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An Assessment of Monensin's Effect Upon the Ruminant Small Intestine: Na^+/K^+-ATPase Activity in Mucosal Biopsies and Net Nutrient Absorption

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An Assessment of Monensin's Effect Upon the Ruminant Small Intestine: Na^+/K^+-ATPase Activity in Mucosal Biopsies and Net Nutrient Absorption

By

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ABSTRACT

An Assessment of Monensin's Effect Upon the Ruminant Small Intestine: Na^+/K^+-ATPase Activity in Mucosal Biopsies and Net Nutrient Absorption

By

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Studies were conducted to 1) examine the effect of monensin upon the Na^+/K^+-ATPase activity in bovine duodenal mucosal biopsies and 2) to examine the effect of monensin supplementation upon net nutrient absorption. Two Angus heifers (233 kg) and two Simmental-crossbred steers (309 kg) were fitted with duodenal cannulas approximately 10 cm from the pylorus. Eighteen biopsies were removed per animal and were examined for Na^+/K^+-ATPase activity, as measured by ouabian-inhibition of O_2 consumption in monensin concentrations of 0, 1, 5 and 10 ppm. Total O_2 consumption of the biopsies increased ($P<.01$) from 5.59 nmol O_2/mg DM/min at 0 ppm to 8.18 nmol O_2/mg DM/min at 10 ppm. Ouabain-sensitive respiration increased ($P<.01$) with increasing monensin concentrations from 2.76 nmol O_2/mg DM/min to 4.9 nmol O_2/mg DM/min at 10 ppm. Monensin addition to the diet of these same animals (300 mg/hd/d) increased ($P<.01$) total O_2 consumption (20%) and ouabian-sensitive respiration (32%). There was no effect ($P>.10$) upon Na^+/K^+-ATPase independent resipiration. In the second study, four crossbred steers (363 kg) were catherized in the portal

potassium were altered by monensin supplementation. Magnesium absorption weas increased ($P<.02$) with monensin supplementation (-.392 g/hr to .243 g/hr). These data indicate that monensin is having an effect upon the small intestine, stimulating the Na^+/K^+-ATPase pump, but that stimulation is not apparent in net nutrient transport studies. It may be that the influx of sodium into the enterocyte is stimulating the pump but not affecting other sodium dependent processes. Altered magnesium absorption is best explained as a ruminal effect with a stimulation of ruminal Na^+/K^+-ATPase.

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Introduction

A growing animal has two types of energy needs, "maintenance" and "gain". Absorbed nutrients are first partitioned to maintenance functions and then to gain. Estimates of the feed energy used by a feedlot steer or heifer for maintenance have ranged from 40 to 63% (Van Es, 1972; Johnson, 1984). This wide fluctuation in maintenance requirements is a serious concern to an industry whose goals include maximum production (e.g., gain) with minimal input (e.g., feed costs). Our goals as animal scientists are: 1) to understand maintenance functions and requirements; and 2) to manipulate and minimize maintenance costs, thereby increasing the feed energy available for gain.

One method of increasing animal efficiency is ionophore supplementation of feedlot rations. Rumensin^{TM}, monensin sodium, is the ionophore used most commonly. Goodrich et al. (1984) examined and summarized data on 16,000 head of cattle and found that monensin supplementation decreased intake 6.4%, increased rate of gain 1.6% and decreased feed/100 kg of gain 7.5%. There was no effect on carcass composition.

The mode of action of these compounds has received considerable attention. In a review article, Schelling (1984) identified seven proposed modes of action for monensin. These modes of action included: 1) modified acid [propionate] production; 2) modified feed intake; 3) change in gas production; 4) modified digestibilities; 5) changes in protein utilization; 6) modified rumen fill and rate of passage; and 7)

other ruminal modes of action. Modifications in these parameters are insufficient to account for the magnitude of the feed efficiency response seen with monensin supplementation (Bergen and Bates, 1984). This suggests that ionophores have effects beyond those observed in the rumen.

Data reported by Davison (1984) indicate that ionophores pass through the rumen unchanged. This might expose the epithelial cells of the intestine to concentrations sufficient to alter their physiological state. Ionophores function by facilitating ion transport (Pressman, 1976). Monensin and lasalocid (another widely used ionophore in beef cattle diets) have affinities for sodium and potassium ions, respectively. This has led to speculation that these ionophores might disrupt the normal sodium and potassium gradients seen across the mucosal cell (Bergen and Bates, 1984).

The balance of sodium and potassium ion concentrations across the intestinal cell is extremely important in nutrient transport processes. Many of the amino acids and all of the glucose absorbed from the intestinal lumen are transported by sodium dependent processes (Munck, 1981). A concentration gradient of high luminal sodium (140 mEq) and low mucosal sodium (14mEq) is maintained by the Na/K^+-ATPase pump. This pump exchanges three sodium ions for two potassium ions, with the hydrolysis of one ATP. Glucose and some amino acids flow with the sodium ions across the mucosa. An ionophore within the epithelial cell membrane would facilitate sodium influx, and might stimulate amino acid or glucose absorption and increasing the Na^+/K^+-ATPase pump activity. Alteration of potassium flux across the cell membrane would affect the mucosal cell and thereby alter nonsodium-linked amino acid transport (Bergen and Bates, 1984).

The effects of ionophores on the intestinal mucosa have not been investigated. Since the ruminal effects are not sufficient to account for the increased efficiency seen with ionophore addition to ruminant diets and since ionophores have been shown to affect glucose transport across other types of cells (Austic and Smith, 1980, Nordenberg et al., 1984) the following studies were conducted to assess the possible alteration of nutrient absorption and gastrointestinal maintenance costs with ionophore addition.

Review of the Literature

Maintenance

An animal's maintenance requirement is defined as the amount of daily dietary intake which will result in neither gain nor loss of body energy (Johnson, 1984). Maintenance costs may be broken down into three areas: environmental factors; animal to animal variations and physiological or compositional changes. Environmental factors include the climate or season. A decline in temperature below the thermoneutral zone may increase maintenance requirements 40-50% (Johnson, 1984). Other environmental factors include: level of alimentation, diet and activity. These may alter maintenance costs 10-30% (Johnson, 1984). Animal to animal variation, including differences in genetics, sex, sweat glands and hair color, may alter the amount of energy partitioned to maintenance by 10 to 30% (Johnson, 1984). Physiological or compositional changes such as lactation, age, vital organ mass and muscle mass may, under different circumstances, contribute a 5 to 15% change in maintenance costs. The greatest changes occur when alterations in protein turnover or ion pumping take place. These physiological factors may alter maintenance costs 20-30%.

