neonatal calves examined by short-term intravenous infusions of glutamine and other nutrients

5.1 Introduction

The role of glutamine as an energy source or a precursor for synthesis of other amino acids and nucleotides such as ATP, purines, and pyrimidines in the intestine of neonates, such as the preruminant calf, is not clear. Since the small intestine of the newborn animal grows quickly (Reeds et al. 1993), utilization of glutamine as a fuel, a characteristic of rapidly proliferating cells (Krebs 1980), is expected. Studies in monogastrics, such as the rat (Kimura 1987) and pig (Darcy-Vrillon et al. 1994), have shown that at birth and during the suckling period intestinal glutamine oxidation is high, whereas glucose and β -hydroxybutyrate oxidations are low. After weaning, glutamine utilization declines in the pig (Darcy-Vrillon et al. 1994) and rat (Windmueller and Spaeth 1980) enterocytes. Intestinal glucose consumption increases, but the extent of glucose metabolism depends on the nutritional status of the animals (Darcy-Vrillon et al. 1994). In ruminants, Wolff et al. (1972) reported utilization of arterial glutamine by the PDV of sheep fed alfalfa. Reynolds and Huntington (1988a and b) found an uptake of β -hydroxybutyrate and glutamine by mesenteric-drained viscera of steers fed alfalfa, suggesting that the tissues of the mesenteric-drained viscera use ketones and glutamine for energy.

Glutamine concentrations in blood and tissues decline markedly during critical illness (Souba et al. 1985a) and research suggests it may be a conditionally indispensable (i.e., essential) amino acid (Grimble 1993). Studies indicate that glutamine may be essential for maintenance of the structural and functional integrity of the small intestinal mucosa (Fink 1991). Glutamine supplementation has been found to increase intestinal protein synthesis and this finding may be one mechanism by which glutamine exerts its protective effect on intestinal mucosa during critical illness (Higashiguchi et al. 1993). Glutamine has also been shown to promote repair of injured intestine (Fox et al. 1988), possibly through its stimulation of proliferative events including enhanced $\text{Na}^+ - \text{H}^+$ exchange and ornithine decarboxylase activity, which has been shown in several cell lines (McCormack et al. 1990).

The objective of this study was to quantify PDV glutamine utilization by neonatal calves and to assess the relative nutritional importance of glutamine, glucose, and acetate for the enterocytes. Energy in mucosal epithelial cells of the PDV can be derived from both luminal and arterial substrates. The relative importance of the two sources is not clear in neonatal calves. It is therefore of interest to first investigate in some detail with an arteriovenous difference model the PDV metabolism by mucosal cells from the blood. Our research is primarily focused on the nutritional intestinal uptake of the neonatal calf, and we are interested in calves receiving whole cow's milk proteins because these contain particularly high quantities of glutamate and glutamine (Davis et al. 1994). In a previous study (Nappert et al. 1993), we showed that diarrheic calves had considerable intestinal malabsorption. A need exists to develop feeding regimens that will support mucosal

regeneration. A better understanding of the nutrients utilized by intestinal tissue would be beneficial in the development of diets for sick calves.

5.2 Methods

5.2.1 Animals, Diet and Experimental Design

Five healthy male Holstein calves were obtained from the University of Saskatchewan dairy barn within a week of birth. The experimental study period was twelve days. On day one, the femoral artery, jugular vein and the portal vein were surgically cannulated in each calf. The calves were fed daily 10% of their body weight as whole cows' milk which was divided equally and consumed as two feeds. On days six, eight, ten and twelve of the experimental period, a series of intravenous infusions were carried out to investigate nutrient metabolism in the calf's intestine by measuring PDV uptake and oxygen consumption. On each of these study days, each calf was infused with four different solutions containing either acetate, glucose, glutamine or saline (control). The order in which the various compounds were infused is shown in Table 5.1. No milk was fed for 16 h before the serial infusions. Infusions on days six and twelve were performed in the postabsorptive state. To mimic work, on days eight and ten, the calves received an electrolyte solution without an energy source just prior to infusion of the various nutrients on the assumption that absorption of electrolytes requires more energy. Calves were euthanatized at the end of the experimental period and a necropsy was performed to confirm proper placement of the surgical catheters. The experimental

Table 5.1. Experimental design and randomization of nutrients infused in the calves.

Study Day	Glutamine	Glucose	Acetate	Saline
1	d	c	b	a
2	b	d	a	c
3	c	a	d	b
4	a	b	c	d

On study days 1 to 4 serial infusions were performed on each calf (n=4). Study days 1, 2, 3 and 4 were days 6, 8, 10 and 12 post surgery, respectively. Letters represent the chronological order in which the compounds were infused on each study day. Serial infusions on study days 1 and 4 were carried out in the postabsorptive state, whereas on study days 2 and 3, animals received a bolus of oral electrolytes prior to the infusion of each nutrient. Saline infusion served as a control.

protocol and the procedures used were approved by the University of Saskatchewan Animal Care Committee, Saskatoon, Canada.

5.2.2 Surgical procedure

The surgical approach used to cannulate the femoral artery, jugular vein and the portal vein has been previously described (Nappert et al. 1996b). Briefly, after an overnight fast, anesthesia was maintained at 2% halothane in 100%O₂ using intermittent positive-pressure ventilation. A bilateral subcostal incision was made parallel to the paracostal arch and the xiphoid. A silastic catheter, 100 cm in length (diameters in mm: 1.57 inside diameter X 3.17 outside diameter), was inserted into the umbilical vein and passed cranially until it could be palpated within the common trunk of the portal vein. An ultrasonic perivascular flowprobe (8R series, Transonic Systems, Ithaca, NY) was placed around the portal vein. A second silastic catheter, 100 cm in length (diameters in mm: 1.01 inside diameter X 2.16 outside diameter), was inserted for 8 cm into the femoral artery toward the aorta. At the completion of surgery, catheters were flushed with 1 mL of heparin (1000 U/mL) and injection caps were changed every three days. One week post-operatively, the heparin strength was increased from 1000 U/mL to 10,000 U/mL.

5.2.3 Infusion studies

Equimolar solutions (200 mmol/L) for L-glutamine, glucose and acetate were prepared using double distilled water. An isotonic L-glutamine solution was prepared (29.22 g/L of glutamine and 2.9 g of sodium chloride/L). When L-glutamine was dissolved, the pH of the resulting solution was adjusted to 7.3 by addition of 1 M

hydrochloric acid. The solution was then filtered through a 0.22-mm Millipore filter into a sterile bottle. The L-glutamine solution was prepared approximately 4 h prior to each infusion. The acetate (16.4 g of sodium acetate/1.3 L), saline (9 g sodium chloride/L) and glucose (36.03 g/L of D-glucose and 2.9 g of sodium chloride/L) solutions were prepared and autoclaved no more than 24 h before use.

In the morning of days 6, 8, 10 and 12 of the experimental period, the prepared acetate, glucose, glutamine, and saline (control) solutions were infused separately as four 1 h infusions in each calf. The solutions were administered intravenously via the jugular vein at a rate of 200 mmol/h. There was a half-hour rest period between each infusion. At the beginning of each one hour infusion in the studies designed to mimic the working state on days 8 and 10, the calves received a 1L oral bolus of an electrolyte solution containing KCl (20 mmol/L) and NaCl (120 mmol/L). Simultaneous arterial and portal blood samples were collected in triplicate in 3 mL heparinized tubes over the last 15 min of each one hour infusion. Portal vein blood flow was measured during blood sampling. Blood samples were placed in an ice water bath and centrifuged to obtain plasma. The plasma was stored at -70°C until assayed. To minimize possible degradation of amino acids and organic acids, an aliquot of plasma was deproteinized prior to freezing by ultrafiltration with a microcentrifuge at 1500 g for 30 min. At the end of each separate infusion, 2.5 mL of blood was collected aseptically from the femoral artery and portal vein in a pre-heparinized plastic syringe. Blood was analyzed immediately for oxygen content.

5.2.4 Biochemical analysis

The blood oxygen content was determined with spectral absorption which uses a co-oxymeter to analyse several hemoglobin derivatives simultaneously (Zwart et al. 1981). Plasma glucose concentration was determined using the hexokinase glucose-6-phosphate dehydrogenase method (Bondar and Mead 1974) and a spectrophotometric autoanalyser. Plasma ammonia concentration was determined using the A-GENT Abbot ammonia method which utilizes the amination of α -ketoglutarate and concomitant oxidation of nicotinamide hypoxanthine dinucleotide phosphate mediated by glutamate dehydrogenase (Gau 1987).

Plasma organic acid and amino acid concentrations were quantitatively determined by RP-HPLC. The RP-HPLC system consisted of a pump (Waters 600 pump, Mississauga, ON), a tunable wavelength absorbance detector (Waters 486), a temperature control module (Waters), an autoinjector (Waters 710 WISP), and a chromatography manager (Waters Millennium version 2.1).

Determination of plasma acetate, pyruvate, lactate and β -hydroxybutyrate concentrations were carried out using the ion exclusion method (Waters, Millipore Corporation, Milford, MA) for the separation of organic acids with slight modifications. Prior to sample deproteinization, 100 μ L of an internal standard of citric acid solution (1 mmol/L) was added to each plasma sample (100 μ L). Plasma was deproteinized by ultrafiltration. Aliquots of the filtrate (20 μ L) were injected into the RP-HPLC. The RP-HPLC was equipped with a reversed-phase column (Shodex KC-811, Showa Denko K.K.,

Tokyo, Japan), packed with a sulfonated rigid styrene divinylbenzene copolymer. Solvents were 0.1% phosphoric acid and DDW. Prior to use, the solvents were filtered through 0.45 mm filters (Scheicher & Schuell, Keene, NH) and degassed under vacuum to remove oxygen and contaminants that could affect chromatographic separation. Organic acids were eluted from the column with a linear gradient from 0 to 30% of 0.1% phosphoric acid in DDW in 20 min, at a flow-rate of 0.7 mL/min at 50°C. The absorbance of the effluent was monitored at 205 nm. The area of the peak was automatically calculated by integration and the retention times of the peaks were checked manually for accuracy. Between runs the system was washed for 20 minutes with DDW. Samples were analyzed in a random order.

Plasma amino acid concentrations were determined using the Pico-Tag Amino Acid method (Waters, Millipore Corporation, Milford, MA) for RP-HPLC (Grimble 1993) with slight modifications. Prior to sample deproteinization and derivatization with the phenylisothiocyanate derivative (Pierce, Rockford, IL), 100 µL of an internal standard of norleucine (200 mmol/L) was added to each plasma sample (100 µL). Plasma was deproteinized by ultrafiltration. Aliquots of the filtrate (25 µL) were transferred into disposable glass tubes (0.5 mL X 6 mm outside diameter, Aldrich Chemical, Milwaukee, WI). The glass tubes were sealed in vacuum vials and dried in a vacuum drier (Pico-Tag work station, Millipore corporation, Milford, MA). After vacuum drying, 10 µL of drying solution, which consisted of methanol-sodium acetate 1M-triethylamine (2:2:1), was added to the glass tubes. Samples were dried to remove excess reagent. Phenylthiocarbamyl-amino acids were formed by adding 20 µL of derivatizing reagent

which consisted of triethylamine-distilled water-phenylisothiocarbamyl-methanol (1:1:1:7) to the dried samples. After vortexing, samples were sealed in vacuum vials for 20 minutes at ambient temperature. Samples were redried in a vacuum drier to remove excess reagent, triethylamine and other volatile products which might affect chromatographic separation. The phenylthiocarbamyl amino acids were then reconstituted with 100 μ L of acetonitrile (Pico-Tag diluent, Waters Corporation, Milford, MA) and a 20 μ L aliquot was injected into the RP-HPLC system.

The column used for amino acid analysis was a Waters Nova-Pak C₁₈ (3.9 mm x 30 cm). Derivatized amino acids were eluted from the column with a 94% linear gradient of a solvent consisting of 140 mmol/L sodium acetate trihydrate (pH 6.4), 3.6 mmol/L of triethylamine, and 0.2 mL/L of stock EDTA solution in acetonitrile over 20 minutes, at a flow-rate of 1 mL/minute at 46°C. The absorbance of the effluent was monitored at 260 nm. The RP-HPLC system was run five minutes in 40% distilled water and 60% acetonitrile, and an additional 20 min at 94% of the sodium acetate, triethylamine, EDTA solution in acetonitrile to clean the column between sample injections (see appendix C: Amino acids measurements by RP-HPLC – Pico-Tag amino acid analysis system). Samples were analyzed in a random order.

5.2.5 Portal-drained viscera nutrient difference and oxygen consumption

The PDV includes the abomasum, small and large intestine, mesenteric fat, pancreas, and spleen. Net flux across the PDV was estimated by multiplying the

arteriovenous concentration differences of a nutrient by the appropriate blood flow. The net flux is an estimate of the combined effect of uptake and release of the nutrient by the PDV. Positive net flux indicates production (or absorption in the case of the PDV when nutrient is fed orally) of a nutrient in excess of uptake. Negative net flux indicates PDV uptake in excess of endogenous production or absorption from the intestinal lumen. Estimates of the PDV net uptake or production were calculated using the following equation:

$$\text{PDV nutrient net flux } (\mu\text{mol/kg}^{0.75}/\text{min}) = [\text{BF} \times (\text{N}_v - \text{N}_a) \times (1 - \text{PCV})] / \text{BW}^{0.75}$$

where BF is the blood flow in the intestine, in liters per min. N_v and N_a are plasma nutrient concentrations, mmol/L, in portal vein and femoral artery respectively. BW is body weight, in kilograms, to the $3/4$ power which is directly proportional to maintenance energy requirements (Kleiber 1975). Packed cell volume (PCV) is assumed to be the same in portal and arterial vessels. The PDV oxygen consumption was calculated from the following equation:

$$\text{PDV oxygen consumption } (\mu\text{mol/kg}^{0.75}/\text{min}) = [\text{BF} \times (\text{N}_v - \text{N}_a)] / \text{BW}^{0.75}$$

To estimate the maximum potential contributions of glucose and glutamine to net oxygen consumption by the gastrointestinal tissues, metabolic quotients were calculated from the ratio of substrate uptake/oxygen uptake, multiplied by the number of oxygen moles required for complete oxidation of 1 mole of the substrate into CO_2 . All lactate was assumed to have come from glucose and this was subtracted from glucose uptake to arrive at the quotient for glucose:

Maximum fraction of oxygen consumption potentially attributable to glucose = $((\text{PDV glucose uptake} \times 6) - (\text{PDV lactate production} \times 3)) / (\text{PDV oxygen uptake})$.

The maximum fraction of oxygen consumption potentially attributable to glucose had also been calculated without considering lactate production = $(\text{PDV glucose uptake} \times 6) / (\text{PDV oxygen uptake})$

Maximum fraction of oxygen consumption potentially attributable to glutamine = $(\text{PDV glutamine uptake} \times 4.5) / (\text{PDV oxygen uptake})$.

5.2.6 Data analysis

The mean arteriovenous differences during each infusion were calculated for each nutrient. To determine if the 30 minute rest period between infusions has any carry-over effect on the measurements of the following infusion, the arterial concentrations of the infused substrate were compared at the end of each infusions and before any infusions. A one way ANOVA was used for these comparisons. To determine if infusion or the presence of electrolytes in the intestinal lumen influenced intestinal metabolism, a two way ANOVA was used to compare the means of PDV nutrient difference during each infusion in the fasting and the working states. Means were compared using Tukey's test. All values were expressed as the mean \pm SEM, and a *p* value of less than 0.05 was considered significant.

5.3 Results

On the day of the surgery, the five calves were 7.5 ± 4.9 (mean \pm SD) days old and weighed 46.2 ± 7.3 kg. One calf was euthanatized a week after the surgery because it

developed septicemia secondary to catheter-related infections. The infusion protocol was successfully performed in four calves, twice with the calves fasted and twice being fed 1L/h of an electrolyte solution at the beginning of each infusion. The four calves were 12.1 ± 2.2 days old at the mid point of the infusion studies (day 9). Twelve days after the surgery, the calves weighed 47 ± 11.3 kg.

In order to determine if there was any carry over effect from the previous infusion, arterial concentrations of the major nutrients were compared with pre-infusion values (Table 5.2). During glutamine infusion, acetate and glucose concentrations were similar to pre-infusion values while glutamine was significantly elevated. Similar results were obtained during acetate infusions, glucose and glutamine concentrations were similar to pre-infusion values and acetate concentrations were significantly elevated. In the case of glucose infusions, glutamine concentrations were similar to pre-infusions values, glucose was significantly elevated and acetate concentrations were actually lower than pre-infusion concentrations. Co-eluting peaks were found for β -hydroxybutyrate. No plasma remained for additional measurements of the β -hydroxybutyrate levels by spectrophotometry. Therefore, the PDV β -hydroxybutyrate uptake could not be determined.