Milligan (1971) has suggested that ion pumping contributes substantially to maintenance costs. The maintenance of sodium and potassium gradients across the cell plasma membrane by Na^+/K^+-ATPase contributes 30-40% of the total energy expenditure of the brain, liver and skeletal muscle (Ismail-Beigi and Edelman, 1971). Other work done in Milligan's group (Gregg and Milligan, 1980a,b; Gregg

and Milligan, 1982a,b; McBride and Milligan, 1984; McBride and Milligan, 1985) confirms this large contribution of Na^+/K^+-ATPase. Muscle biopsies from calves and lambs of different ages show that young animals have a much greater total oxygen uptake indicating a greater Na^+/K^+-ATPase activity and greater energy costs. Similar results have been shown in hepatocytes (McBride and Milligan, 1985). Data in muscle, liver and duodenal biopsies indicate that lactation also increases the aerobic energy consumed and energy requirements of the tissue involved (Milligan and McBride, 1985).

Cold exposure (Gregg and Milligan, 1982a), level of energy intake (McBride and Milligan, 1985), and age of the animal alter energy costs. This effect of physiological state of the animal alters maintenance costs (Milligan and McBride, 1985).

Na^+/K^+-ATPase

Enzyme Characteristics

Experiments with various tissues, frog skin, nerves, muscle, red blood cells and enterocytes, have shown that sodium is transported from the cytosol to interstitial fluid against an electrochemical gradient (Skou, 1965). This active transport of sodium was shown to be ATP-dependent (Hoffman, 1960), dependent on extracellular potassium concentrations (Taylor, 1962), inhibited by cardiac glycosides (Skou, 1965) and showed a coupling between sodium efflux and potassium influx (Taylor, 1962). These and other sets of data lead to the proposal of a transport system for sodium that fulfilled the following criteria (Skou, 1965):

1) located in the cell membrane
2) have greater affinity for Na^+ than K^+ inside the cell membrane
3) have greater affinity for K^+ than Na^+ outside the cell membrane
4) be able to catalyze the hydrolysis of ATP and convert the energy to a transport process
5) be able to hydrolyze ATP at a rate dependent on internal Na^+ concentration and external K^+ concentration
6) be found in all cells which have active Na^+/K^+ linked active transport
7) have a close correlation between the effect of cardiac glycosides and cation transport

A Na^+/K^+ATP-hydrolyzing enzyme system was identified and characterized by Skou (1957, 1960, 1961, 1963, 1964) in nerves, brain, and kidneys. This enzyme requires ATP, Na^+, K^+ and Mg^+. Further work identified a similar enzyme system in intestine (Taylor, 1962), red blood cells, liver, muscle and many other tissues. The differences in the enzyme ATPases isolated from the various tissues include differential sensitivity to cardiac glycoside concentrations, different activities at different pH's and differential sensitivity to Mg^+ concentrations (Skou, 1965).

The stoichiometry of the Na^+-K^+ exchange was determined to be 2-3 Na^+ ions for 2 K^+ ions (Sen and Post, 1961). In several studies with phospholipase A and C, additives of detergents (DOC and lauryl sulfate) and additions of fatty acids (oleic and stearic acids)

it became obvious that the enzyme system required a stable, organized lipoprotein structure (Glynn, 1962; Skou, 1962).

The enzyme has also been shown to be inhibited by high concentrations of oligomycin (Van Groningen and Slater, 1962; Jobsis and Vreman, 1963), sulfhydryl inhibitors (Skou, 1963) and cardiac glycosides (Skou, 1963). It is unaffected by 2,4-dinitrophenol, aldosterone and insulin (Bonting et al., 1961). More recent evidence indicates that insulin stimulates the Na^+/K^+-ATPase in skeletal muscle (Moore, 1983).

The enzyme is composed of two noncovalently bound subunits (Harris and Stahl, 1984). These are an α-subunit that contains the catalytic site and a β-subunit. Ouabain acts to modify the α-subunit's conformation (Anner, 1985) and thereby inhibits enzyme function. The proposed mechanism of enzyme action of the ATPase involves protonation of an NH_2 group on the α-subunit (Jorgensen et al., 1982). At this time the enzyme is in the E_2 form which has a high affinity for K^+ and a low affinity for Na^+ and ATP. Deprotonation or the E_1 form has a high affinity for Na^+ and ATP but low affinity for K^+. This protonation-deprotonation reaction may cause a change in tertiary or quarternary structure causing a "flip-flop" of the α-subunit from a hydrophilic to hydrophobic environment. The suggested function of the β-subunit is to orient the α-subunit (Skou, 1982).

Na^+/K^+-ATPase Activity

In the intestine Na^+/K^+-ATPase has been identified in the basolateral membrane of the intestinal cell (Fugita et al., 1971; Parkinson et al., 1972). It has also been shown to be associated with

intestinal electrolyte transport, glucose transport in rat ileum, and maintenance of an electrochemical gradient across the enterocyte (Charney et al., 1975).

Data presented by Quigley and Gotterer (1968) indicate that the Na^+/K^+-ATPase in rat intestinal mucosa is associated with, but not stimulated by amino acid addition. Increased sodium and potassium concentrations did stimulate ATPase activity 8 fold over control concentrations. More recent data also indicate that increased intracellular sodium concentrations stimulate the activity of the Na^+/K^+-ATPase (Marunaka, 1986). Other stimulation of the pump was seen by Charney et al. (1975) when adrenal steroids, methylprednisolone acetate and deoxycorticosterone acetate injections were given to rats. Jejunal, ileal and colon segments were examined for ATPase activity. Their data indicate that where gluco and mineralocorticoid treatments increased water and electrolyte transport, the Na^+/K^+-ATPase also increased in activity. This is unlikely to be a direct effect of adrenal steroids on the pump but may be secondary to an enhancement of sodium absorption. Silva et al. (1975) examined the effect of aldosterone and adrenalectomy upon the colon mucosa. Adrenalectomy reduced Na^+/K^+-ATPase activity 50%, and replacement of aldosterone increased activity although not quite to control levels.

Another finding of this group (Silva et al., 1975) was a progressive decrease in Na^+/K^+-ATPase activity from the duodenum to the colon. In rats, duodenal Na^+/K^+-ATPase activity was 14.69 μmol Pi/mg protein/hr, jejunal 11.35 μmol Pi/mg protein/hr, ileal 7.72 μmol Pi/mg/protein/hr and colon 4.48 μmol Pi/mg protein/hr (Pi=inorganic

phosphate). This decreasing gradient of activity is in agreement with data of Charney et al. (1974) and later data of Murray and Wild (1980).

Fasting has been shown to decrease Na^+/K^+-ATPase activity in rat jejunum (Murray and Wild, 1979) and sheep duodenum (McBride, 1984). This would seem to indicate that the enzyme may be inducible. Data presented by Schiffl and Loeschke (1977) indicate that adaptation responses in the cecal mucosa to diet differences, are due to an induction of more ATPase molecules per unit of basolateral cell membrane.

In summary, the Na^+/K^+-ATPase couples the flow of cations against their electrochemical gradients $[Na^+]$ and the flow of cation along their gradients $[K^+]$ to ATP hydrolysis, maintaining critical sodium and potassium balances in the cell. The enzyme is located on the basolateral membrane of the intestinal cell and has been shown to react to changes in diet, diet composition and hormone concentrations (Skou, 1982).

Na^+/K^+ Activity in Ruminant Duodenal Biopsies

The available data in ruminants on duodenal mucosal Na^+/K^+-ATPase include two studies done by McBride (1984). Total oxygen consumption, ouabain-sensitive and ouabain-insensitive respiration rates were measured for the duodenal mucosa of nonlactating and lactating cows (McBride and Milligan, 1984). In this study 55% of the total mucosal respiration of cows at peak lactation was attributed to Na^+/K^+-ATPase, ouabain-sensitive respiration. In mid-lactation and during the dry period the portion of oxygen consumption inhibited by ouabain (Na^+/K^+-ATPase) declined to 34-35%. Total oxygen

consumption rates ranged from 6.49 µl O_2/mg DM/hr (pregnant, nonlactating) to 8.20 µl O_2/mg DM/hr at 22-25 weeks into lactation. The ouabain-sensitive portion of this oxygen consumption ranged from 2.45 µl O_2/mg DM/hr (pregnant, nonlactating) to 3.99 µl O_2/mg DM/hr at 5 weeks of lactation. These data indicate that Na^+/K^+-ATPase accounted for a major portion of duodenal maintenance costs, and that these costs change with different physiological states.

In another study McBride (1984) examined the effect of digestible energy intake (starvation, low and high energy) on the Na^+/K^+-ATPase in sheep mucosal biopsies. In this study there was no significant difference in total mucosal oxygen consumption but ouabain-sensitive respiration was different ($P<.01$). During starvation biopsies consumed 1.48 nmol O_2/mg DM/min. At low energy intake 2.69 nmol O_2/mg DM/min were consumed and at high energy intakes 3.69 nmol O_2/mg DM/min were consumed. These results indicate that the Na^+/K^+-ATPase activity contributes significantly to the magnitude of energy consumption by the mucosa and that this portion of energy is variable with level of digestible energy intake.

Mineral Absorption

The major factors that influence mineral availability in the digestive tract of ruminants are dry matter content, pH and location in the tract (Cragle, 1973). The diet influences all of these factors. The primary effect of pH is on mineral solubility. A decrease in pH will allow a specific mineral to become more ionized allowing for absorption across membranes (Georgievskii, 1981). This is particularly true of calcium and magnesium.

Calcium

There is some disagreement as to where in the ruminant intestinal tract calcium is absorbed. Chandler and Cragle (1962) described net absorption of calcium from the abomasum. Other workers (Phillipson and Storry, 1965; Liebholz, 1974) have also demonstrated calcium absorption from the intestine. Absorption estimates vary from 10-50% depending on the age of the animal being investigated and the diet fed (Leibholz, 1974; Georgievskii, 1981). Calcium absorption may be inhibited by excess phosphates and magnesium ions and stimulated by vitamin D (Georgievskii, 1981).

The mechanism of calcium absorption by the small intestine has been actively investigated. There are four proposed mechanisms of intestinal calcium absorption (Wasserman, 1981). In one model the calcium diffuses across the brush border and down a gradient as a free ion (Rasmussen et al., 1979). It then complexes with binding proteins (such as mitochondrial binding proteins) maintaining low intracellular ion concentrations. Calcium extrusion is via an energy demanding process across the basolateral membrane. The pumps on the basolateral membrane are considered to be one of two types. A calcium stimulated ATPase has been found on the brush border and basolateral membrane (Melancon and DeLuca, 1970; Ghijsen et al., 1982). A second possible mechanism is a calcium-sodium exchange mechanism or a sodium-calcium-dependent ATPase found on the basolateral membrane (Birge et al., 1972, 1974; Martin and DeLuca, 1969).

The third proposed mechanism involves endocytosis. Luminal calcium is incorporated into membrane vesicles at the brush border membrane (Wasserman, 1981) and moves into the cell cytosol where it

associates with lysosomes. Secondary lysosomes are formed, where calcium binding would occur, and eventually calcium is released by exocytosis. This mechanism involves mitochondrial accumulation of calcium from the cytosol with subsequent transport to the basolateral membrane (Wehringer et al., 1978).

Finally, a paracellular pathway has been proposed. This pathway allows for calcium transport in either direction and is thought to be the primary mechanism of calcium secretion into the intestine. The flux of calcium would depend on the direction of solvent flow and the electrochemical potential gradient (Wasserman, 1981).

1α,25-dihydroxycholecalciferol (Vitamin D) may be a factor in each of these proposed mechanisms. Calcium-binding protein, thought to be induced by vitamin D, is likely to be extremely important in absorption of calcium from the intestine but may not be the only mechanism. Vitamin D has also been shown to increase the activity of calcium-ATPase (Melancon and DeLuca, 1970; Ghijsen and Van Os, 1982) and to modify the hydrocarbon side chains of the phospholipids present in the brush border membrane (Goodman et al., 1972; Max et al., 1978). Both of these actions would increase calcium absorption from the intestine and would fit with one or more of the proposed models.

The absorbed calcium moves to the liver through the portal vein where it forms new compounds and is released into the circulation (Georgievskii, 1981). Feed and endogenous calcium is eliminated primarily through the gastrointestinal tract, although negligible amounts of calcium are excreted in the urine (Georgievskii, 1981). Normal plasma calcium values for cattle range from 6.4 to 10.9 mg/dl (Georgievskii, 1981).

The critical balance of calcium absorption and excretion is mediated through parathyroid hormone. A decrease in plasma calcium level stimulates an increased parathyroid hormone release which stimulates increased 1α,25 $(OH)_2D_3$ production in the kidney. 1α,25 $(OH)_2D_3$ acts on the gut to produce calcium binding protein and increases intestinal absorption (DeLuca, 1979).

Phosphorus

Phosphorus absorption in ruminants is thought to occur in the small intestine (Bruce et al., 1966; Pfeffer et al., 1970; Grace et al., 1974; Ben-Ghedalia et al, 1975; Kay and Pfeffer, 1970), primarily the duodenum. There is recent evidence of some ruminal absorption of phosphorus through a Na^+/K^+-ATPase mechanism (Breves et al., 1986) but this is not thought to be as significant as intestinal absorption.