PDV uptake for acetate and pyruvate was significantly different between fasted and electrolyte fed calves. However, there were no significant differences in PDV amino acids uptake between the two groups. Therefore, data for the PDV organic acids uptake

are reported separately for fasted and electrolyte fed calves (Table 5.3), but the PDV amino acids uptake from both groups were combined (Table 5.4).

PDV uptake of glutamine (Figure 5.1) and production of ammonia (Figure 5.2) was significantly greater during glutamine infusion in fasted and electrolyte fed calves. Ammonia is produced when glutamine is deaminated. During glutamine infusion in fasted and electrolyte fed calves, the PDV glutamine uptake ($29.9 \pm 11.2 \mu\text{mol/kg}^{0.75}/\text{min}$) was significantly higher ($p = 0.004$) than the PDV ammonia production ($7.0 \pm 0.5 \mu\text{mol/kg}^{0.75}/\text{min}$). Although the arterial ammonia level ($109.5 \pm 11.4 \mu\text{mol/L}$) was highest during glutamine infusion, no signs of nervous dysfunction were observed. Assuming glutamine was either completely deaminated or partially deaminated to glutamate only 12 or 24% respectively of the glutamine was deaminated by the PDV. PDV uptake of the amino acid histidine (Figure 5.3) was significantly affected by infusion. Uptake was significantly greater during glutamine infusion and this was associated with an increase in delivery of this amino acid to the intestine. The uptake of leucine, and lysine uptake was marginally affected by infusion ($p = 0.06$) with the greatest absolute uptakes during glutamine infusion (Table 5.4).

Despite the increase in arterial acetate concentration during infusion of this nutrient, there was no increase in uptake by the PDV (Table 5.3). Over all infusions, acetate production by the PDV was significantly higher in fasted than in electrolyte fed calves. The increase in PDV glucose uptake during glucose infusion was not statistically significant. There was no effect of infusion on lactate production but the PDV uptake of

electrolyte fed calves was significantly different from the net lactate production in fasted calves.

The PDV of electrolyte fed calves used significantly less oxygen than fasted calves, values for oxygen consumption were 38.6 ± 2.1 and $49.8 \pm 5.0 \mu\text{mol/kg}^{0.75}/\text{min}$ respectively. There was some tendency ($p = 0.06$) for PDV oxygen consumption to change between infusions (Table 5.5). Oxygen consumption was lowest during glucose infusions and highest during glutamine infusions. The maximal fraction of oxygen consumption potentially attributable to glutamine oxidation was significantly affected by

Table 5.2. The arterial concentrations of the infused substrate at the end of infusion with acetate, glucose, glutamine or saline and before any infusions in four calves. Data from fasted calves and calves fed oral electrolytes were pooled. Values are means \pm SE. Means with different superscripts in the same row are significantly ($P < 0.05$) different.

	Infusion Type				
	Before any infusion	Acetate	Glucose	Glutamine	Saline
Arterial concentration					
Acetate (mmol/L)	0.5 ± 0.1^b	0.7 ± 0.07^c	0.3 ± 0.05^a	0.4 ± 0.06^b	$0.2 \pm 0.03^{a,b}$
Glucose (mmol/L)	4.1 ± 0.1^b	$3.6 \pm 0.2^{a,b}$	7.0 ± 0.3^d	$4.7 \pm 0.2^{a,b,c}$	$3.8 \pm 0.1^{b,c}$
Glutamine (mmol/L)	0.4 ± 0.04^a	0.3 ± 0.01^a	0.3 ± 0.02^a	3.5 ± 0.4^b	0.3 ± 0.03^a

Table 5.3. PDV organic acids production (positive values) or uptake (negative values) and arterial concentration in four neonatal calves during infusion of acetate, glucose, glutamine or saline. Calves were either fasted or fed oral electrolytes during the infusion. Values are means \pm SE. Means with different superscripts in the same row are significantly ($P < 0.05$) different. * Indicate a significant overall difference between fasted and fed states.

Organic acids			Infusion Type:			
			Acetate	Glucose	Glutamine	Saline
Acetate	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	Fasted	9.8 ± 7.1	7.7 ± 4.0	6.5 ± 1.9	7.6 ± 3.1
		Fed*	-1.3 ± 2.0	5.6 ± 4.5	-2.0 ± 5.1	5.1 ± 1.9
	Arterial concentration mmol/L	Fasted	0.7 ± 0.1^a	0.3 ± 0.1^b	0.4 ± 0.05^b	0.2 ± 0.05^b
		Fed	0.7 ± 0.1^a	0.2 ± 0.05^b	$0.4 \pm 0.1^{a,b}$	0.3 ± 0.05^b
Glucose	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	Fasted	-5.3 ± 5.1	-8.5 ± 3.5	-3.3 ± 2.7	-1.9 ± 3.1
		Fed	-4.1 ± 1.3	-5.0 ± 3.8	-10.5 ± 1.2	-3.5 ± 1.5
	Arterial concentration mmol/L	Fasted	4.3 ± 0.4^a	7.0 ± 0.5^b	5.0 ± 0.4^a	4.2 ± 0.2^a
		Fed	0.3 ± 0.2^a	7.0 ± 0.4^b	4.5 ± 0.1^a	3.8 ± 0.1^a
Pyruvate	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	Fasted	1.2 ± 0.3	0.8 ± 0.4	0.6 ± 0.4	1.1 ± 0.3
		Fed*	0.5 ± 0.2	0.2 ± 0.2	-0.2 ± 0.8	-1.1 ± 0.6
	Arterial concentration mmol/L	Fasted	0.1 ± 0.02	0.2 ± 0.01	0.3 ± 0.01	0.4 ± 0.01
		Fed	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02
Lactate	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	Fasted	8.8 ± 2.6	13.4 ± 6.1	5.4 ± 4.0	15.2 ± 5.9
		Fed	10.2 ± 2.8	5.2 ± 2.4	-6.4 ± 8.3	-8.8 ± 9.1
	Arterial concentration mmol/L	Fasted	$1.1 \pm 0.2^{a,b}$	$1.1 \pm 0.2^{a,b}$	1.6 ± 0.2^b	0.8 ± 0.1^a
		Fed	1.6 ± 0.2	1.2 ± 0.1	1.8 ± 0.2	1.4 ± 0.2

Table 5.4. PDV nutrient production (positive values) or uptake (negative values) and arterial concentration in four neonatal calves during different types of infusion. Values are means \pm SE. Means with different superscripts in the same row are significantly ($P < 0.05$) different.

Nutrients		Infusion Type:			
		Acetate	Glucose	Glutamine	Saline
Alanine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	2.8 \pm 0.6	0.7 \pm 0.8	0.9 \pm 1.3	4.0 \pm 1.8
	Arterial concentration mmol/L	0.17 \pm 0.02 ^a	0.17 \pm 0.02 ^a	0.32 \pm 0.02 ^b	0.16 \pm 0.02 ^a
Arginine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	1.3 \pm 0.4	0.6 \pm 0.4	-0.2 \pm 0.4	1.5 \pm 0.5
	Arterial concentration mmol/L	0.13 \pm 0.01 ^a	0.11 \pm 0.01 ^a	0.22 \pm 0.03 ^b	0.13 \pm 0.01 ^a
Aspartate	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0 ^a	0.1 \pm 0.1 ^b	-0.7 \pm 0.4 ^a	0 ^a
	Arterial concentration mmol/L	0.01 \pm 0 ^a	0.01 \pm 0 ^a	0.03 \pm 0 ^b	0.01 \pm 0 ^a
Cystine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0.4 \pm 0.2	-0.3 \pm 0.6	-0.3 \pm 0.3	0.1 \pm 0.2
	Arterial concentration mmol/L	0.02 \pm 0.01 ^a	0.06 \pm 0.01 ^b	0.05 \pm 0 ^{a,b}	0.03 \pm 0 ^{a,b}
Glycine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	1.7 \pm 0.5	-0.1 \pm 1.0	-1.7 \pm 2.1	1.1 \pm 0.6
	Arterial concentration mmol/L	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Glutamate	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	-0.3 \pm 0.1	-0.4 \pm 0.2	-1.0 \pm 0.4	-0.2 \pm 0.1
	Arterial concentration mmol/L	0.05 \pm 0.01 ^a	0.04 \pm 0.01 ^a	0.1 \pm 0.01 ^b	0.04 \pm 0 ^a
Glutamine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0 \pm 0.6 ^a	-0.8 \pm 1.2 ^a	-29.9 \pm 11.2 ^b	0.9 \pm 1.0 ^a
	Arterial concentration mmol/L	0.3 \pm 0.02 ^a	0.26 \pm 0.02 ^a	3.49 \pm 0.38 ^b	0.31 \pm 0.03 ^a

Ammonia	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	2.7 ± 0.3^a 0.02 ± 0^a	3.3 ± 0.2^a 0.04 ± 0^a	7.0 ± 0.5^b 0.11 ± 0.01^b	3.9 ± 0.5^a 0.04 ± 0^a
Histidine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	0.5 ± 0.1^{ab} 0.06 ± 0.01^a	0.1 ± 0.3^{ab} 0.05 ± 0.01^a	-0.7 ± 0.4^a 0.1 ± 0.01^b	0.7 ± 0.2^b 0.06 ± 0^a
Isoleucine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	0.7 ± 0.3 0.1 ± 0.01	0.4 ± 0.4 0.06 ± 0.01	-0.1 ± 0.5 0.1 ± 0.02	1.1 ± 0.3 0.1 ± 0
Leucine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	1.4 ± 0.4 0.15 ± 0.02	0.7 ± 0.6 0.1 ± 0.01	$-0.3 \pm 0.7^*$ 0.16 ± 0.02	1.8 ± 0.4 0.15 ± 0.01
Lysine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	0.9 ± 0.3 0.1 ± 0.01^{ab}	0.2 ± 0.5 0.08 ± 0.01^a	$-1.0 \pm 0.7^*$ 0.15 ± 0.02^b	0.9 ± 0.4 0.1 ± 0.01^{ab}
Methionine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	0.4 ± 0.1 0.03 ± 0.01	0 ± 0.2 0.02 ± 0.01	0.1 ± 0.2 0.03 ± 0.01	0.3 ± 0.1 0.03 ± 0
Phenylalanine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	0.7 ± 0.2 0.08 ± 0.01	0.1 ± 0.3 0.07 ± 0.01	0 ± 0.3 0.08 ± 0.01	0.8 ± 0.2 0.07 ± 0
Proline	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$ Arterial concentration mmol/L	2.1 ± 0.6 0.16 ± 0.02	-0.2 ± 0.8 0.13 ± 0.02	-0.2 ± 0.9 0.2 ± 0.02	1.7 ± 0.3 0.15 ± 0.02

Serine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0.9 ± 0.4	0.7 ± 0.2	0 ± 0.3	0.1 ± 0.3
	Arterial concentration mmol/L	0.1 ± 0.01^a	0.08 ± 0.01^a	0.14 ± 0.02^b	0.1 ± 0.01^a
Threonine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0 ± 0.4	0 ± 0.3	0.2 ± 0.7	-2.0 ± 2.0
	Arterial concentration mmol/L	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
Tyrosine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	0.9 ± 0.3	0.1 ± 0.4	-0.2 ± 0.4	0.6 ± 0.3
	Arterial concentration mmol/L	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Valine	PDV net flux $\mu\text{mol/kg}^{0.75}/\text{min}$	2.1 ± 1.4	0.8 ± 1.9	-0.5 ± 2.1	2.7 ± 2.5
	Arterial concentration mmol/L	0.36 ± 0.04	0.32 ± 0.04	0.39 ± 0.05	0.33 ± 0.04

($p = 0.06$).

Figure 5.1. Arterial glutamine concentration and PDV production (positive values) or uptake (negative values) in four calves infused with acetate, glucose, glutamine or saline. Values are mean \pm SE. Means with different superscripts are significantly ($p < 0.05$) different.

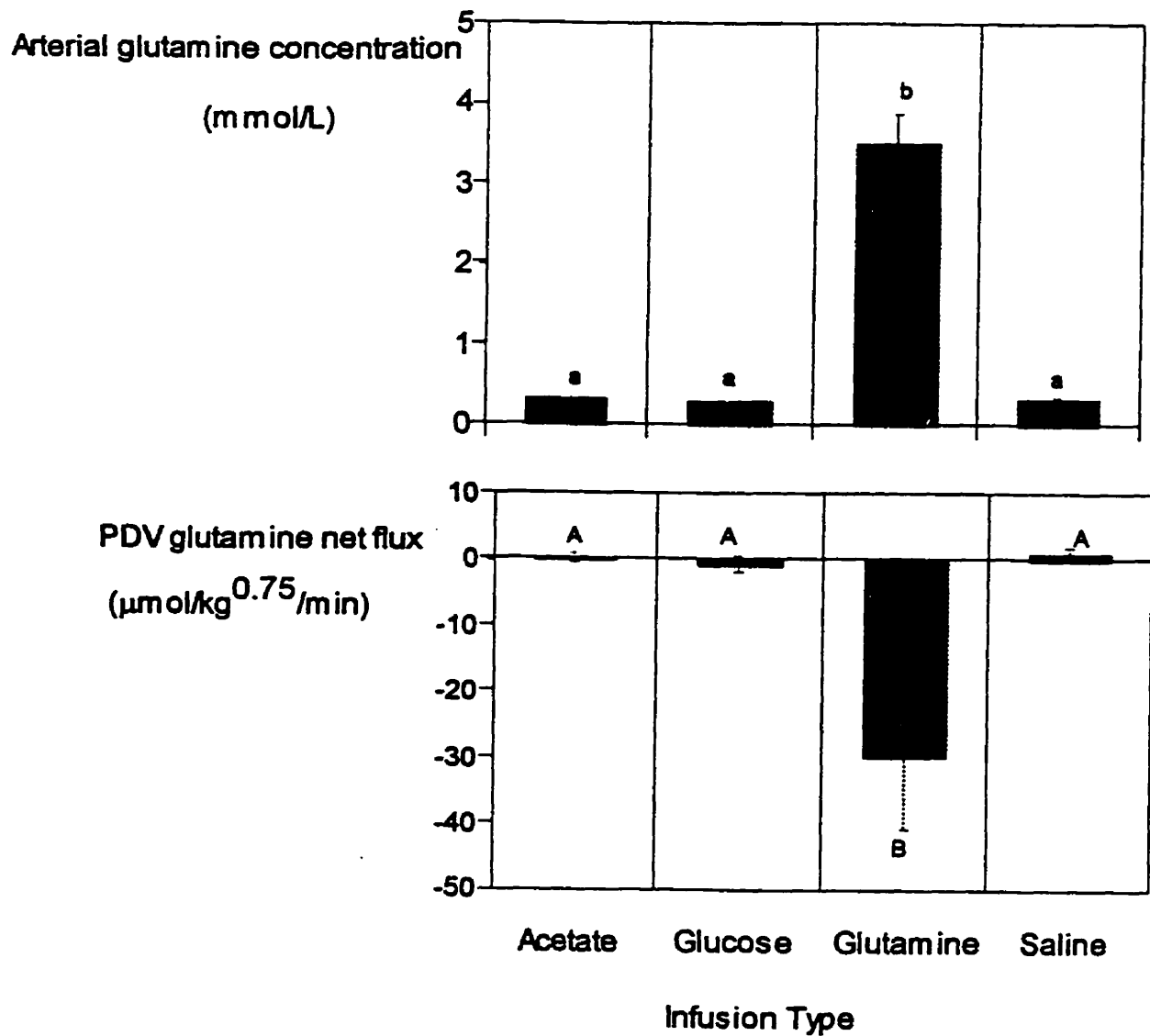
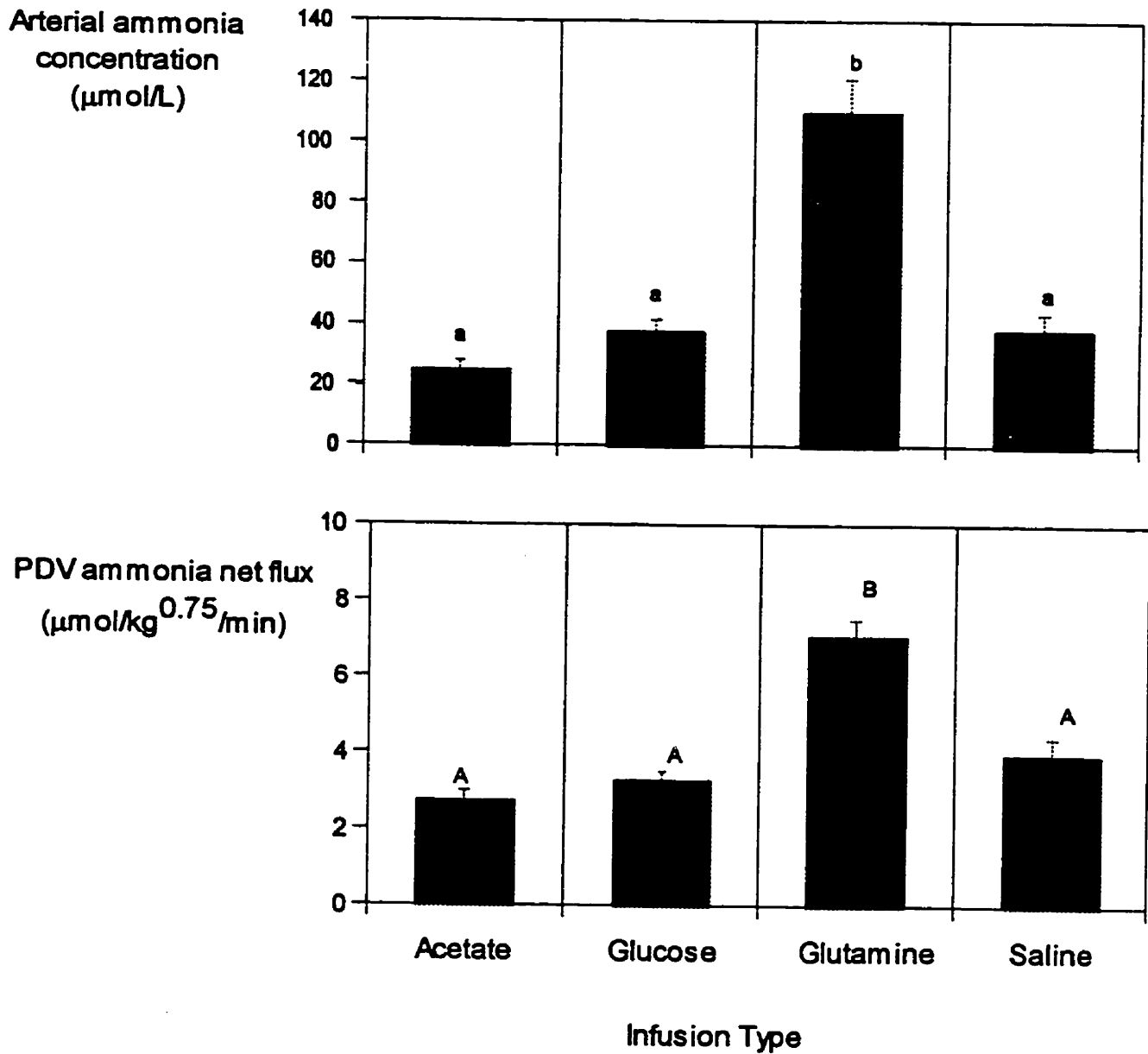


Figure 5.2. Arterial ammonia concentration and PDV production in four calves infused with different substrates. Values are means \pm SE. Means with different superscripts are significantly ($p < 0.05$) different.



infusion. During acetate, glucose, and saline infusions, vascular glucose was more important than glutamine as a source of energy for the intestine. Oxidation of vascular glutamine was low, except during glutamine infusion.

5.4 Discussion

The main function of the intestine is to absorb nutrients arising from intestinal digestion. However, it also uses substrates for its own metabolic needs. This study reports for the first time the net uptake and release of nutrients by the PDV of the calf *in vivo*. Very little information is available on the metabolite removal from the vascular side by the developing intestine. The present study also examined the extent to which metabolism is limited by the supply of substrate and delineates the importance of circulating glutamine and glucose as respiratory substrates for the calf's intestine.

In spite of normal food intake within four days post surgery, the calves did not gain weight over the two-week period. There was no evidence of infectious inflammatory process to explain this lack of weight gain; however, abdominal surgery is known to increase nutrient demands (Ralston and Naylor 1991). The calves may have grown if they were fed above average nutrient demands (Naylor 1991) and the study had been performed over a longer period of time. The 30 minute rest period, followed by a 45 minute infusion period before any samples were collected prevented any carry-over effects on the measurements because the arterial concentrations of glucose, glutamine, and acetate were only higher during the infusion of these substrates (Table 5.2).

In the small intestine of rats, glutamine from the vascular side may be metabolized via two principal routes, forming Δ^1 -pyrroline-5-carboxylate or α -ketoglutarate (Windmueller and Spaeth 1974) (Figure 2.2.5). Glutamine carbon appears in venous blood in CO₂ (57%), in citrulline (6%), proline (5%), and organic acids (18%), predominantly citric acid and lactic acid (Ardawi, 1987). In the same model, glutamine nitrogen appears in venous blood in citrulline (34%), alanine (33%), ammonia (23%), and proline (10%) (Windmueller and Spaeth 1974). Uptake of glutamine by rat intestine, from either the luminal or the vascular side, gives rise to similar products which support the conclusion that utilization of plasma glutamine is a property of enterocytes, the absorptive cells of the mucosa (Windmueller and Spaeth 1974 and 1980).

Our study confirms that glutamine has a role in the PDV function of neonatal calves and demonstrates that its uptake and metabolism is substrate limited. Glutamine infusion resulted in increased PDV glutamine uptake in association with increased PDV ammonia production and a tendency for oxygen consumption to increase. The PDV glutamine uptake was fourfold higher than PDV ammonia production, a significant difference. Therefore, for every mole of glutamine consumed, 0.24 moles of ammonia were produced. In the late-pregnant and peak-lactating rats, for every mole of glutamine consumed, 1-2 moles of ammonia were produced (Ardawi, 1987). In germ-free rats, Windmueller found for every mole of glutamine consumed, only 0.4 moles of ammonia were produced (Windmueller and Spaeth, 1974). In the dog, ammonia generation was almost zero unless glutamine was perfused, in which case its generation was about 1:1

with respect to glutamine disappearance (Weber et al., 1988; Cersosimo et al., 1989). Differences in intestinal metabolic activity between species may explain the variation in ammonia production from glutamine catabolism. In the dog, alanine and glutamate were released under basal conditions and especially when glutamine was infused (Weber et al., 1988). In our study, there was net PDV uptake of glutamate and no significant change in PDV alanine production. Studies in rats (Windmueller and Spaeth 1974 & 1978) and human (Darmaun et al., 1986) showed net release of alanine but little glutamate production during glutamine infusion.

An important aspect of glutamine nitrogen metabolism in the intestine of rats is the production of citrulline (Windmueller and Spaeth, 1978). In another study using the same experimental model (Chapter 6.0), we were unable to show any increase in citrulline production in calves. Although arginine is known to be released from the small intestine of pre- and post-weaning pigs (Wu et al., 1994), we were unable to demonstrate a change in net production of arginine by the PDV of neonatal calves during glutamine infusion. However, we did document a marked increase in PDV ammonia production so this appears to be the major nitrogen metabolite in calves.

A concern exists about the potential for ammonia intoxication during the intravenous infusions of glutamine. However, the ammonia from glutamine metabolism released into the portal blood should be extracted by the calf's liver before it reaches the systemic circulation. As a result the arterial ammonia concentrations were less than half the portal vein concentrations. This helps explain the absence of signs of toxicity during

glutamine infusions. Other studies have failed to demonstrate any toxicoses associated with glutamine-supplemented parenteral nutrition. Such studies have been carried out in human volunteers (Lowe et al. 1990), dogs (Souba et al. 1985a), and rats (Salloum et al. 1993).

Intestinal protein synthesis should be associated with uptake of indispensable amino acids. In our study, the uptake of histidine was significantly increased during glutamine infusion but this may have been a response to the higher arterial concentrations of this nutrient. During glutamine infusion, there was also a tendency for less production, or a switch from net production to net utilization for the indispensable amino acids phenylalanine, valine, isoleucine, methionine, and lysine in association with a slight increase in arterial concentrations of these amino acids (Table 5.4). Perhaps this uptake indicates that glutamine is being used for protein synthesis. This would also explain why only a small part of the glutamine nitrogen is released from the PDV as nitrogenous waste products. This is a potentially important finding in the context of mucosal repair in diarrheic calves. Uptake of the indispensable amino acids from the circulation are usually unimportant in the small intestine of adult rats (Windmueller and Spaeth 1974, 1978, and 1980) and pigs (Wu et al. 1994) suggesting that these nutrients are usually supplied from the intestinal lumen. However, intestinal supply of amino acids should have been limited in these calves because food was withheld for 16 hours prior to infusion, so there should be more reliance on arterial amino acids in our model.

The maximum fraction of oxygen consumption attributable to the oxidation of glucose or glutamine has some analogies with the respiratory quotient (Table 5.5). For

these calculations, we assume that all glucose or glutamine taken up by the PDV is oxidized to CO₂. In reality, not all glucose or glutamine taken up by the PDV is oxidized to CO₂ and large proportions of their carbons are utilized for synthesis of amino acids and other organic metabolites (e.g. alanine, aspartate, ornithine, citrulline, proline, etc) (Hartmann and Plauth, 1989). During glutamine infusion, more glucose and glutamine are removed than could possibly be oxidized (total greater than 1), so some of the glutamine must be used for purposes other than oxidation. During saline and acetate infusion glucose utilization probably accounts for the majority of PDV oxygen consumption. Glutamine played a very minor role. During glucose infusion, the PDV oxygen consumption was lowest. The fraction of glucose potentially oxidized exceeded 1 when lactate production was not included in the formula. This is presumably because anaerobic conversion of glucose to lactate generated ATP without using oxygen.

Future studies should measure the intestinal oxygen consumption in diarrheic calves during glucose infusion, because infusing glucose may reduce the intestine's oxygen requirement. Diarrheic calves often have bacteremia (Fecteau et al. 1997). The high frequency of septicemia in calves greater than 1 week of age may be explained by injury to their gastrointestinal tract, which predisposes to translocation of opportunistic bacteria that reside in the intestine. Countercurrent blood circulation in the intestinal villi makes them extremely susceptible to damage from lack of oxygen when blood flow is low (Phillips 1985). Therefore, infusing glucose may reduce gastrointestinal injury by reducing the need for intestinal oxygen consumption. The increased oxygen consumption during glutamine infusion suggested an increased metabolic activity of the intestine probably

related to tissue protein synthesis. Therefore, the greater oxygen consumption and the absolute uptake of some indispensable amino acids in our study suggest glutamine may enhance proliferation of the intestinal epithelium.

In our study, acetate had no effect on PDV metabolism. D-glucose decreased oxygen consumption, presumably because more glucose was metabolized to lactate. Glutamine infusion resulted in increased glutamine uptake by the PDV of calves. This was associated with increased ammonia production and a tendency for oxygen, glucose and indispensable amino acid consumption to increase. This indicates increasing glutamine supply increases the metabolic rate of the PDV. Glutamine supplementation could provide a new approach to promote intestinal healing the treatment of enteritis in neonatal calves. However, a number of questions concerning the optimum dose, route by which glutamine is to be administered, and the requirement for other nutrients for optimal effect have yet to be established. Therefore, studies on the effect of long-term intravenous glutamine infusions, route of administration and dosage by the calf's intestine should be performed before there is sufficient information to properly base any recommendation that glutamine supplementation is safe and effective in clinical therapies of calves.

Table 5.5. Oxygen consumption by PDV and net flux of glucose, lactate and glutamine in four neonatal calves during infusion with acetate, glucose, glutamine or saline.

Infusion Type:	PDV nutrient uptake $\mu\text{mol/kg}^{0.75}/\text{min}$ (mean \pm SE for 4 animals)				Maximum fraction of oxygen consumption potentially attributable to:		
	Glucose	Lactate	Glutamine	Oxygen	Glucose + Lactate ¹	GLUCOSE ²	GLN ³
Acetate	-4.7 \pm 2.2	9.5 \pm 2.3	-0.1 \pm 0.6 ^b	-48.7 \pm 7.3	-0.2 \pm 0.4	0.7 \pm 0.3	-0.1 \pm 0.1 ^e
Glucose	-6.7 \pm 2.6	9.1 \pm 3.3	-0.8 \pm 1.2 ^b	-34.8 \pm 3.5	0.1 \pm 1.0	1.4 \pm 0.8	0 \pm 0.2 ^e
Glutamine	-6.6 \pm 1.7	0.2 \pm 4.3	-29.9 \pm 11.2 ^a	-52.0 \pm 4.0	0.9 \pm 0.3	0.9 \pm 0.2	1.6 \pm 0.6 ^d
Saline	-2.7 \pm 1.6	3.7 \pm 5.8	0.9 \pm 1.0 ^b	-40.9 \pm 6.1	0.7 \pm 0.7	0.5 \pm 0.3	-0.1 \pm 0.1 ^e

Within a column means with different letters are significantly different ($p < 0.05$).

- ¹ Maximum fraction of oxygen consumption potentially attributable to glucose consumption = ((PDV glucose uptake x 6) - (PDV lactate production x 3))/(PDV oxygen uptake).
- ² Maximum fraction of oxygen consumption potentially attributable to glucose consumption = (PDV glucose uptake x 6)/(PDV oxygen uptake).
- ³ Maximum fraction of oxygen consumption potentially attributable to glutamine consumption = (PDV glutamine uptake x 4.5)/(PDV oxygen uptake).

6.0 Determination of nutrient uptake in portal-drained viscera during intravenous glutamine infusions in neonatal calves

6.1 Introduction

The small intestine has a central role in glutamine metabolism in normal and in pathological states. Glutamine has been the subject of considerable investigation, because its concentrations in blood and tissues decline markedly during critical illness (Wilmore, et al., 1988), and research suggests it may be a conditionally indispensable (i.e., essential) amino acid (Grimble 1993). Studies indicate that glutamine may be essential for maintenance of the structural and functional integrity of the small intestinal mucosa (Wilmore, et al., 1988; Fink, 1991). Glutamine supplementation has been found to increase intestinal protein synthesis and this finding may be one of the mechanisms by which glutamine exerts its protective effect on gut mucosa during critical illness (Higashiguchi, 1993). Glutamine has also been shown to promote repair of injured intestine (Fox et al. 1988), possibly through its stimulation of proliferative events including enhanced $\text{Na}^+ - \text{H}^+$ exchange and ornithine decarboxylase activity, which has been shown in several cell lines (McCormack et al. 1990).

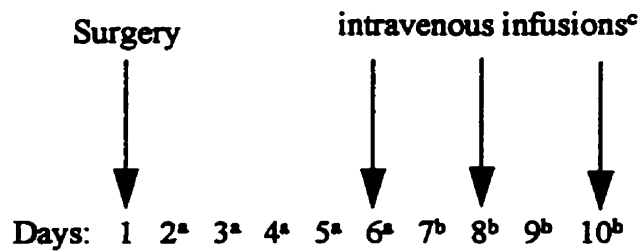
Arteriovenous difference measurements across the tissues drained by the nonhepatic splanchnic organs of dogs (Souba, 1991), sheep (Heitmann and Bergman 1978), and humans (Owen, et al., 1981) indicate that the small intestine is the principal organ of glutamine consumption. Furthermore, glutamine serves as an important source of energy for both enterocytes (Ashy, et al., 1988) and colonocytes (Ardawi, 1986) and

has trophic effects on the intestinal mucosa (Souba, et al., 1985). In adult rats (Windmueller and Spaeth, 1980) and pigs (Darcy-Vrillon, *et al.*, 1994), *in vitro* measurements using intestinal mucosal scrapings or isolated enterocytes have found that glutamine, glucose, and ketone bodies represent the major oxidative substrates of the small intestine. As an energy source glutamine's role may be magnified, at birth and during the suckling period, as intestinal glutamine oxidation is high, whereas glucose and β -hydroxybutyrate oxidation are very low in rats (Kimura, 1987) and pigs (Darcy-Vrillon, *et al.*, 1994). After weaning, intestinal glucose consumption increases, and glutamine utilization declined in enterocytes of rats (Windmueller and Spaeth, 1980) and pigs (Darcy-Vrillon, *et al.*, 1994).

In a previous study with calves (Nappert et al. 1997), we showed that a short-term intravenous infusion of 200 mmol/L of glutamine over a period of an hour resulted in increased PDV glutamine uptake in association with increased PDV ammonia production and a tendency for oxygen consumption to increase. The objectives of this study were to quantify further PDV glutamine utilization by neonatal calves, and to determine if glutamine uptake could be further stimulated either by longer term intravenous infusion or by chronic oral supplementation. A better understanding of the nutrients used by intestinal tissues may allow us to develop feeding regimens that will support intestinal regeneration.

Figure 6.1. Feeding protocol.

- ^a** 10% of the calf's body weight as whole cows' milk.
- ^b** 10% of the calf's body weight as whole cows' milk plus 30 g of glutamine twice a day in 1 L of water.
- ^c** Glutamine infusions (200 mmol/L/h) were carried out over a 5 h period prior to feeding after an overnight fast.



6.2 Methods

6.2.1 Animals, Diet and Experimental Design

Four healthy male Holstein calves were obtained from the University of Saskatchewan dairy barn within a week of birth. The study period was 10 days (Figure 6.1). On day 1, the femoral artery, jugular vein and the portal vein were surgically cannulated in each calf. In the feeding protocol, the calves were fed daily 10% of their body weight as whole cows' milk which was divided equally and consumed as two feeds. On days 7 to 10, the calves were fed milk plus 30 g of glutamine twice a day and two additional feeds of 30 g of glutamine in 1L of water. On days 6, 8 and 10 of the experimental period, after an overnight fast, a five hours intravenous infusion of glutamine was carried out to investigate nutrient metabolism in the calf's intestine by measuring PDV uptake and oxygen consumption. Calves were euthanatized at the end of the experimental period and a necropsy was performed to confirm proper placement of the surgical catheters. The experimental protocol and the procedures used were approved by the University of Saskatchewan Animal Care Committee, Saskatoon, Canada.