Phosphate, in the rat, is absorbed by active transport (Harrison and Harrison, 1961), is stimulated by vitamin D and is dependent upon sodium. The proposed model indicates uphill transport of phosphate across the brush border energized by the downhill transport of sodium. A common membrane component present in the brush border for phosphate and sodium transport has been proposed (Wasserman, 1981). Phosphate then diffuses through the cytosol, without entering the cytoplasmic pool of phosphate, and diffuses through the basolateral membrane (Peterlik and Wasserman, 1978). This process has also been demonstrated to be saturable (Scott et al., 1984; Care et al., 1980).

Once absorbed inorganic phosphates undergo transformation to organic phosphorus compounds (Georgievskii, 1981). Tissue phosphorus incorporation is variable and dependent on the state of the tissue

(i.e., growing, lactation). Normal plasma phosphorus values range from 3.5 to 7.8 mg/dl (Georgievskii, 1981).

The major route of phosphorus excretion is the digestive tract (Care et al., 1980). Dietary phosphate and salivary phosphate are available for absorption or reabsorption in the gastrointestinal tract (Care et al., 1980). A second route of excretion of phosphate is the urine (Georgievskii, 1981; Scott and Buchan, 1985). The extent to which these routes are utilized is dependent upon the diet. Generally, concentrate feeding increases urinary excretion of phosphorus and decreases salivary secretion as compared to hay feeding (Scott and Buchan, 1985). Whether this shift is a form of feed effect or a digestibility effect or both is unclear. Earlier work by the same authors indicates that digestibility of the diet may affect renal response to phosphorus (Scott et al., 1984).

Parathyroid hormone affects phosphate balance as well as calcium balance. Phosphate elimination through the urine is increased with increasing parathyroid hormone through decreased reabsorption (McIntosh and Tomas, 1978). $1\alpha,25(OH)_2D_3$ has also been suggested to affect phosphate absorption in the intestine but it is not known if this is a direct effect on phosphorus or a secondary effect of calcium absorption (Care et al., 1980). Calcium is not essential for phosphorus absorption (Wasserman, 1981) but does affect the amount of absorbed phosphorus (Care et al., 1980). Excess calcium, magnesium or iron may result in the formation of insoluble phosphate compounds while a decrease in the Ca/P ratio below 0.3 will also decrease phosphate absorption (Care et al., 1980).

Magnesium

Magnesium in blood is found either ionized (65%) or protein (albumin) bound (35%) and is in dynamic equilibrium (Georgievskii, 1981). There is disagreement as to the site of magnesium absorption. Until 1970 the concensus was that magnesium was absorbed by either diffusion (ordinary or facilitated) or active transport in the duodenum and upper large intestine (Martens and Rayssiquier, 1980). Since that time evidence for absorption of magnesium from the rumen epithelium has mounted. Ben-Ghedalia et al. (1975) demonstrated that the forestomachs (before the pylorus) absorbed magnesium. Tomas and Potter (1976) further defined the site of absorption to be the rumen. With the development of cannulation techniques, magnesium transport across isolated rumen epithelium has been actively studied. Active transport is the proposed mechanism (Martens and Rayssiquier, 1980) based upon evidence of transport across a concentration gradient, dinitrophenol inhibition, saturation kinetics, temperature sensitivity and competive inhibition. Additional evidence of inhibition of magnesium transport exists when Na^+/K^+-ATPase inhibitors, such as ouabain, are added. Care et al (1984) using a dorsal rumen pouch demonstrated: 1) net magnesium absorption against an electrochemical gradient, 2) sensitivity to K^+/Na^+ ratio (increased K^+/Na^+ leads to decreased net efflux) and 3) an inverse relationship between calcium concentrations in the rumen and net absorption rate of magnesium. Martens (1985a) further demonstrated that magnesium efflux was ATP dependent supporting the conclusion that magnesium transport is an active transport process, that it is not sensitive to changes in water

absorption or osmotic pressure (Martens, 1985b), and that magnesium transport is a saturable process (Martens, 1983; Gabel and Martens, 1985).

Absorbed magnesium is deposited in bone and muscle tissue. The major mechanisms for elimination of nonabsorbed and endogenous magnesium is elimination from the gastrointestinal tract (Georgievskii, 1981). Urinary excretion of magnesium is small but does play a significant role in magnesium homeostasis. Magnesium levels in the plasma range from 1.8 to 2.7 mg/dl (Georgievskii, 1981).

Potassium

Normal serum potassium levels in growing cattle are 18-20 mg/dl (Georgievskii, 1981). Potassium is absorbed by all segments of the digestive tract, particularly the rumen, abomasum and duodenum. The fraction of absorbed potassium that each of these areas account for is diet dependent (Paquay, 1969).

Absorption mechanisms for potassium within the rumen have been investigated by Scott (1974). His findings indicate that potassium absorption from the rumen is dependent on the concentration of potassium present in the rumen fluid. In one of his studies he infused sheep with various levels of potassium through rumen cannulas. At the lowest level of potassium infused, rumen potassium levels reached 40 mEq/l. The absorption rate at this concentration was 6 mEq/hr. At the highest level, rumen concentrations reached 110 meq/L and absorption rates were 20 mEq/hr. The mechanism by which potassium is absorbed through the rumen wall is proposed to be via a concentration gradient established by higher potassium in the rumen fluid than in the blood. This concentration gradient is likely to

exceed the electrical potential gradient so that net movement of potassium is to the blood stream (Scott, 1967).

There is also omasal absorption of potassium (Engelhardt and Hauffe, 1975). Ten percent of the potassium entering the omasum was absorbed. The mechanism by which the absorption occurs has been suggested to be similar to that of the rumen epithelium (Engelhardt and Hauffe, 1975).

The mechanism of potassium absorption from the duodenum, jejunum and ileum are not elucidated for the ruminant animal but are well investigated in the rabbit, rat, cat and dog. Rabbit ileum data indicate that potassium transport across the mucosal membrane is a passive process driven by an electrochemical gradient (Schultz et al., 1974). This evidence further suggests that the mucosal membrane is essentially impermeable to potassium and that intracellular potassium levels are maintained by the pump at the basolateral membrane. All the potassium moving across the epithelium appears to be moving extracellularly through tight junctions (Schultz et al., 1974).

Potassium is eliminated primarily through the kidneys, with a small percentage eliminated in the feces (Ward, 1966). At the level of the kidney, aldosterone and deoxycorticosterone are the mechanisms for maintaining homeostasis. As the concentration of sodium in the plasma decreases relative to potassium, aldosterone acts to decrease urinary sodium and increase potassium excretion thus maintaining a Na^+/K^+ balance (Georgievskii, 1981).