6.2.2 Surgical procedure

The surgical approach used to cannulate the femoral artery, jugular vein and the portal vein has been previously described (Nappert, *et al.*, 1997). Briefly, after an overnight fast, the calves were anesthetized with 2% halothane in 100%O₂ using intermittent positive-pressure ventilation. A bilateral subcostal incision was made parallel

to the paracostal arch. A silastic catheter, 100 cm in length (internal diameter: 1.57 mm, outside diameter: 3.17 mm), was inserted into the umbilical vein and passed cranially until it could be palpated within the common trunk of the portal vein. An ultrasonic perivascular flowprobe (8R, Transonic Systems, Ithaca, NY) was placed around the portal vein. A second silastic catheter, 100 cm in length (diameters in mm: 1.01 inside diameter X 2.16 outside diameter), was inserted 8 cm into the femoral artery toward the aorta. At the completion of surgery and every three days thereafter, catheters were flushed with 1 mL of heparin (1000 U/mL) and injection caps were changed. One week post-operatively, the heparin strength was increased from 1000 U/mL to 10,000 U/mL.

6.2.3 Infusion studies

An isotonic L-glutamine solution was prepared (29.22 g/L of glutamine and 2.9 g of sodium chloride/L) using double distilled water. When the L-glutamine was dissolved, the pH of the resulting solution was adjusted to 7.3 by addition of 1 M hydrochloric acid. The solution was then filtered through a 0.22-mm Millipore filter into a sterile bottle. The L-glutamine solution was prepared approximately 4 h prior to each infusion.

During the morning of days 6, 8, and 10 of the experimental period, the glutamine solution was administered intravenously via the jugular vein at a rate of 200 mmol/h for a 5 h period. Simultaneous arterial and portal blood samples were collected in duplicate in 3 mL heparinized tubes every hour for five hours. Portal vein blood flow was measured during blood sampling. Blood samples were placed in an ice water bath and centrifuged to obtain plasma. The plasma was stored at -70°C until assayed. To minimize possible

degradation of amino acids and organic acids, an aliquot of plasma was deproteinized prior to freezing by ultrafiltration (ultrafree-MC 10,000 NMWL, Millipore corporation, Milford, MA) with a microcentrifuge (Miofuge 11 Centrifuge, Beckman, Instruments, Inc., Palo Alto, CA) at 1500 g for 30 min. Blood (2.5 mL) was also collected aseptically every hour for five hours from the femoral artery and portal vein in a pre-heparinized plastic syringe (Smooth-E™, Radiometer America Inc., Westlake, OH) and analyzed immediately for oxygen content.

6.2.4 Biochemical analysis

Blood oxygen contents were determined by spectral absorption with a co-oxymeter (Ciba Corning Diagnostics Corp., Medfield, MA) (Zwart, *et al.*, 1981). Plasma glucose concentrations were determined using the hexokinase glucose-6-phosphate dehydrogenase method (Bondar and Mead, 1974) and a spectrophotometric autoanalyser (Abbott Spectrum system, North Chicago, IL). Plasma ammonia concentrations were determined using the A-GENT Abbot ammonia method (Abbot Laboratories, Abbot Park, IL) which utilizes the amination of α -ketoglutarate and concomitant oxidation of nicotinamide hypoxanthine dinucleotide phosphate mediated by glutamate dehydrogenase (Gau, 1987).

Plasma organic acid and amino acid concentrations were quantitatively determined by reverse-phase high performance liquid chromatography (RP-HPLC). The RP-HPLC system consisted of a pump (Waters 600 pump, Mississauga, ON), a tunable wavelength absorbance detector (Waters 486), a temperature control module (Waters), an autoinjector (Waters 710 WISP), and a chromatography manager (Waters Millennium version 2.1).

Determination of plasma acetate, pyruvate, lactate and β -hydroxybutyrate concentrations were carried out using the ion exclusion method (Waters, Millipore Corporation, Milford, MA) for the separation of organic acids with slight modifications. Plasma amino acid concentrations were determined using the Pico-Tag Amino Acid method (Waters, Millipore Corporation, Milford, MA) for RP-HPLC (Heinrikson and Meredith, 1984) with slight modifications (see section 5.2.4 Biochemical analysis).

6.2.5 PDV nutrient difference and oxygen consumption

The portal-drained viscera includes the abomasum, small and large intestine, mesenteric fat, pancreas, and spleen. Net flux across the PDV was estimated by multiplying the arteriovenous concentration differences of a nutrient by the appropriate blood flow. The net flux is an estimate of the combined effect of uptake and release of the nutrient by the PDV. Positive net flux indicates production or absorption of a nutrient in excess of uptake. Negative net flux indicates PDV uptake in excess of endogenous production or absorption from the intestinal lumen. Estimates of the PDV net uptake or production were calculated using the following equation:

$$\text{PDV nutrient net flux } (\mu\text{mol/kg}^{0.75}/\text{min}) = [\text{BF} \times (\text{N}_v - \text{N}_a) \times (1 - \text{PCV})] / \text{BW}^{0.75}$$

where BF is the blood flow in the intestine, in liters per min. N_v and N_a are plasma nutrient concentrations, mmol/L, in portal vein and femoral artery respectively. BW is body weight, in kilograms, to the $3/4$ power which is directly proportional to maintenance energy requirements (Kleiber 1975). Packed cell volume (PCV) is assumed to be the same in portal and arterial vessels. The PDV oxygen consumption was calculated from the

following equation: PDV oxygen consumption ($\mu\text{mol}/\text{kg}^{0.75}/\text{min}$) = $[\text{BF} \times (\text{N}_v - \text{N}_i)]/\text{BW}^{0.75}$

To estimate the maximum potential contributions of glucose and glutamine to net oxygen consumption by the gastrointestinal tissues, metabolic quotients were calculated from the ratio of substrate uptake/oxygen uptake, multiplied by the number of oxygen moles required for complete oxidation of 1 mole of the substrate into CO_2 . All lactate was assumed to have come from glucose and this was subtracted from glucose uptake to arrive at the quotient for glucose:

Fraction of oxygen consumption attributable to glucose = $((\text{PDV glucose uptake} \times 6) - (\text{PDV lactate production} \times 3))/(\text{PDV oxygen uptake})$. The fraction of oxygen consumption attributable to glucose had also been calculated without considering lactate production = $(\text{PDV glucose uptake} \times 6)/(\text{PDV oxygen uptake})$.

Fraction of oxygen consumption attributable to glutamine = $(\text{PDV glutamine uptake} \times 4.5)/(\text{PDV oxygen uptake})$.

6.2.6 Data analysis

The mean arteriovenous differences were calculated for each nutrient every hour during each infusion. Data were analyzed using repeated measures ANOVA with factors for day of infusion ($n=3$) and sampling time (0 to 5 h). Differences between means were calculated using Tukey's test. All PDV nutrient differences were expressed as the mean \bullet SE, and a p value of less than 0.05 was considered significant.

6.3 Results

On the day of the surgery, the four calves were 4 ± 1 (mean \pm SD) days old and weighed 45.5 ± 0.7 kg. The infusion protocol was successfully performed in the four calves. Ten days after the surgery, the calves weighed 51 ± 1 kg. There was no evidence of infectious inflammatory process in any calf at necropsy.

The mean PDV glutamine, and oxygen uptake and ammonia production were not significantly affected by feeding glutamine orally on days 7 through 10. Therefore, the means of the PDV nutrient uptake for each experimental day were pooled together. Prior to any infusion, the mean PDV nutrient uptake in $\mu\text{mol}/\text{kg}^{0.75}/\text{min} \pm \text{SE}$ was 1.54 ± 1.34 for glutamine and 1.06 ± 0.77 for glucose (Table 6.1). The arterial concentration of glutamine (Figure 6.2) and ammonia (Figure 6.3) were significantly higher during glutamine infusion. No detectable amounts of acetate (< 0.5 mmol/L) were found in the portal vein or the femoral artery in any calves during the infusions. Co-eluting peaks were found for β -hydroxybutyrate. No plasma remained for additional measurements of the β -hydroxybutyrate levels by spectrophotometry. Therefore, β -hydroxybutyrate uptake was not determined.

During glutamine infusion, there was an absolute increase in PDV uptake of glutamine (Figure 6.2) associated with an increased PDV production of ammonia in a time-dependent fashion ($p < 0.001$) (Figure 6.3). Ammonia is produced when glutamine is deaminated. Although the arterial ammonia concentrations were higher during glutamine

Table 6.1 Oxygen consumption by portal-drained viscera and net flux of glucose, lactate and glutamine in four neonatal calves during intravenous glutamine infusion.

Time of infusion (h)	PDV nutrient uptake				Fraction of oxygen consumption attributable to:		
	$\mu\text{mol/kg}^{0.75}/\text{min}$ (mean \pm SE for 4 animals)						
	Glucose	Lactate	Glutamine	Oxygen	Glucose + Lactate ¹	Glucose ²	GLN ³
0	-1.1 \pm 0.8	-5.1 \pm 4.8	-1.5 \pm 1.3	-43.2 \pm 3.8	0.5	0.1	0.2
0.5	-2.7 \pm 0.9	3.3 \pm 4.9	-9.6 \pm 11.8	-38.9 \pm 3.1	0.2	0.4	1.1
1	-4.4 \pm 0.9	2.8 \pm 4.9	-2.0 \pm 14.4	-34.5 \pm 3.0	0.5	0.8	0.3
2	-1.6 \pm 1.0	-0.3 \pm 5.0	-11.3 \pm 15.5	-38.7 \pm 2.6	0.3	0.2	1.3
3	-1.4 \pm 0.8	5.7 \pm 4.3	-11.7 \pm 23.5	-37.8 \pm 2.9	-0.2	0.2	1.4
4	-1.8 \pm 0.7	-1.3 \pm 5.4	-8.0 \pm 16.5	-30.9 \pm 3.5	0.5	0.3	1.2
5	-2.9 \pm 0.7	-0.8 \pm 5.0	-27.7 \pm 17.1	-37.1 \pm 3.7	0.5	0.5	3.4

¹ Fraction of oxygen consumption attributable to glucose (when there is PDV lactate production) = ((PDV glucose uptake x 6) - (PDV lactate production x 3))/(PDV oxygen uptake).

² Fraction of oxygen consumption attributable to glucose = (PDV glucose uptake x 6)/(PDV oxygen uptake).

³ Fraction of oxygen consumption attributable to glutamine = (PDV glutamine uptake x 4.5)/((PDV oxygen uptake).

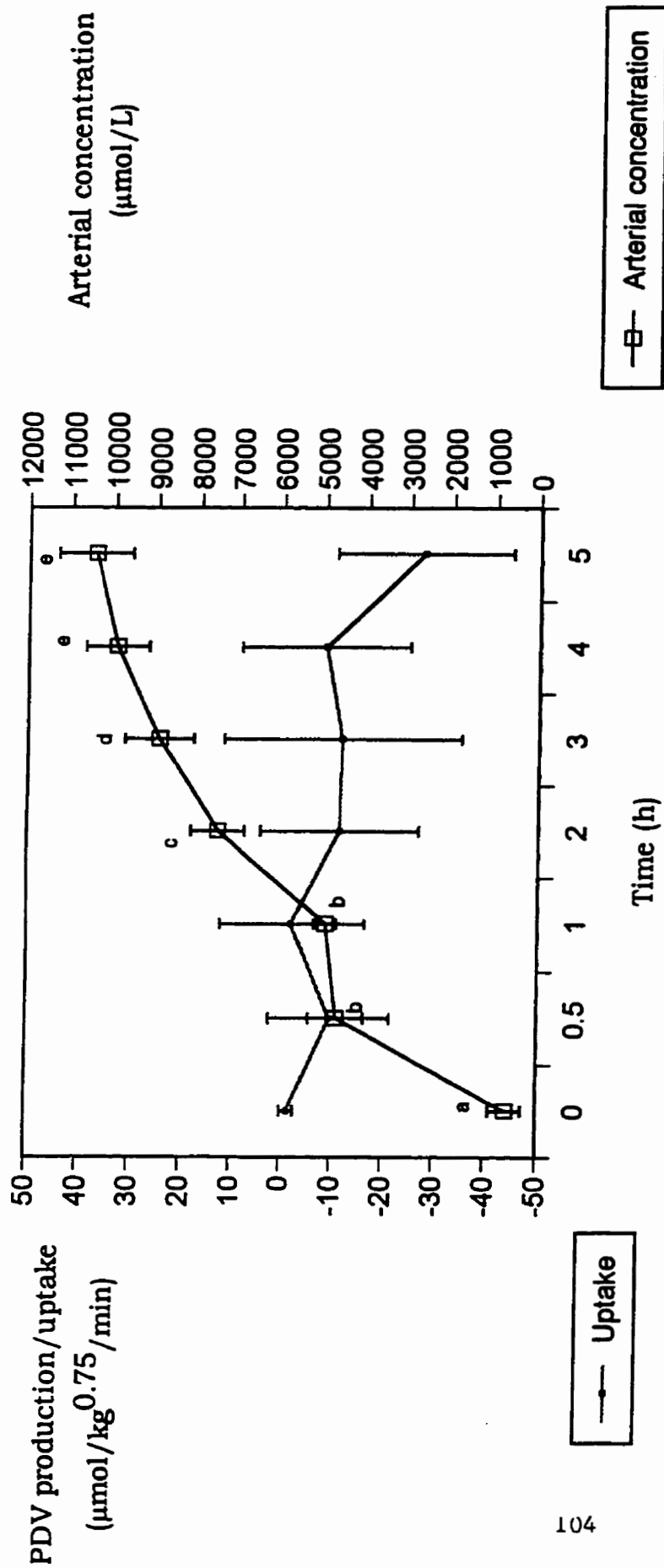


Figure 6.2 PDV glutamine production (positive values) or uptake (negative values) and arterial glutamine concentration in four calves during glutamine infusion. Values are means \pm SE. Means with different superscripts are significantly ($p < 0.05$) different.

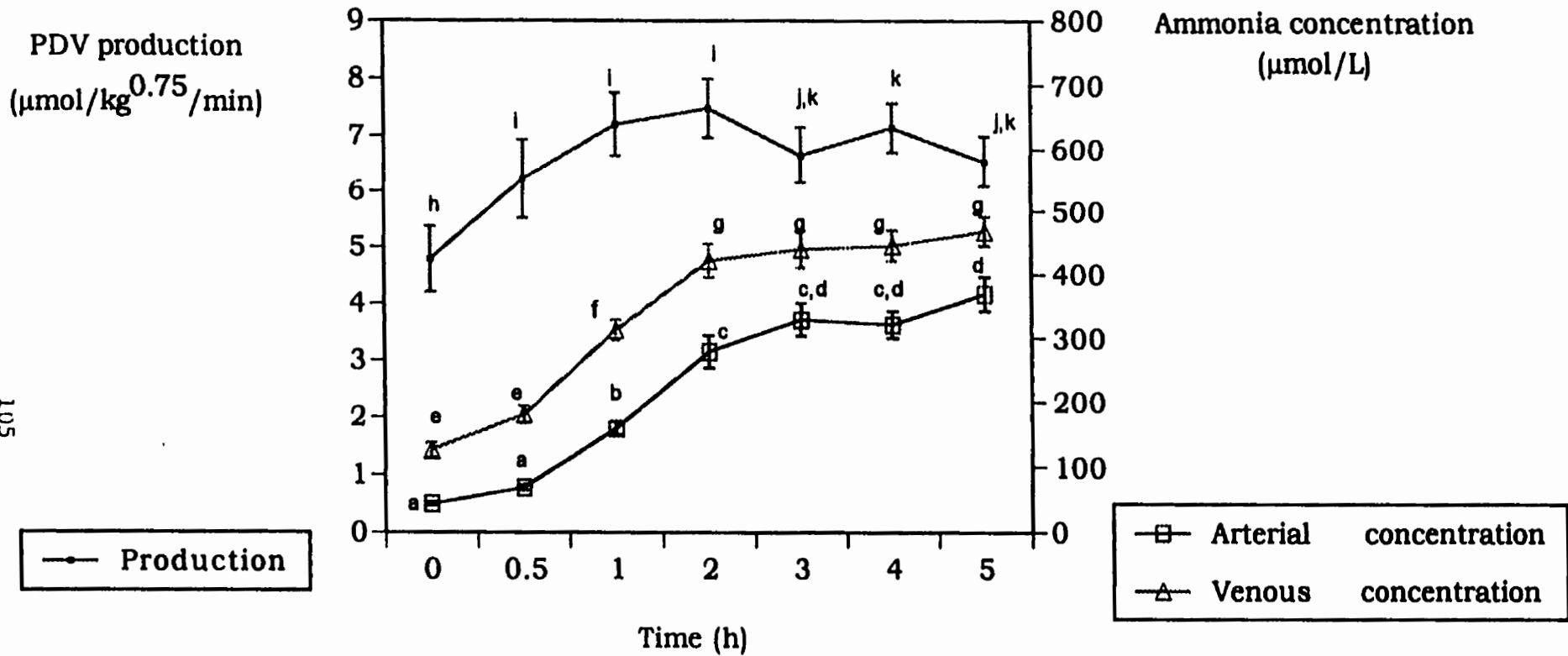


Figure 6.3 PDV ammonia production, and arterial and portal ammonia concentrations in four calves during glutamine infusion. Values are means \pm SE. Means with different superscripts are significantly ($P < 0.05$) different.

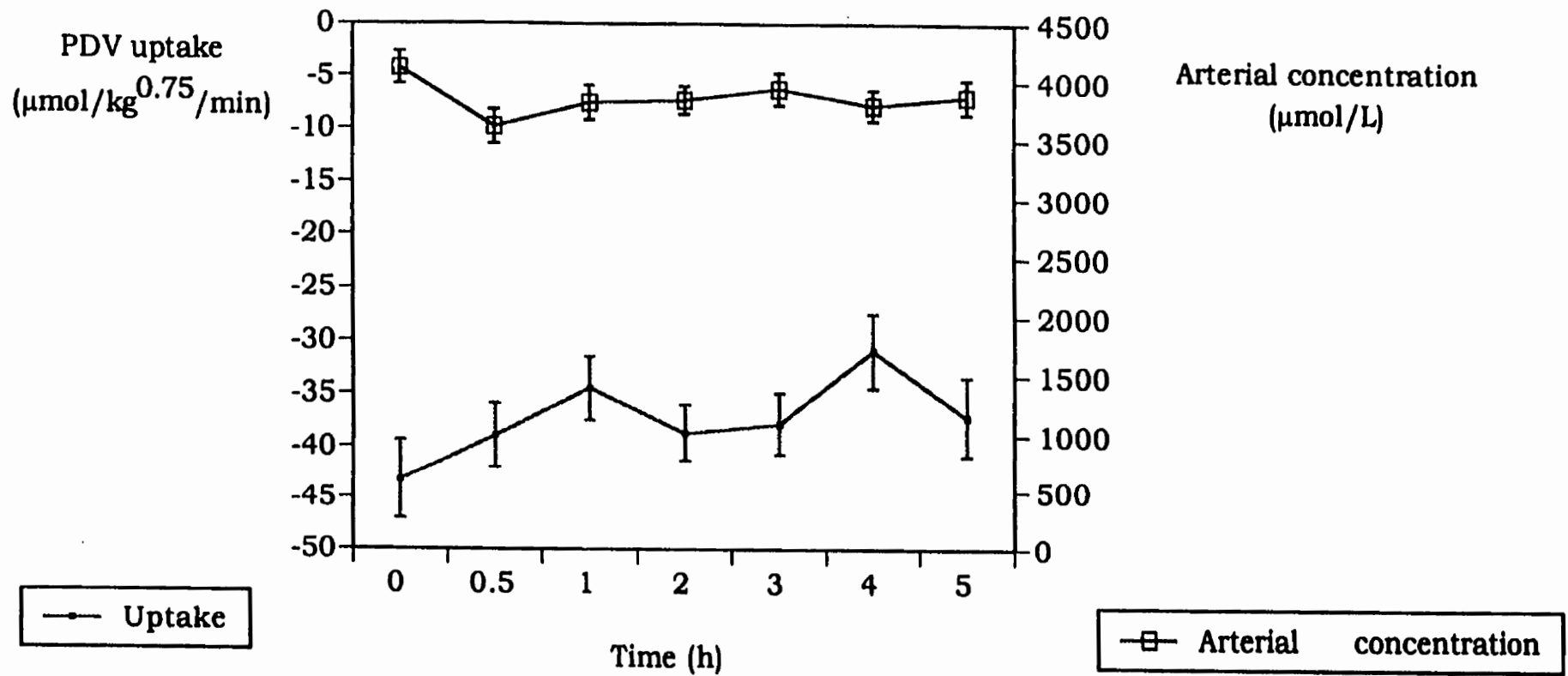


Figure 6.4 PDV oxygen uptake and arterial oxygen concentration in four calves during glutamine infusion. Values are means \pm SE.

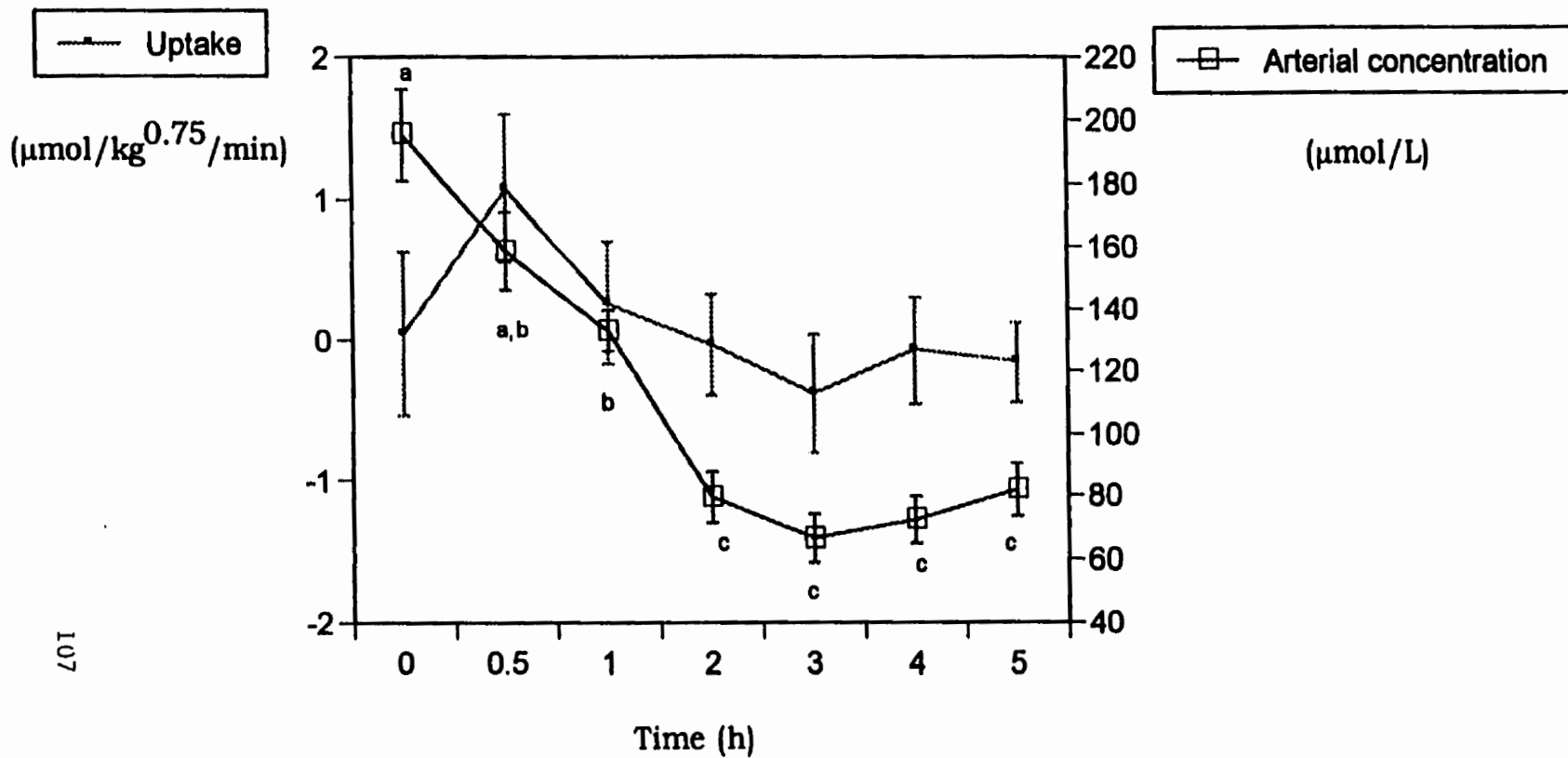


Figure 6.5 Arterial leucine concentration and PDV leucine production in four calves during glutamine infusion. Values are means \pm SE. Means with different superscripts are significantly ($P < 0.05$) different.

infusion, no signs of nervous dysfunction were observed. PDV uptake of oxygen (Figure 6.4) was significantly decreased in a time-dependent fashion ($p < 0.006$) during glutamine infusion and this was associated with a decrease in delivery of oxygen to the PDV.

Oxygen consumption was lowest 4 h after the beginning of the infusion and highest prior to glutamine infusion (Table 6.1). The maximal fraction of oxygen consumption potentially attributable to glucose or glutamine oxidation was not significantly affected by glutamine infusions. PDV uptake of leucine (Figure 6.5) was decreased in a time-dependent fashion during glutamine infusion and this was associated with a decrease in delivery of leucine to the intestine. Overall, the PDV uptake of the other indispensable amino acids or the production of citrulline and arginine (Figure 6.6) were not affected by glutamine infusion. Arterial glucose concentration was highest and net PDV glucose uptake was greatest at the one hour sampling period (Figure 6.7).

6.4 Discussion

Very little information is available on the metabolite removal from the vascular side by the developing intestine of the calf *in vivo*. In the small intestine of rats, glutamine from the vascular side may be metabolized via two principal routes, forming Δ^1 -pyrroline-5-carboxylate or α -ketoglutarate (Windmueller and Spaeth, 1974) (Figure 2.2.5). Glutamine carbon appears in venous blood in CO_2 (57%), in citrulline (6%), proline (5%), and organic acids (18%), predominantly citric acid and lactic acid (Windmueller and Spaeth, 1974). In the same model, glutamine nitrogen appears in venous blood in citrulline (34%), alanine (33%), ammonia (23%), and proline (10%) (Windmueller and Spaeth, 1974). Uptake of glutamine by rat intestine, from either the luminal or the

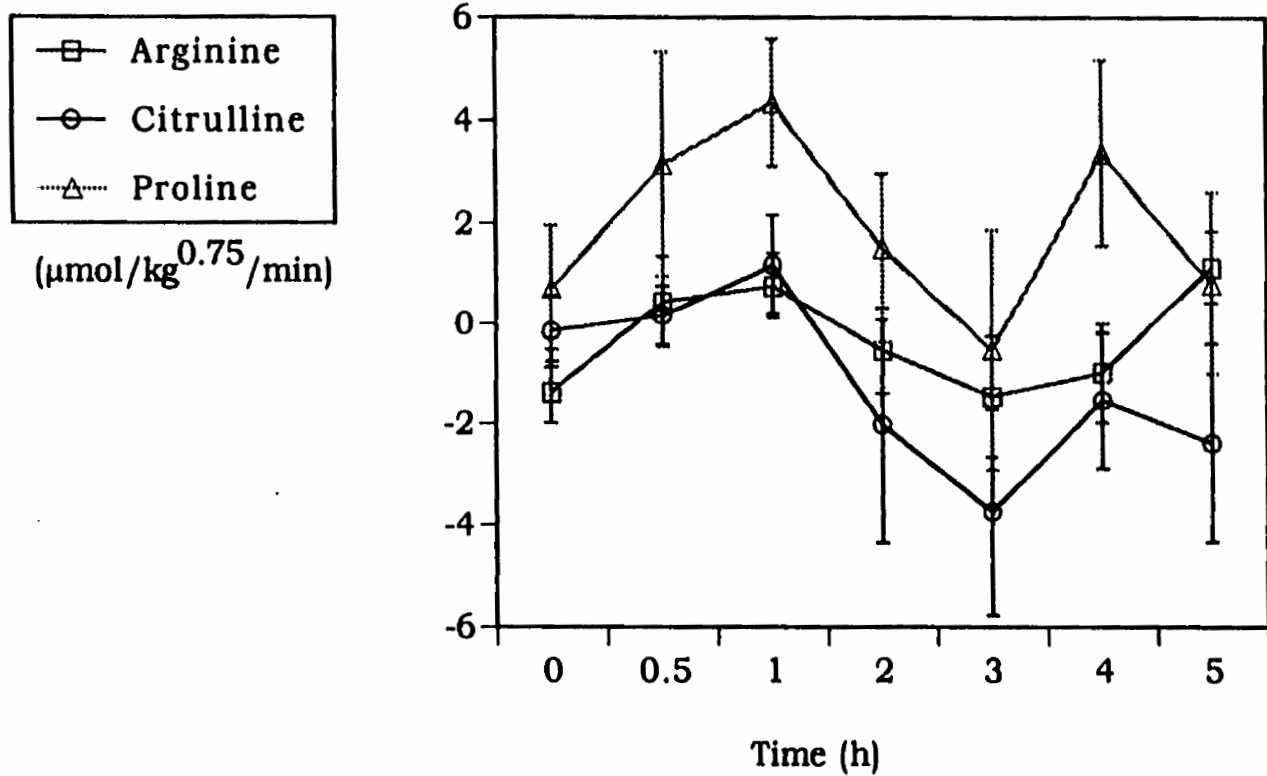


Figure 6.6 PDV production (positive values) or uptake (negative values) of arginine, citrulline and proline in four calves during glutamine infusion. Values are means \pm SE.

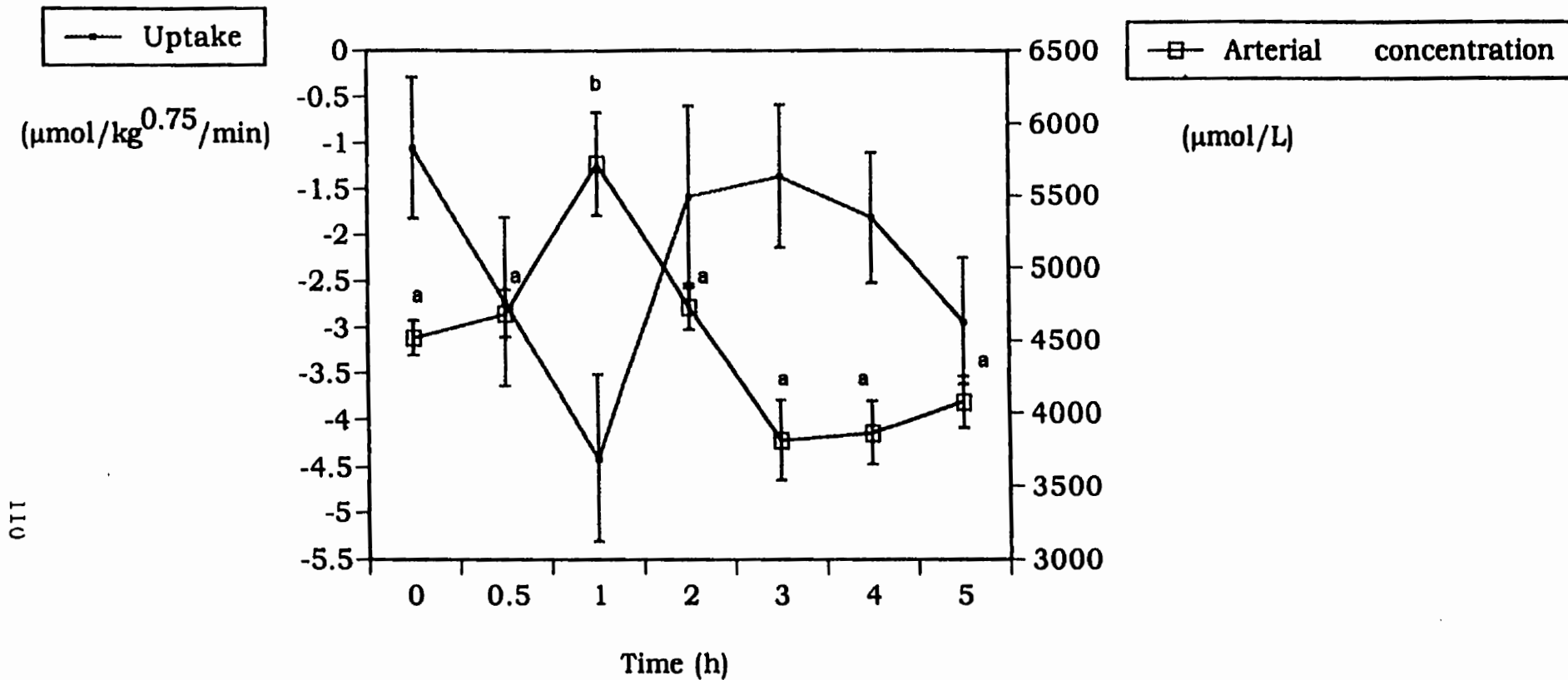


Figure 6.7 Arterial glucose concentration and PDV glucose uptake in four calves during glutamine infusion. Values are means \pm SE. Means with different superscripts are significantly ($P < 0.05$) different.

vascular side, gives rise to similar products which support the conclusion that glutamine is used by the enterocytes (Windmueller and Spaeth, 1974 and 1980).