Absorption of Sodium and Chloride from the Forestomach

Sodium and chloride are absorbed by all segments of the ruminant forestomach (Edrise et al., 1986). These minerals are also actively

secreted into each of these compartments from the plasma or saliva. The omasum is the part of the stomach that has the greatest absorption rate of sodium and the greatest secretion of chloride ions. Engelhardt and Hauffe (1975) estimated that 25% of the sodium entering the omasum was absorbed. This is in agreement with Edrise et al. (1986) who estimated 40-60% of the sodium was absorbed. Data from studies measuring chloride absorption across the rumen and omasal epithelium (Engelhardt and Hauffe, 1975) show absorption across the rumen and a net secretion of chloride in the omasum (Edrise et al., 1986). This increase in concentration in the omasum does not appear to be related to backflux from the abomasum but rather a permeability of the omasal epithelium (Engelhardt and Hauffe, 1975).

The greatest absorption of sodium and chloride is through the intestine (see Transcellular Sodium and Chloride Absorption by the Small Intestine). Sodium balance is maintained by aldosterone and deoxycorticosterone action on the convoluted tubules of the kidney. Aldosterone promotes retention of sodium ions and secretion of potassium in the urine. Normal blood sodium concentration in cows and calves range from 260-280 mg/dl and normal chloride values are 101-113 mEq/L (Georgievskii, 1981).

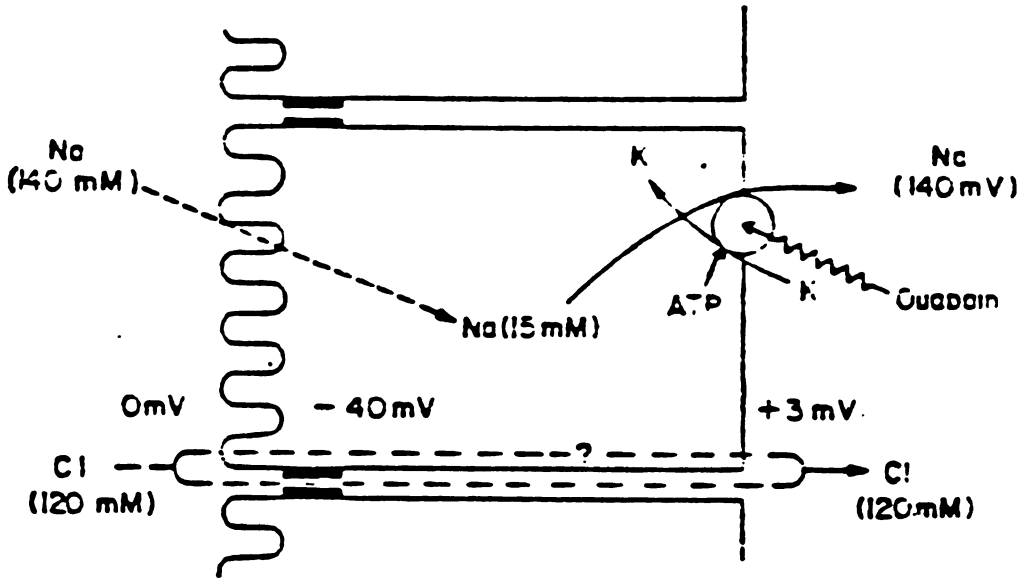
Transcellular Sodium and Chloride Absorption by the Small Intestine

Sodium absorption may be accomplished by three processes. These are: 1) active absorption not coupled to absorption of other solutes but associated with passive chloride absorption (electrogenic), 2) absorption coupled with transport of nonelectrolytes and 3) neutral sodium chloride absorption (Schultz, 1981).

Electrogenic sodium transport is postulated to occur as follows (Figure 1). Sodium absorption across the apical membrane occurs due to the differences in chemical concentration (mucosal solution - 140 mM, 0 mV and cellular solution 15 mM and -40 mV) and electrical potential. Sodium extrusion across the basolateral membrane in to plasma or serosal solutions is energetically unfavorable (serosal sodium 140 mM and + 3 mV) and is therefore coupled to ATP hydrolysis. This is the role of the Na^+/K^+-ATPase enzyme complex found in the basolateral membrane of most all animal cells. The diffusion of chloride is coupled to the electrical potential difference generated by the active absorption of sodium and the positive difference between mucosal and serosal solutions (+3 mV). This diffusion occurs across the "leaky" epithelium.

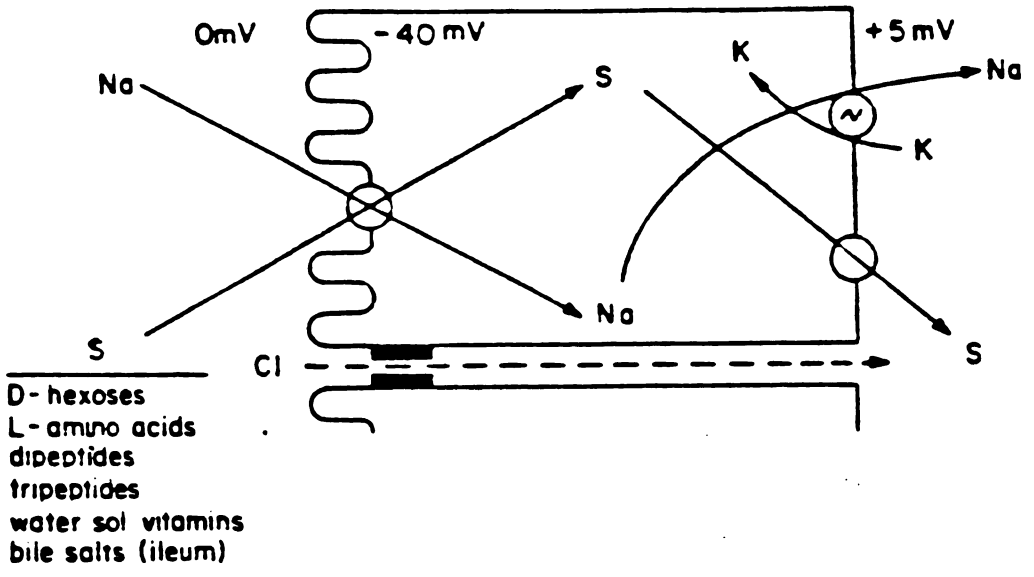
Coupled sodium absorption (i.e., organic solute absorption coupled to sodium uptake) is the proposed mechanism to account for D-hexose, L-amino acid, di and tripeptide, water soluble vitamin and bile salt absorption (Figure 2). The downhill movement of sodium across the apical membrane energizes the uphill movement of solutes across "carrier mechanisms" located in the mucosal membrane. The Na^+/K^+-ATPase mechanism actively extrudes sodium across the basolateral membrane maintaining low intracellular sodium activity which allows continued entry of organic solute and maintaining the electrochemical potential for sodium across the mucosal membrane. Solute transport from the cell solution to the serosal solution occurs down a concentration gradient. Evidence presented by Bihler and Cybulsky (1973) and Danisi et al. (1976) using intact intestinal preparations