In accordance with our previous experiment, the present study delineates the importance of circulating glutamine and glucose as respiratory substrates for the calf's intestine. Glutamine infusion resulted in a marginally increased PDV glutamine uptake in association with an increase in PDV ammonia production. However the PDV oxygen consumption did not appear altered by glutamine infusion. The plateau in arterial blood ammonia concentrations after three hours of infusion suggests that excess ammonia is probably removed by the liver through the urea cycle (Prior and Gross, 1994). Presumably portal ammonia concentrations gradually increase until they are sufficiently high that hepatic removal of ammonia is driven at the same rate as intestinal production (Figure 6.3). The PDV glutamine uptake was usually higher than the PDV ammonia production during the infusion. If all ammonia production comes from the conversion of glutamine to glutamate, it can be calculated that approximately 60% (range 24- 88%) of the circulating glutamine was catabolized to yield glutamate. However, there was no net release of glutamate into the portal circulation. Instead there was usually net uptake of glutamate. This is consistent with studies in rats (Windmueller and Spaeth, 1974 and 1978) and pigs (Wu et al., 1994) that showed little glutamate production during glutamine infusion. The remaining glutamine could be used for tissue protein synthesis or it may have diffused into the intestinal lumen or its carbon skeleton could be used for energy.

A concern exists about the potential for ammonia intoxication during the intravenous infusions of glutamine. However, the ammonia from glutamine metabolism released into the portal blood should be extracted by the calf's liver before it reaches the systemic circulation. As a result the arterial ammonia concentrations were less than half the portal vein concentrations. This helps explain the absence of signs of toxicity during glutamine infusions. Other studies have failed to demonstrate any toxicity associated with glutamine-supplemented parenteral nutrition. Such studies have been carried out in human volunteers (Lowe et al., 1990), dogs (Souba et al., 1985), rats (Windmueller and Spaeth, 1978), and pigs (Wu et al., 1994).

An important aspect of glutamine metabolism in the intestine of rats (Windmueller and Spaeth, 1978; Henselee and Jones, 1982) and pigs (Wu et al. 1994) is the production of citrulline, ornithine or proline. We were unable to show any increase in citrulline and proline production in calves (Figure 6.6). Ornithine was not determined. In suckling pigs, the rate of citrulline synthesis from glutamine was found to be low in enterocytes, but was enhanced 10-fold in cells from weaned pigs due to the induction of pyrroline-5-carboxylate synthase (Wu et al. 1994). The induction of citrulline synthesis from glutamine in calves may be age-dependent. Although arginine is known to be released from the small intestine of pre- and post-weaning pigs (Wu, 1994), we were unable to demonstrate a change in net production of arginine by the PDV of neonatal calves during glutamine infusion (Figure 6.6). However, we did document a marked increase in PDV ammonia production so this appears to be the major nitrogen metabolite in calves.

Intestinal protein synthesis should be associated with uptake of indispensable amino acids. During the previous short-term infusion study, there was a tendency for less production, or a switch from net production to net utilization of the indispensable amino acids phenylalanine, valine, isoleucine, methionine, and lysine in association with a slight increase in arterial concentrations of these amino acids. Neither chronic oral supplementation with glutamine, or infusion for periods longer than an hour, significantly increased the PDV uptake of indispensable amino acids. The vascular supply of leucine and PDV uptake declined during glutamine infusion suggesting that its supply became limiting in our study (Figure 6.5). The PDV oxygen consumption decreased during glutamine infusion, suggesting that there was no stimulation of intestinal metabolism. Glutamine infusion did not increase the PDV uptake of indispensable amino acids in adult rats (Windmueller and Spaeth, 1974, 1978, and 1980) and pigs (Wu et al., 1994) suggesting that these nutrients may be supplied from the intestinal lumen. Food was withheld for 16 hours prior to infusion, so there should be more reliance on arterial amino acids in our model but in the growing animal the supply to the intestine may be limited. Thus glutamine supplementation may require the provision of a mixture of amino acids to be effective. This would explain the absence of a beneficial affect of simple glutamine supplementation of oral electrolyte solutions in diarrheic calves deprived of milk.ⁱ Therefore, more studies in the feeding state or during intravenous infusion of all the indispensable amino acids should be performed.

The fraction of oxygen consumption attributable to the oxidation of glucose or glutamine has some analogies with the respiratory quotient (Table 6.1). When the

combined total for these fractions is less than one, some other metabolite is also being oxidized. When the total is greater than one, some of the glucose or glutamine must be used for purposes other than oxidation. During glutamine infusion, glutamine utilization usually accounted for the majority of PDV oxygen consumption and the fraction exceeds one so presumably there is some use of these substrates for synthetic rather than oxidative purposes. However, there is no increase oxygen consumption during glutamine infusion to suggest an increased metabolic activity of the intestine.

In our study, vascular acetate was not removed by the PDV. Glutamine infusion resulted in an absolute increased glutamine uptake by the PDV of calves. This was associated with increased ammonia production. Glutamine supplementation could provide a new approach to promote intestinal healing in the treatment of enteritis in neonatal calves. However, a number of questions concerning the optimum dose, route by which glutamine is to be administered, and the requirement and route of administration for other nutrients (e.g., leucine) for optimal effect have yet to be established. Therefore, more studies should be performed before there is sufficient information to properly base any recommendation that glutamine supplementation is safe and effective in clinical therapies of calves.

7.0 GENERAL DISCUSSION AND CONCLUSIONS

7.1 Discussion and Future Directions

For a long time, veterinarians have been interested in the most appropriate method of providing nutritional support for the diarrheic calf. The approaches that have been tried include, nothing orally for a few days and feeding a comparable volume of a bicarbonate-rich rehydration solution, rehydration solutions that contain dextrose as the principal energy source in amounts varying from about 5 to 75% of the calf's maintenance energy requirement, mucilage-based rehydration solution, and multiple feeds of small volumes of whole cow's milk with acetate-based rehydration solutions in separate feedings. A definitive answer is not yet in hand and, in any case, probably varies with the situation. However, research on PDV nutrient utilization is providing information that can guide our choice.

Early studies were somewhat empirical - diarrheic calves were fed different diets and the results observed. More recently, nutritional requirements across the portal-drained viscera of preruminant calves were determined using an arteriovenous difference technique. In the current studies, a bilateral subcostal approach was used to reach the portal area to provide access for proper placement of an ultrasonic transit time flow probe around the portal vein. The application of an ultrasonic flow probe provided consistent measurements of blood flow. The umbilical vein was used as an entry point for the portal vein catheter. The femoral artery was also catheterized. Using blood flow measurements

and arterio-venous differences we could measure net nutrient uptake. This model has been performed successfully on healthy calves, but the study may have implications in the development of treatment to promote intestinal healing in diarrheic calves.

The small intestine is usually the major site of glutamine utilization in the mammalian body. However, the role of glutamine as an energy source or a precursor for synthesis of other amino acids and nucleotides such as ATP, purines, and pyrimidines in the intestine of the preruminant calf is not clear. Therefore, PDV metabolism was investigated by intravenous infusion of possible nutrients in four neonatal calves. The experimental design consisted of a series of infusions conducted on four different study days in each calf. On the study days, four separate one hour infusions of acetate, glucose, glutamine, saline (control) were administered intravenously via the jugular vein at a rate of 200 mmol/L/h in a different order. Venous and arterial blood was collected over the last 15 min of each one hour infusion. Our study shows that during acetate, glucose, and saline infusions, glucose was a greater source of energy than glutamine for the gut. However, during glutamine infusion, PDV glutamine uptake increased significantly which was associated with a rise in ammonia production. There was net PDV production of acetate during all the infusions. There was some tendency for PDV oxygen consumption to change between infusions. Oxygen consumption was lowest during glucose infusions and highest during glutamine infusions. If the same holds true in diarrheic calves, this work has interesting implications.

Diarrheic calves often have bacteremia. The high frequency of septicemia in preruminant calves may be explained by injury to their gastrointestinal tract, which predisposes to translocation of opportunistic bacteria in the intestine. The countercurrent blood circulation in the intestinal villi make them extremely susceptible to damage from lack of oxygen when blood flow is low. Oxygen leaves the arterioles for the venous circulation as blood passes towards the tip of the villi. In consequence, arterial oxygen content is reduced at the villous tip. This effect is enhanced when flow rates are low, as in shock, and the villous tip can become anoxic and slough. Because intravenous glucose has a tendency to reduce the PDV oxygen requirement, it might be possible to spare the villi from some of this anoxic damage by adding glucose to intravenous fluids. The use of intravenous glucose supplementation should be recommended when bicarbonate is included in the fluids to correct acidosis, because glucose is initially converted to lactic acid. Therefore, glucose supplementation should only be required when the diarrheic calves are in shock, and tissue metabolism is failing.

Considering the results of our study on the short-term intravenous infusion of nutrients, we decided to quantify further PDV glutamine utilization, and to determine if glutamine uptake could be further stimulated either by longer term intravenous infusion or by chronic oral supplementation. Feeding glutamine orally did not alter the PDV glutamine uptake. Glutamine infusion did not increase the PDV uptake of indispensable amino acids. Neither chronic oral supplementation with glutamine, or infusion for periods longer than an hour, further increased PDV glutamine uptake. Arterial leucine concentration and PDV uptake declined during glutamine infusion suggesting that its

supply became limiting. Thus various nutrients combined with glutamine supplementation might be most successful, because glutamine supplementation increases the use of other amino acids.

Intestinal malabsorption is believed to occur in many types of bovine diarrhea, especially those associated with villous atrophy caused by viral infection. Physiological studies have demonstrated that the ability of the calf's intestine to digest and absorb in the healthy and diarrheic state is different. In fact, our previous study, using breath hydrogen excretion as the marker, showed that carbohydrate malabsorption rather than a specific lactose maldigestion was a significant problem in diarrheic calves. Therefore, the intestinal villous atrophy makes it unlikely that any one nutrient will have unimpaired absorption. Rather than searching for one golden nutrient, we should concentrate on means of feeding the calf within the limits of its impaired digestive and absorptive function.

Another problem in diarrheic calves is that any undigested nutrients promote bacterial overgrowth and exacerbate diarrhea. To date, the most promising food source for diarrheic calves is whole cow's milk. It has the advantages of being a good source of both carbohydrates and proteins. It is also rich in glutamate and glutamine. However, to avoid problems with malabsorption it is important to tailor milk feeding to absorptive ability. It is also important not to feed electrolytes that contain large amounts of citrate or bicarbonate that will inhibit milk clotting. Ideally, there should be a gradual release of nutrients into the small intestine as the milk clot breaks down. This makes it less likely that the intestine's impaired absorptive ability will become saturated. The exact amount of

milk to feed a given calf is difficult to know. In general, recumbent calves that lack a suckle reflex may have ileus and should be treated intravenously. Calves with rapidly progressing signs of dehydration also should be treated intravenously because fluid losses may be too rapid to be replaced orally. As intestinal motility and the suckle reflex return milk is gradually introduced. Bicarbonate-alkalinizing ORT is acceptable if the calf is kept from milk and feed with large amount of glucose. Acetate-based solutions, which do not inhibit milk clotting, should be used in calves that are receiving milk. Many calves seem to tolerate 1 L of milk given two to four times a day. To some extent this pattern of feeding mimics natural suckling. Calves usually suck small amounts at frequent intervals. However, following a period of diarrhea the udder may become over distended. Traditionally this has been handled by some combination of milking out the dam and giving the calf a large feed of electrolytes immediately before re-introduction to the dam. Feeding small volumes may be particularly important with milk replacer fed calves. The majority of milk replacers do not clot in the abomasum, so large feeds will be even more likely to flood the intestines.

For the future of ORT, the addition of glutamine will be of interest. Investigations in laboratory animals have demonstrated that glutamine feeding improves growth and repair of the small intestinal mucosa and helps maintain intestinal immune function. Glutamine stimulates also sodium uptake by the mucosal cells of the small intestine. Results of one trial indicated that substituting glutamine (40 mmol/L) for glycine in ORT solutions did not cause improvement of diarrheic calves or decrease the time needed for mucosal healing. However, in another study, high-glucose (378 mmol/L) ORT solutions

containing glutamine (30 mmol/L) were more effective in correcting plasma, extracellular fluid, and blood volume in diarrheic calves than high-glucose amino acid-free solutions. Our improved understanding of intestinal physiology suggests some reasons for the failure of the first study. Feeding glutamine seems to have a general trophic effect and increase the uptake of a variety of nutrients. It may be that glutamine supplementation will only be effective as part of a more balanced diet that supplies the breadth of nutrients required for the synthesis of new intestine. In human medicine, use of cereal-based rehydration fluids causes dramatic improvements, compared with conventional glucose electrolyte oral rehydration treatment, by reducing the duration and severity of diarrhea. Therefore, additional research is needed to determine the requirements for intestinal healing in diarrheic calves.

7.2 Conclusion

The purpose of this thesis was to determine which nutrients are used by the intestine of the calf and to determine if simple supplementation with these nutrients would promote intestinal metabolism. Glutamine has a role in intestinal mucosal function and metabolism in preruminant calves. Glutamine supplementation could provide a new approach to promote intestinal healing when treating diarrheic calves. However, additional studies must be performed before there is sufficient information to properly recommend that glutamine supplementation is safe and effective in clinical treatment.

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APPENDICES

- Appendix A. Determination of calf plasma lactic acid, pyruvic acid, butyric acid and acetic acid by RP-HPLC.**
- Appendix B. Calibration of calf plasma lactic acid, pyruvic acid, butyric acid and acetic acid by RP-HPLC.**
- Appendix C. Amino acids measurements by RP-HPLC – Pico•Tag Amino Acid Analysis System.**
- Appendix D. Calibration of amino acids measurements by RP-HPLC.**
- Appendix E. Protocol for glutamine colorimetric assay.**

Appendix A. Determination of calf plasma lactic acid, pyruvic acid, butyric acid and acetic acid by RP-HPLC.

Sample preparation: Take fresh plasma 100 μ L and 100 μ L of citric acid (1mM)¹ to ultrafiltration unit, mix and centrifuge at 1500 g for 30 min. Store the filtrates at -20°C.

Chromatography:

Instrument: Waters 600 controller with Waters 486 tunable absorbance detector.

Column: Ionpak KC-811 protected by a Ionpak KC810P precolumn

Temperature: 50 °C

Gradient Table

	Time (min)	flow rate (mL/min)	% pump B 0.1% H₃PO₄²	% pump D H₂O³	curve
1		0.7	0	100	
2	1.0	0.7	0	100	6
3	20.0	0.7	30	70	6
4	25.0	0.7	0	100	6
5	39.5	0.7	0	100	6
6	50.0	0.0	0	100	6

¹ Citric acid solution 0.02 M: weigh 42.02 mg of citric acid (MW: 210.4) and dissolve it by 10 mL phosphoric acid solution (0.025 M; MW: 98.0; density: 1.7 g/mL => 1.44 mL/L H₂O). Mix 0.5 mL of citric acid solution (0.02 M) with 9.5 mL of water => CITRIC ACID SOLUTION (1mM).

² 588 L H₃PO₄ is diluted by 500 mL H₂O, filtered through 0.45 μ m filter and degassed under vacuum by sonicator.

³ DD water is filtered through 0.45 μ m filter and degassed under vacuum by sonicator.

Appendix B. Calibration of calf plasma lactic acid, pyruvic acid, butyric acid and acetic acid by RP-HPLC.

Solutions:

Pyruvic acid: 110 g = 1000 mM/L \Rightarrow 0.06 g/200 mL DDW \Rightarrow 2.5 mmol/L
 Lactic acid: 112.1 g = 1000 mM/L \Rightarrow 3.75 g/L DDW = 20 mM/L (purity 60%)
 if the density is 1.31 g/mL then 0.71 mL/250 mL \Rightarrow 20 mM/L.
 Butyric acid: 126.1 g = 1000 mM/L \Rightarrow 0.1 g/10 mL DDW \Rightarrow 80 mM/L.
 Acetic acid: 82.03 g = 1000 mM/L \Rightarrow 0.328 g/200 mL DDW \Rightarrow 20 mM/L.

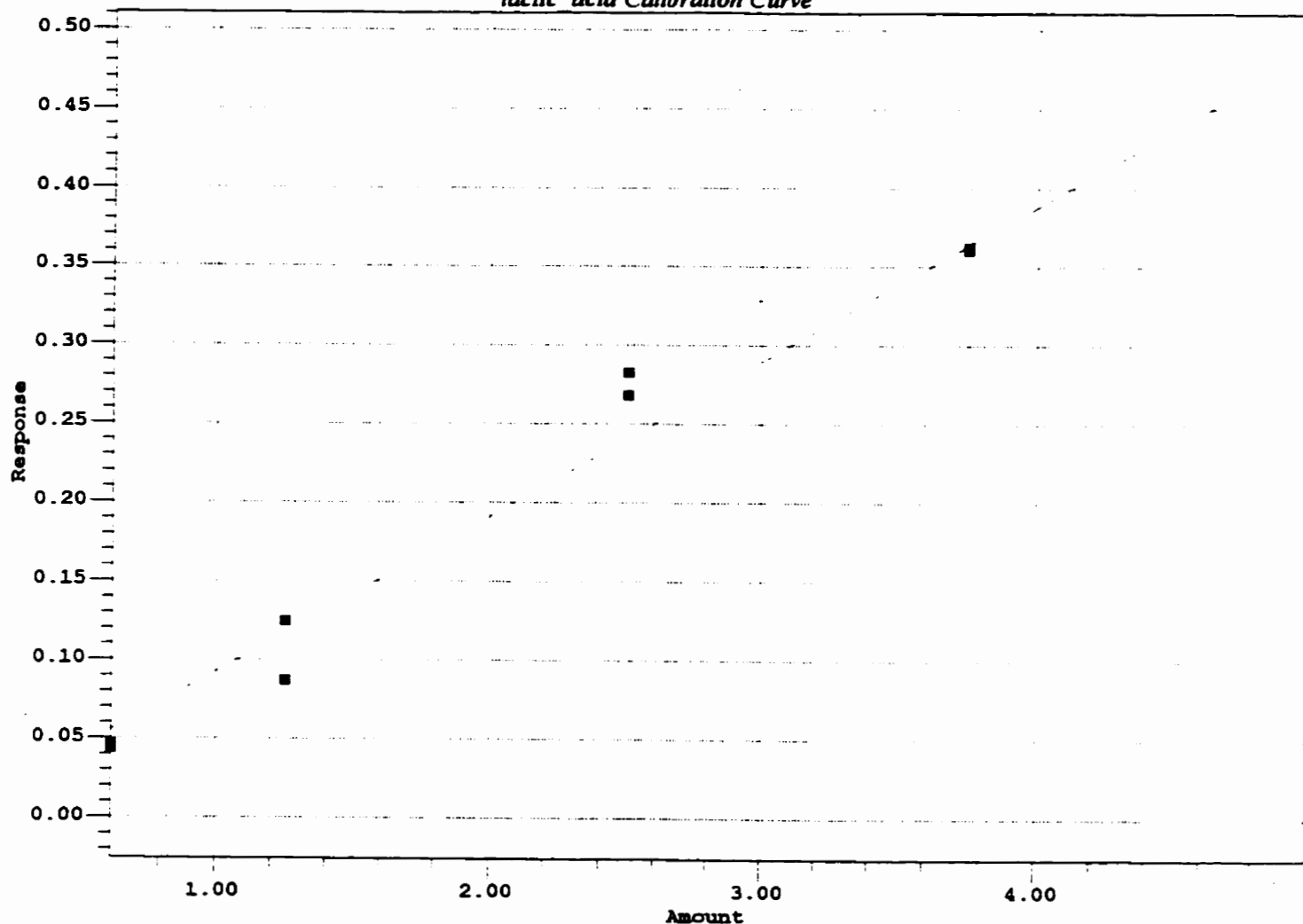
Standard solutions and dilutions (mmol/L) used for HPLC calibration.

	vial #1	vial #2	vial #3	vial #4	vial #5	retention time
Internal Standard (100 L)	1	1	1	1	1	
Dilution:	400 μ L: 0 DDW	300 μ L:100 μ L DDW	200 μ L:200 μ L DDW	100 μ L:300 μ L DDW	50 μ L:450 μ L DDW	
pyruvic acid	0.625	0.469	0.3125	0.156	0.078	10.95
lactic acid	5	3.75	2.5	1.25	0.625	13.88
butyric acid	20	15	10	5	2.5	14.67
acetic acid	5	3.75	2.5	1.25	0.625	15.91

Accuracy

Accuracy of the assay was determined by spiking a plasma sample with lactic acid. A plasma sample was analysed in triplicate for lactic acid concentration. The sample was then spiked with 20 mmol/L of lactic acid and re-analysed (n=5) for lactic acid concentration. Lactic acid recovery from plasma was 93% which meets the criteria for acceptable recovery.

lactic acid Calibration Curve



lactic acid Calibration Information

Processing Method	organic	System	Waters_HPLC
Channel	486	Date	13-FEB-97
Type	LC	Name	lactic_acid
Retention Time	13.880 min	Order	1
A	-0.006354	B	0.098572
C	0.000000	D	0.000000
E	0.000000	F	0.000000
R	0.991880	R ²	0.983825
Standard Error	0.022620		

lactic acid Point Table

#	Amount	Response	Calc. Amount	% Deviation	Manual	Ignore?
1	0.625000	0.043371	0.504456	-19.287	No	No
2	0.625000	0.046466	0.535847	-14.264	No	No
3	1.250000	0.087120	0.948276	-24.138	No	No
4	1.250000	0.124831	1.330855	6.468	No	No
5	2.500000	0.268018	2.783467	11.339	No	No
6	2.500000	0.282882	2.934256	17.370	No	No
7	3.750000	0.360389	3.720562	-0.785	No	No
8	3.750000	0.362358	3.740538	-0.252	No	No
9	5.000000	0.472659	4.859519	-2.810	No	No

lactic_acid Point Table

#	Amount	Response	Calc. Amount	% Deviation	Manual	Ignore?
10	5.000000	0.475882	4.892225	-2.156	No	No

Table 'lactic_acid Average Table' contains no data.

Appendix C. Amino acids measurements by RP-HPLC – Pico•Tag Amino Acid Analysis System.

- a. **Sample Preparation:** Mix 100 μ L of sample with 100 μ L internal standard solution (norleucine; 200 nmol/mL). Take the sample into ultrafiltration device and centrifuge at 1500 g for 30 min. Pipette 25 μ L of filtered sample into 6 x 50 mm sample tube and vacuum dry in Work Station. Add 10 μ L of drying solution (a 2:2:1 mixture of methanol:1M sodium acetate:triethylamine) to sample tube, vortex to mix, and vacuum dry at Work Station.
- b. **Derivatization:** The amino acids are derivatized with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids. Add 20 μ L of derivatization reagent (a 7:1:1:1 mixture of methanol:triethylamine:H₂O:PITC) to each dried sample. Vortex to mix. Let stand 20 minutes at room temperature, then vacuum dry in Work Station.
- c. **Analysis by Pico.Tag RP-HPLC**
 Instrument: Waters 600 Controller
 Detector: Waters 486 Tunable absorbance detector
 Column: 30 cm Pico.Tag column
 Temperature: 46°C
 Inject: 20 L

Gradient Table for Amino acids

Time	Flow	%Eluent	%H ₂ O	%CH ₃ CN	Curve
	1	94	0	6	
1	1	94	0	6	6
21	1	56	16	28	5
21.5	1	0	40	60	6
22	1.5	0	40	60	6
26	1.5	0	40	60	6
26.5	1.5	94	0	6	6
39	1.5	94	0	6	6
39.5	1	94	0	6	6

- d. **Eluent preparation:**
 Stock EDTA solution (weigh 100 mg EDTA, add 100 mL DDW, dissolve well and store in refrigerator.
 Sodium acetate trihydrate (weigh 19.0 g), add 1L water. Add 0.5 mL triethylamine under stirring, add 0.2 mL stock EDTA solution. Titrate to pH 6.4 with acetic acid. Filter the solution and degas by sonicating under vacuum.

Appendix D. Calibration of amino acids measurements by RP-HPLC.

SOLUTION I: 200 μ L Amino acid standard + 525 μ L glutamine solution (0.29 g of glutamine in 10 mL of DDW (200 mmol/L); dilution 1:10) + 525 μ L DDW.

SOLUTION II:

Take 1000 μ L of solution I + 680 μ L of DDW:

Name	Solution I (μmol/L)	Solution II (μmol/L)	Name	Solution I (μmol/L)	Solution II (μmol/L)
Ammonia	2500	1488	L-Tyrosine	2500	1488
L-Aspartic acid	2500	1488	L-Valine	2500	1488
L-Glutamic acid	2500	1488	L-Methionine	2500	1488
L-Serine	2500	1488	L-Cystine	1250	744
Glutamine	8400	5000	L-Cysteine	8400	5000
Glycine	2500	1488	L-Isoleucine	2500	1488
L-Histidine	2500	1488	L-Leucine	2500	1488
L-Arginine	2500	1488	L-Phenylalanine	2500	1488
L-Threonine	2500	1488	L-Lysine	2500	1488
L-Alanine	2500	1488			
L-Proline	2500	1488			

Solution III: Standard amino acid solutions and dilutions ($\mu\text{mol/L}$) for HPLC calibration.

Solution II (μL)	500	450	400	350	300	250	200	150
DD Water (μL)	0	50	100	150	200	250	300	350
vial #:	1	2	3	4	5	6	7	8
Glutamine	5000	4500	4000	3500	3000	2500	2000	1500
Solution II (μL)	100	80	75	60	50	40	20	15
DD Water (μL)	400	420	425	440	450	460	480	485
vial #:	9	10	11	12	13	14	15	16
Glutamine	1000	800	750	600	500	400	200	150

SOLUTION IV: Mix 100 μL of sample with 100 μL of internal standard "Norleucine". Pipette 25 μL of filtered sample into 6*50 mm sample tube and vacuum dry in Work Station.

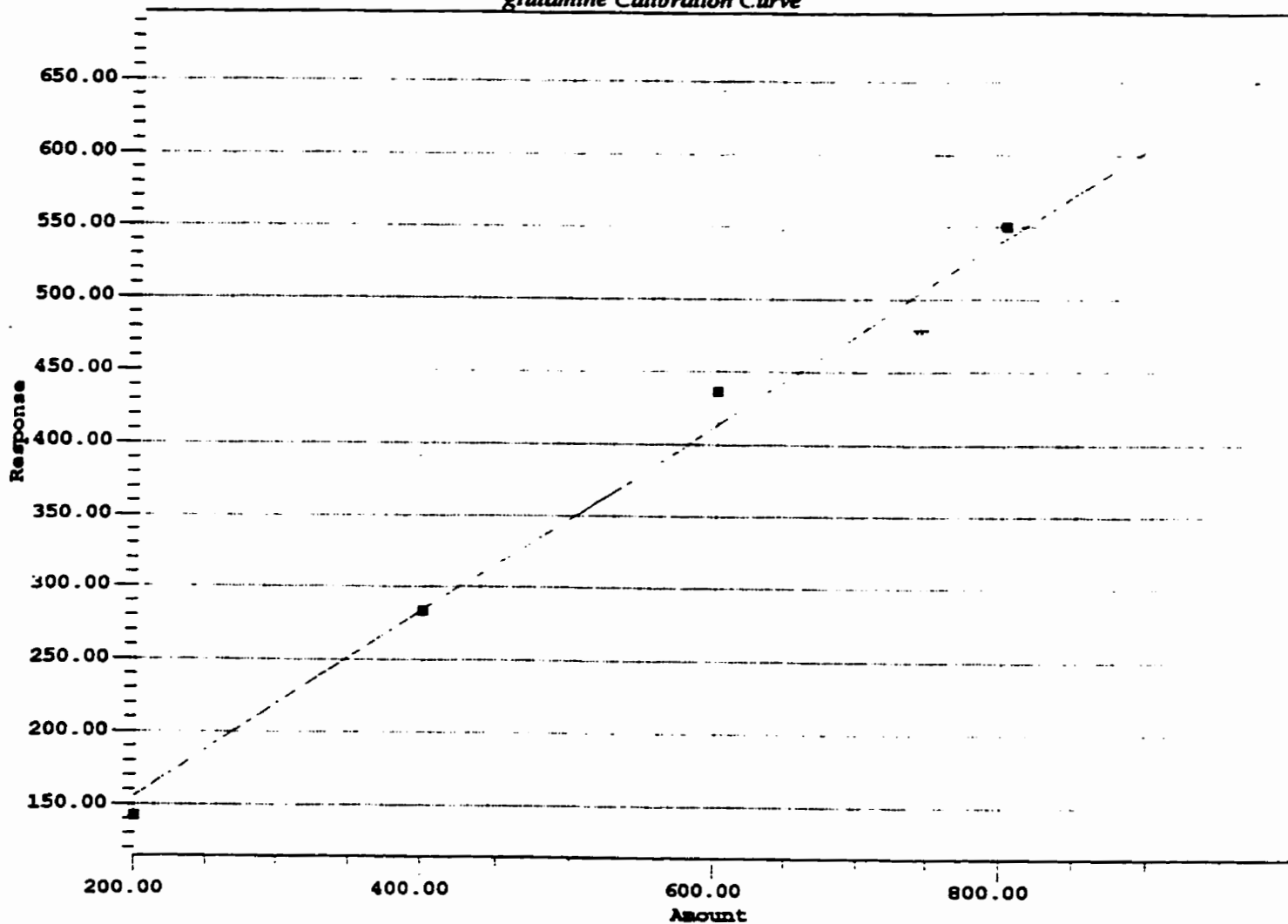
Method precision:

The sensitivity and the accuracy of our results improved dramatically with RP-HPLC analysis in comparison with the spectrophotometric method (Appendix E). When samples are analysed in triplicate the same day, there is usually no major variation in the glutamine concentration.

Injection Number	glutamine concentration $\mu\text{mol/L}$
1	286.42
2	274.45
3	276.08
Average	278.98
Standard deviation	6.49

We kept also few plasma samples for 6 months at $-70\text{ }^{\circ}\text{C}$, and the glutamine concentration did not vary significantly during this period of time.

glutamine Calibration Curve



glutamine Calibration Information

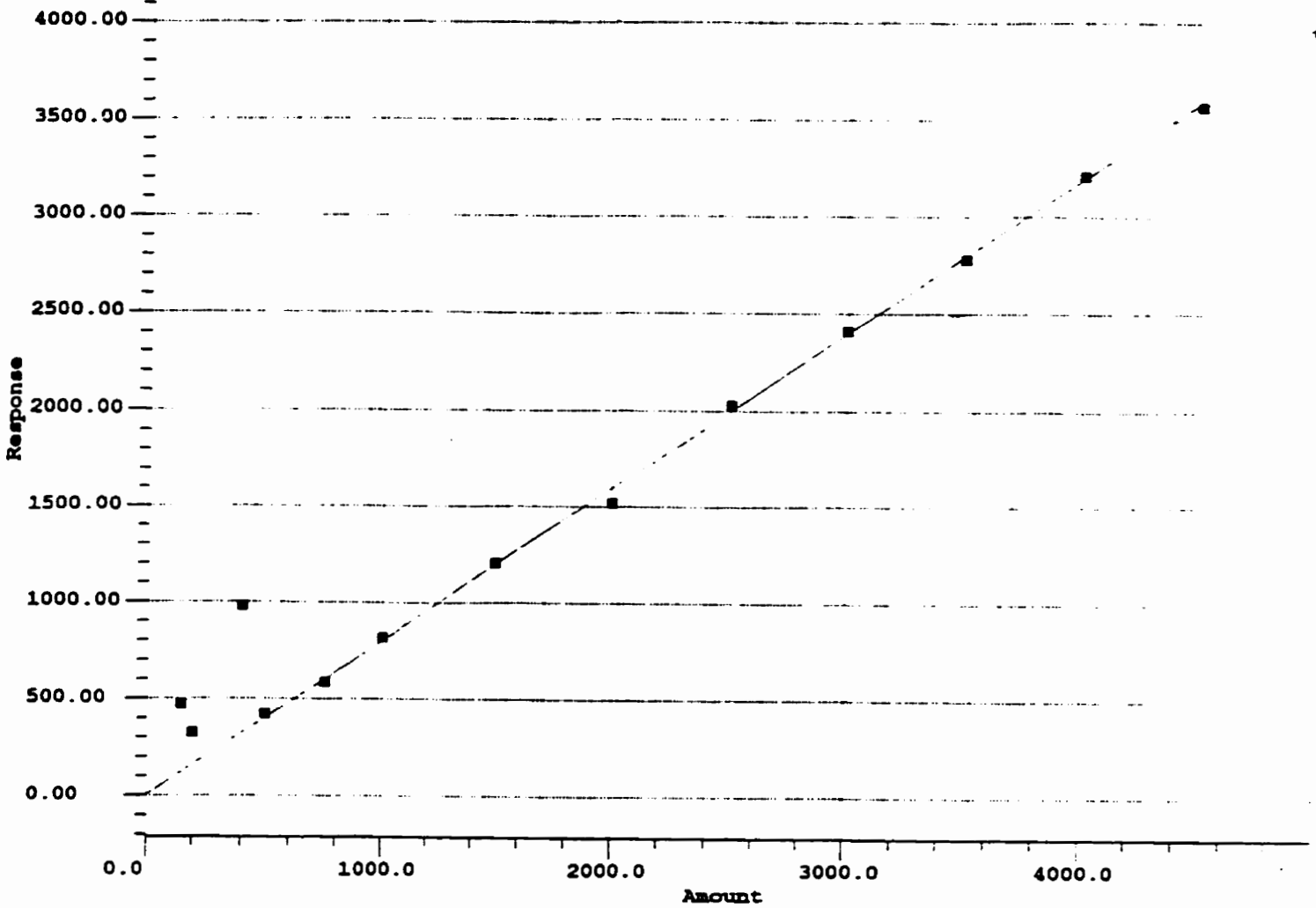
Processing Method	DTD88122_P27_5r_High-Guard_gln	System	Waters_HPLC
Innuel	486	Date	14-MAR-95
ie	LC	Name	glutamine
ention Time	6.260 min	Order	1
	26.900328	B	0.643630
	0.000000	D	0.000000
	0.000000	F	0.000000
	0.996701	R^2	0.993412
andard Error	19.138618		

glutamine Point Table

#	Amount	Response	Manual	Ignore?
1	200.000000	142.486664	No	No
2	400.000000	283.559631	No	No
3	600.000000	436.388458	No	No
4	800.000000	550.119568	No	No
5	1000.000000	652.836365	No	No