Figure 1. A Model for Electrogenic Sodium Transport



Schultz, 1981

Figure 2. Cellular Model for Na-coupled Absorption of Organic Solutes (S) by Small Intestine



Schultz, 1981

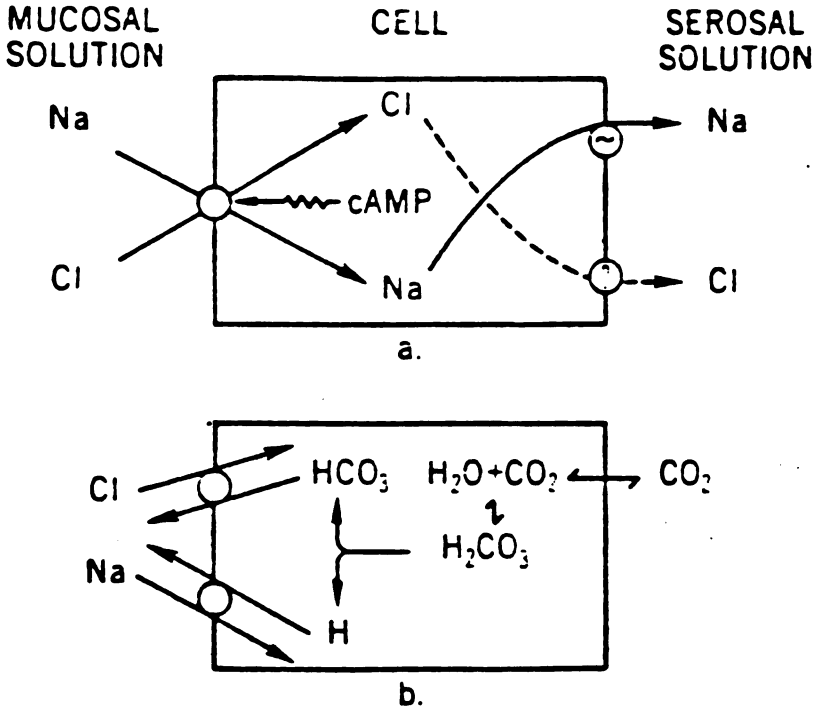
and Murer et al. (1974) using isolated membrane vesicles indicate the presence of membrane carriers on the basolateral membrane to facilitate sugar and amino acid diffusion into serosal solutions. This process is independent of sodium transport.

In summary, organic solute transport into the epithelial cell is mediated by sodium transport in two ways. First the concentration gradient that exists for sodium and second the electrical potential difference created across the membrane by sodium transport. Alterations of sodium transport might alter transport of organic solutes or result in compensation by other mechanisms specifically the Na^+/K^+-ATPase pump.

Several models have been proposed to account for sodium chloride absorption. The first of these is shown in Figure 3a. Sodium chloride is absorbed at the apical membrane by a one for one neutral mechanism. The "downhill" movement of the sodium ion energizes the "uphill" transport of chloride. Sodium is extruded through the basolateral membrane by the Na^+/K^+-ATPase pump and chloride is tranported with the energetically favorable gradient into the serosal solution. The mechanism of chloride extrusion is unknown (Frizzell et al., 1979).

A second model (Figure 3b) involves two antiport processes; a Na-H exchange and a $Cl\text{-}HCO_3$ exchange. The movement of sodium down the concentration gradient at the apical membrane energizes the movement of hydrogen from the cell against a gradient. This creates a basic cell solution causing HCO_3 movement out of the cell which generates energy to pull chloride into the cell. Presumably sodium and chloride transport into the serosal solution would occur much the same as is

Figure 3. Models for Chloride Absorption



a. Direct symport model

b. Dual Na-H and Cl-HCO_3 antiport model

Schultz, 1981

postulated for the first model. This model would tightly couple sodium chloride transport to intracellular pH regulation.

Copper

Absorption of copper has been shown to occur in the large intestine (Stevenson and Unsworth, 1978) in sheep fed either fresh forage or hay concentrate diets. In this study there was net secretion of copper in the abomasum and forestomach, and no net absorption between the duodenum and ileum. These data are contrary to those found in calves or mature cows. In these studies (Chapman and Bell, 1963; Ivan and Grieve, 1976), the duodenum, jejunum and ileum contributed roughly the same amount of absorption. Secretion of copper in these studies was shown to occur primarily before the duodenum (most likely the abomasum) and along the small intestine. In the calves and mature cows the large intestine did not absorb appreciable quantities of copper.

The mechanism of copper absorption is largely uninvestigated. There is some evidence that copper may be absorbed in a passive process (Bremner and Davies, 1980) or in a saturable transport system (Neethling et al., 1968, as cited by Bremner and Davies, 1980). The disparity in evidence may be due to the concentration used. At low doses a saturable process seems to be indicated while at higher doses a passive process appears to fit the evidence.

The factors that appear to affect copper absorption are: 1) the chemical form of copper in the digesta and at sites of absorption, 2) the level of molybdenum and sulfur in the diet, 3) cadmium and zinc

levels in the diet, 4) the protein level of the diet and 5) the presence of a protozoal population in the rumen. Bremner (1970) examined the changes in the forms of zinc, manganese, and copper in the intestinal tract of sheep. His data indicate that soluble copper concentrations increased when digesta moved from the rumen to the ileum. The solubility of copper was less than 20% in the rumen and this was attributed to binding the insoluble rumen residue. Copper solubility in the abomasum was very low. As the pH increased over 6 in the intestinal tract, copper became increasingly soluble. In this study Bremner was unable to determine what copper was complexed with and how the presence of a copper complex would affect absorption.

Molybdenum and sulfur are known to affect copper availability. Molybdenum-copper complexes appear to be absorbed through the intestine and transported through the blood and are excreted through the urine and bile (Marcilese et al., 1969) as a complex. Copper-sulfur interactions have also been shown to decrease copper absorption through the formation of insoluble copper-sulfide complexes (Huisingh et al., 1973). When molybdenum, sulfur and copper interact, copper availability is further reduced. It is thought that thiomolybdate derivatives may be formed in the rumen which interfere with copper absorption (Bremner and Davies, 1980).

Cadmium and zinc levels in the diet are also thought to affect copper availability. Indirect evidence implicates cadmium. In a study with ewes and lambs, Mills and Dalgarno (1972) observed a decreased copper retention with high levels of cadmium in forage. In studies using bull calves, Ivan and Grieve (1976) have found decreased net

copper absorption with increasing zinc levels in the diet. This inhibition was shown to occur in the small intestine and rumen.

Ivan and Veira (1981) examined the effect of dietary protein level on the solubility of copper in the rumen and abomasum of sheep. Their data indicate that increasing dietary protein level from 7% to 19% decreased copper solubility in the rumen from 23.5% to 15% (this was not significantly different at $P>.05$). Abomasal copper solubility decreased significantly ($P<.05$). The authors speculated in a later paper (Ivan et al, 1986) that this was due to degradation of soluble protein to amino acids thereby increasing the availability of sulfur containing amino acids to bacterial and protozoal metabolism. This metabolism results in the production of sulfide which interacts with copper to form an insoluble complex.

In the same study the role of protozoa in copper availability was examined. Rams were maintained either faunated or ciliate-free and fed a high copper diet. The presence of ciliate protozoa decreased liver copper accumulation 38-50%. This indicated a decrease in absorption of copper. The mechanism by which the ciliate protozoa appeared to alleviate a copper toxicity is related again to the degradation of protein in the rumen and the availability of sulfur containing amino acids to protozoal metabolism.

Copper in the plasma is found bound to ceruloplasmin (Georgievskii, 1981), and levels range from 8-120 µg/dl in the blood of adult cattle, calves and sheep.

Zinc

There is disagreement as to where in the digestive tract of ruminants zinc is absorbed. Miller and Cragle (1965) reported that 35%

of dietary zinc was absorbed between the mouth and abomasum. Secretions of zinc in the intestinal tract (proximal duodenum) in this study amounted to 280% of intake. There was also a small amount of net absorption throughout the small intestine. Work by Grace (1975) indicates that zinc is secreted rather than absorbed in the forestomach of ruminants, and that the small intestine, particularly the upper segment is the primary site of zinc absorption. Other work utilizing labeled zinc indicated that the rumen actively secretes zinc with little absorption taking place. The abomasum was the primary site of zinc absorption with about 40% of the zinc from rumen digesta absorbed. Active absorption of zinc took place along the small intestine as did secretion (Georgievskii, 1981). It seems that zinc is absorbed to some degree from all segments of the tract from the rumen through the colon. Zinc is also secreted from all of these segments. The small intestine, however, is likely to contribute the greatest amount of absorption (Bremner and Davies, 1980).

The mechanism of zinc absorption across the mucosal cell is not known. There is some evidence that zinc, like other minerals, interacts with a protein molecule at the apical membrane which after chelation enhances transport (Freeman et al, 1971 as cited by Ashmend et al, 1985). In any case, zinc absorption into the mucosal cells is rapid, but passage into the blood stream is slow (Georgievskii, 1981). Zinc in plasma is found loosely bound to albumin (transport) and tightly bound to globulins. Normal plasma zinc levels in whole blood range from 250-500 µg/dl in dairy cattle to 200-300 µg/dl in calves (Georgievskii, 1981). Zinc excretion is through intestinal secretions,

pancreatic juices and saliva. There is little zinc in urine (Georgievskii, 1981).

Selenium

Selenium is absorbed in the lower segments of the small intestine of sheep and is secreted in small amounts through the intestine (Georgievskii, 1981). Absorption is a rapid process that moves against a concentration gradient. Data indicate that selenium is absorbed as Se-amino acid complexes by the same mechanism as sulfur-containing amino acids (Georgievskii, 1981). Selenium in blood is carried by albumin and globulins and enters the red blood cell rapidily via an oxygen-dependent active transport system. Normal blood selenium levels in cattle are .09-.1 μg/ml.

Selenium excretion occurs through the kidneys, gastrointestinal tract and lungs. The primary routes in ruminants are feces and urine (30-35% each) and exhaled air (2-3%). As selenium intake increases the urinary portion of selenium excretion increases (Georgievskii, 1981).

Lipid Absorption

Lipid absorption in the ruminant animal begins in the rumen with short-chain fatty acids (SCFA) transported across the ruminal epithelium. The ability of the ruminal epithelium to transport SCFA's is inversely proportional to the chain length. The order is butyric > propionic > acetic (Sutton et al, 1963). To some extent this process is pH dependent with a decrease in pH increasing the overall rate of absorption (Sutton et al, 1963) suggesting that the epithelium is more permeable to undissociated acid forms (Stevens, 1970).

These ruminally absorbed SCFA's, or volatile fatty acids (VFAs), are transported into the portal vein with a very minor amount absorbed

into the lacteals and transported through the lymphatic system (Kiddle et al., 1951, Annison, 1965). SCFA's may also appear in the portal blood from omasal (Gray et al, 1954) and abomasal (Johnston et al., 1961) absorption.

Long and medium chain fatty acids are also absorbed prior to the small intestine (Wood et al., 1963) but the quantitative significance of this process appears to be limited (Noble, 1981). The composition of digesta found in the various points of the gastrointestinal tract have been found to vary markedly from that found in the rumen (Lennox et al., 1968). Digesta present in the rumen, abomasum and upper duodenum have been found to be similar in composition (Bath and Hill, 1967; Lennox et al, 1968) and distribution of lipid, despite changes in diet composition. In this area of the tract, digesta is comprised of unesterified fatty acids, small amounts of phospholipids and very little triglyceride. The unesterified fatty acids were found to be closely associated with the particulate fraction while phospholipids were more evenly distributed with the aqueous and particulate phases (Smith and Lough, 1973).

In the lower duodenum and jejunum the majority of the lipid is largely esterified. Rumen and abomasal lipid consisted of stearic acid primarily while jejunal lipid contains a greater proportion of phospholipids and esterified fatty acids. This source of lipid has been found to be bile lipid secretions.

Johnson et al. (1974), in a study investigating the site of lipid absorption in the sheep intestine, showed limited hydrolysis of

esterified fatty acids and 20% of the fatty acids were absorbed unesterified from the upper jejunum. A further 60% of the total lipids were absorbed from the middle and lower jejenum. These absorbed acids were derived from the hydrolysis of phospholipids and neutral lipids. By the ileum, lipid absorption was almost complete.

The mechanism of lipid absorption in the ruminant is much the same as that for the monogastric, although the ruminant has been shown to be very efficient at lipid digestion and in fact has a greater ability to digest dietary fat than does the monogastric (Carroll, 1958; Carroll et al., 1958; Andrews and Lewis, 1970).