Table 'glutamine Average Table' contains no data.

glutamine Calibration Curve



glutamine Calibration Information

Processing Method	calamin5	System	Waters_HPLC
Channel	486	Date	30-DEC-97
Type	LC	Name	glutamine
Retention Time	6.504 min	Order	1
A	0.000000	B	0.799109
C	0.000000	D	0.000000
E	0.000000	F	0.000000
R	0.985389	R ²	0.970992
Standard Error	213.758351		

glutamine Point Table

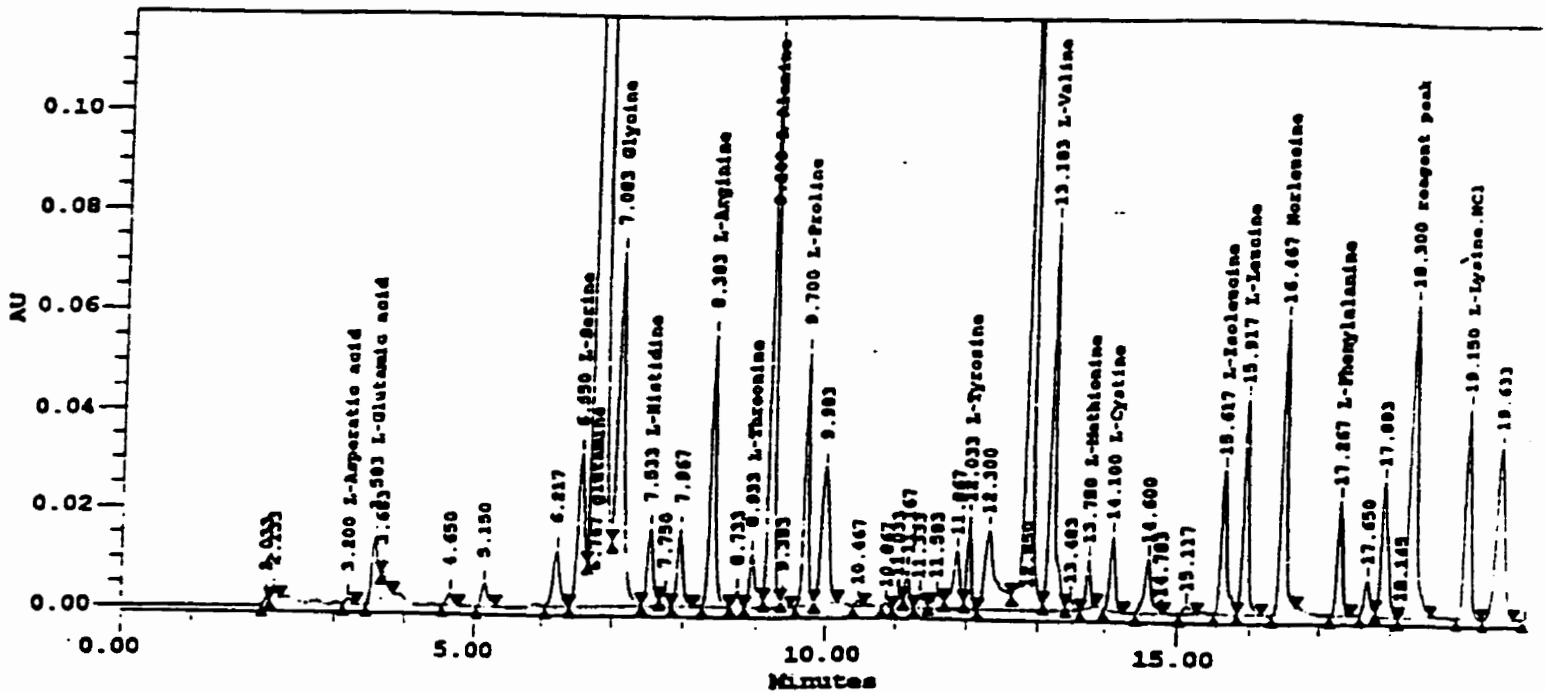
#	Amount	Response	Calc. Amount	% Deviation	Manual	Ignore?
1	150.000000	469.808235	587.915329	291.944	Yes	No
2	200.000000	324.668224	406.287953	103.144	Yes	No
3	400.000000	977.540285	1223.288305	205.822	No	No
4	500.000000	421.854489	527.906287	5.581	Yes	No
5	750.000000	584.660782	731.641148	-2.448	Yes	No
6	1000.000000	818.948167	1024.827038	2.483	Yes	No
7	1500.000000	1204.141489	1506.855750	0.457	Yes	No
8	2000.000000	1518.588650	1900.353124	-4.982	Yes	No
9	2500.000000	2028.592563	2538.569095	1.543	Yes	No

glutamine Point Table

#	Amount	Response	Calc. Amount	% Deviation	Manual	Ignore?
10	3000.000000	2411.556301	3017.807719	0.594	Yes	No
11	3500.000000	2777.576354	3475.843113	-0.690	Yes	No
12	4000.000000	3209.372962	4016.190911	0.405	Yes	No
13	4500.000000	3571.017266	4468.750518	-0.694	Yes	No
14	5000.000000	3953.718160	4947.660221	-1.047	Yes	No

Table 'glutamine Average Table' contains no data.

**Chromatogram from the portal vein sample of a calf
after an intravenous infusion of 200 mmol of glutamine over an hour.**



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount
1	L-Asperatic acid	3.200	7206	1173	6.717 μmol/L
2	L-Glutamic acid	3.583	83726	12961	49.635 μmol/L
3	L-Serine	6.550	196861	31048	152.807 μmol/L
4	glutamine	6.767	3800870	551328	2852.636 μmol/L
5	Glycine	7.083	523128	71836	299.110 μmol/L
6	L-Histidine	7.533	95143	15828	100.254 μmol/L
7	L-Arginine	8.383	290306	55059	199.365 μmol/L
8	L-Threonine	8.933	68884	10969	69.979 μmol/L
9	L-Alanine	9.200	553865	116285	395.683 μmol/L
10	L-Proline	9.700	223105	51280	213.242 μmol/L
11	L-Tyrosine	12.033	78388	18612	47.938 μmol/L
12	L-Valine	13.183	400359	78448	266.323 μmol/L
13	L-Methionine	13.750	44148	9963	33.489 μmol/L
14	L-Cystine	14.100	82887	15361	67.233 μmol/L
15	L-Isoleucine	15.617	162005	28968	93.980 μmol/L
16	L-Leucine	15.917	222668	43803	152.408 μmol/L
17	Norleucine	16.467	298114	59189	200.000 μmol/L
18	L-Phenylalanine	17.267	118367	23376	83.961 μmol/L
19	reagent peak	18.300	462315	63279	
20	L-Lysine.HCl	19.150	248031	42633	97.663 μmol/L

NOTE TO USERS

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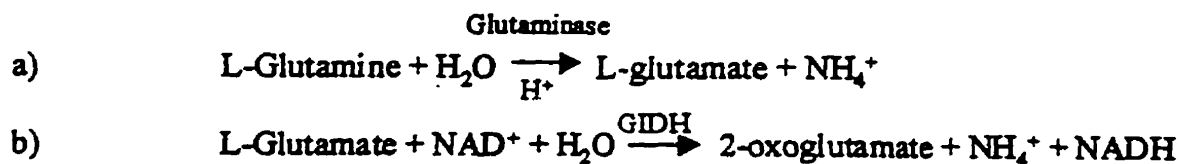
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Appendix E. Protocol for Glutamine Colorimetric Assay

Most of the colorimetric methods used for the determination of glutamine are based on the estimation of glutamate or NH_3 after preliminary hydrolysis by boiling with acid or treatment with glutaminase (Lund 1985; Mecke 1985). Acid hydrolysis has been found not specific, because many tissues yield ammonia on acid hydrolysis. Therefore, values which are too high are obtained regardless of whether glutamine or ammonia is determined. However, enzymatic hydrolysis with glutaminase from *E. coli* followed by spectrophotometric determination of glutamate using glutamate dehydrogenase, has the advantage of simplicity and specificity (Lund 1985). Glutamine can be determined by chromatographic analysis. This method requires expensive equipment. They are recommended only if a large number of analysis are carried out routinely. At first, we decided to measure L-glutamine with an UV-method with glutaminase and glutamate dehydrogenase.

Principle:



Solutions:

Tris Buffer 2: Mix 12.11 g Trizma base in 800 mL water. Adjust pH to 7.7 using concentrated HCl. Dilute to 1000 mL.

Perchloric Acid: Dilute 43.5 mL HClO₄ to 100 mL with water.

Assay Mixture 8: For every 10 samples mix: 0.139 g hydroxylamine hydrochloride, 0.103 g ADP-NA₃, 5 mL Tris buffer 1 (0.8 M; pH 7.7 – as above), 1 mL disodium hydrogen arsenate (0.2 M), 0.05 mL manganese sulfate (0.2 M), 1 mL NaOH (2M).

Preparation of enzyme:

Reconstitute the 5 mg vial of L-glutamine synthetase, lyophilized, from Sigma, in 12 mL of Tris buffer 2.

Treatment of samples:

1. Blood is collected in 7.5 mL heparinized venoject tubes. Mixed and put on ice.
2. Blood is then spun down for fifteen minutes at 2500 rpm. The refrigerated centrifuge temperature is set for between 2 and 4 °C.
3. 1.6 mL of serum is extracted and 0.2 mL of perchloric acid solution is added. Mix well and centrifuge for 10 minutes at 3000 rpm, leaving the centrifuge with the same temperature settings as above.
4. Carefully bypassing the lipid bilayer with the Eppendorf pipetor, extract 0.8 mL supernatant, leaving behind the white precipitate that has formed. Add 0.1 mL potassium hydroxide solution (10 mmol). Mix well and allow to sit in an ice bath for 15 minutes. Centrifuge at 3000 rpm for 10 minutes with the same temperature settings as before.
5. Extract 0.5 mL of supernatant. To this tube, add 0.7 mL of assay mixture 8 and 0.1 mL of enzyme. Mix. The blank is made here by using 0.5 mL double distilled water. Add the assay mixture and enzyme and proceed to incubation.

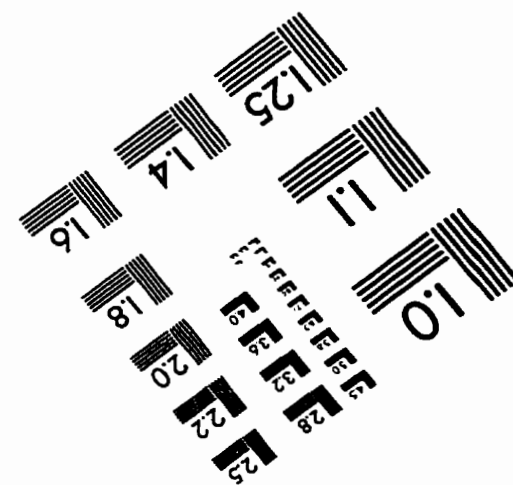
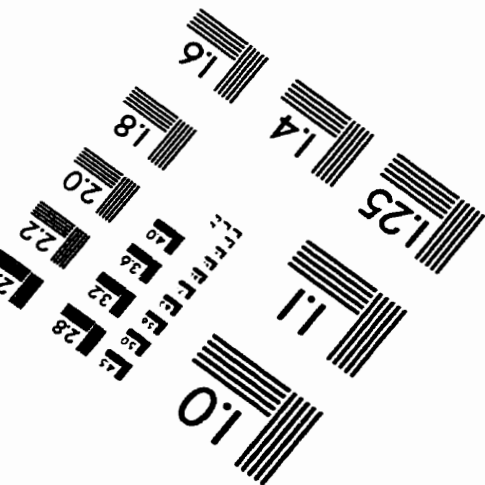
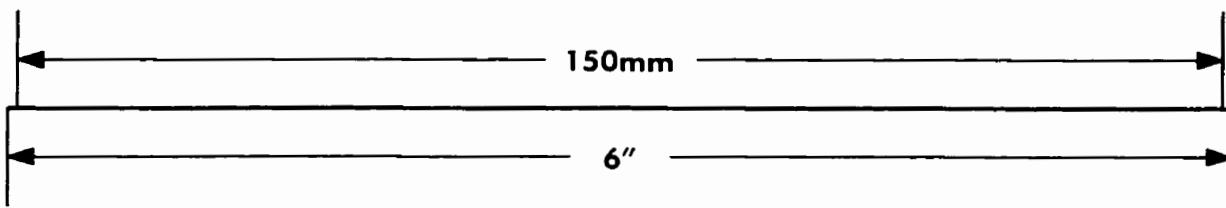
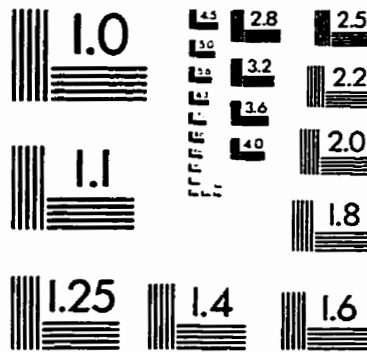
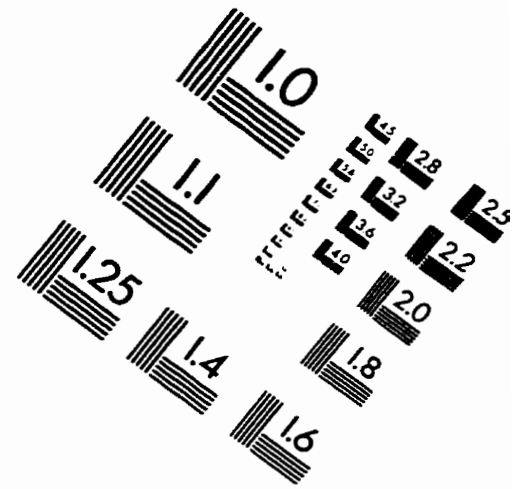
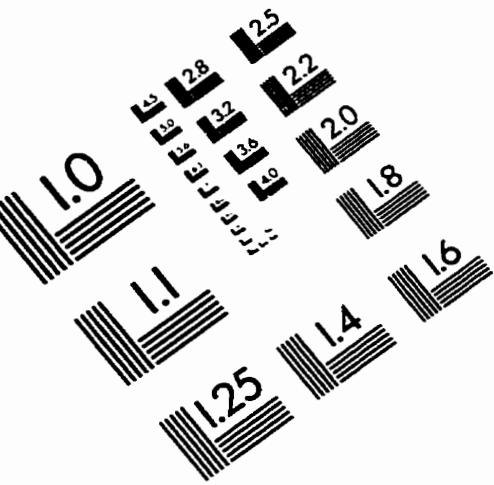
6. Incubate in a water bath at 37 °C for 30 minutes.
7. Add 1 mL colour reagent, mix and read in a spectrophotometer set at 500 nm, within 30 minutes of addition of the colour reagent.

Treatment of Standards:

1. Optimally, the best results come from a fresh set of standards. The standards remain reasonably linear from 0-8 mmol/L.
2. Use 0.5 mL standard. To it add 0.7 mL assay mixture 8 and 0.1 mL enzyme. Mix well.
3. Incubate in a water bath at 37 °C for 30 minutes.
4. Add 1 mL colour reagent and mix gently. Read in a spectrophotometer set at 500 nm no later than 30 minutes after addition of the colour reagent.

Sources of error: In several calves, the amount of glutamine measured in the portal vein was sometimes higher than in the femoral artery during glutamine infusions. We found this assay was disturbed by air bubbles which form in the cuvettes after addition of 0.7 mL of assay mixture 8. After several modifications of this assay, it was impossible to get similar results in triplicates. Therefore, we decided to quantitatively determine plasma glutamine and amino acid concentrations by reverse-phase high performance liquid chromatography using the Waters Pico-Tag Amino Acid Analysis System and the phenylisothiocyanate (PITC) derivative.

IMAGE EVALUATION TEST TARGET (QA-3)



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