The major steps in mucosal uptake of dietary lipid include: 1) intraluminal digestion, 2) diffusion across the unstirred water layer, 3) membrane permeation, 4) metabolism and lipoprotein assembly, 5) exit from the mucosal cell (Thomson and Dietschy, 1981). Digestion proceeds in the small intestinal lumen with the interaction of bile and pancreatic secretions. In the ruminant the pancreatic duct and bile duct empty into the duodenum through a common channel. This process is a continuous one because of the relatively constant flow of digesta entering the duodenum (Noble, 1981). The absorption rate of digested lipid is controlled by the rate of diffusion across the unstirred water layer and penetration through the apical membrane (Thomson and Dietschy, 1981). The unstirred water layer (UWL) consists of a series of water "layers" each becoming progressively more stirred until it blends with the water phase. Transport of molecules across this layer is dependent on the thickness of the UWL, the diffusion constant of the molecule to be transported and the concentration gradient present

between the bulk phase (luminal lipid) and the cell membrane (Thomson and Dietschy, 1981). It is at this point that bile acids and bile salt concentrations play a critical role in the absorption of lipid across the apical membrane of the mucosal cell. Micelle formation is dependent on bile salt concentration. Unless a critical level of bile salt/lipid is reached micelles will not form. Since long chain fatty acids and monoglycerides have low water solubilities, and since the formation of micelles may increase solubility of lipid 100-1000 times (Higgens and Barrnett, 1971), diffusion across the UWL is increased by micelle formation. However, there is also, an increase in diffusional resistance with increased size of the bile acid-lipolytic monomer but this is overcome by the great increase in the concentration gradient (Higgins and Barrett, 1971). This is perhaps better seen using the following equation (Thompson and Dietschy, 1981) that describes movement across the UWL.

$J = C(C_1-C_2)D/d$ where,

J = rate of movement of solute

C_1 = concentration of solute at the bulk phase (digesta)

C_2 = concentration of solute at the aqueous - membrane interface

D = diffusion constant of the molecule

d = functional thickness of the UWL.

If $(C_1-C_2)D$ is increased by solubilization and d is constant, then J will increase.

There are three theories that may help explain the events that occur during lipid uptake from the micelle into the membrane (Thomson

and Dietschy, 1981). One is that the micelle simply is taken up in a pinocytosis-like action. This appears to be unlikely because experiments in which uptake rates of various fatty acids were measured indicated that the constituent parts of the micelle were absorbed at rates independent of each other (Hoffman, 1970; Hoffman and Simmonds, 1971). The second possibility is that the micelle interacts with the membrane in a collision which excludes water. The fatty acids within the micelle moves into the cell and the bile acids return to the UWL (Thomson and Dietschy, 1981). Experimental evidence does not fit this model (Westergaard and Dietschy, 1976). In the third model, which best fits the evidence, the micelle acts as the solubilizer from which molecules pass into the microvillus membrane (Westergaard and Dietschy, 1976). Actual uptake of the fatty acid is determined by the concentration in the micelle which is in equilibrium with the fatty acid concentration in the aqueous solution (Westergaard and Dietschy, 1976).

Once inside the mucosal cell the resynthesis of triglyceride occurs primarily through the α-glycerophosphate pathway (Noble, 1981). Phospholipid resynthesis mechanisms are not well defined in the ruminant intestine, but the evidence suggests that the major pathway is the reacetylation of l-lysolecithin (Subbiah et al., 1969). Cholesterol is produced primarily by the small intestine in the ruminant (Scott and Cook, 1975) in a process which is sensitive to changes in diet and other factors (Scott and Cook, 1975).

Following resynthesis within the smooth endoplasmic reticulum, the absorbed or synthesized lipid is incorporated into chylomicrons or lipoproteins (VLDL's) and is secreted from the Golgi apparatus in

secretory vesicles. These vesicles migrate toward the basolateral membrane where reverse pinocytosis is thought to occur. Microtubules are thought to have an integral role in this process (Barrowman, 1984). The chylomicra then pass through the lamina propria to the lacteal. This process is not clear. There is evidence that the chylomicra pass through interendothelial gaps (Casley-Smith, 1962) into the lymphatic endothelium. Once inside the lacteal the lipid moves through the intestinal lymphatic system until it is eventually released into the plasma through the lymphatic duct (Noble, 1981).

The flow of lymph through the lymphatic system has been shown to be related to the amount of fluid absorption through the intestine, elevation of portal venous pressure and increased plasma dilution (Barrowman, 1984). In general an increase in intestinal fluid in the mucosa increases fluid pressure which stimulates lymph flow. In the ruminant animal there is virtually continuous lipid absorption and lymph flow under normal dietary conditions (Noble, 1981).

Harrison et al. (1974) examined the composition of lymph from sheep fitted with thoracic duct catheters. Seventy-three percent of the lipid present was associated with the VLDL fraction and 27% with the chylomicrons. The major classes of lipid present were triglycerides, phosopholipids and cholesterol with triglycerides making up the greatest amount. The relative proportions of the lipid classes do not appear to be altered with different dietary regimes, but the concentrations of triglycerides, phospholipids and cholesterol in thoracic lymph as well as lymph flow rate may be altered (Noble, 1981).

The distribution between lymphatic lipid absorption and direct portal absorption is known to be in favor of lymphatic absorption. Generally speaking, long chain fatty acids are incorporated into triglycerides and are transported to the lymphatic ducts as chylomicrons (McDonald et al., 1980). There is evidence in sheep that in the absence of bile, a substantial amount of the lipid absorbed does not appear in the lymph (Noble, 1981). This suggests that lipid absorption through the portal vein might be of more importance than lymphatic absorption in the ruminant. However, in later absorption studies with labeled fatty acids, no labeled acid could be detected in the jugular blood casting doubt on this hypothesis (Harrison and Leat, 1972).

Glucose Absorption

The ion gradient hypothesis of coupled sugar-sodium absorption was first proposed by Crane (1961). His hypothesis was that the energy required for establishment of a concentration gradient for sugar transport is derived from the flow of sodium ions down a concentration gradient into the cell. He also postulated that a membrane carrier or transport site was involved with sugar absorption and that this carrier had different affinities for sugar depending on the presence or absence of sodium. Since 1961, evidence has mounted that confirms this hypothesis (Kimmich, 1981).

The sodium that is absorbed is extruded across the basolateral membrane via a ouabain-sensitive energy dependent mechanism (Schultz, 1981). This mechanism functions to maintain low concentrations of sodium intracellularly and also acts to maintain the electrochemical

potential difference for sodium across the apical membrane (Schultz, 1981).

Use of membrane vesicles has confirmed Crane's sodium-dependent glucose transport hypothesis and has also provided evidence that there is another pathway of glucose transport across the cell (Stevens et al., 1984). In membrane vesicles from steers and rabbits (Kaunitz et al., 1983) the so called diffusive pathway was examined. This pathway exhibits first-order kinetics between 0 and 20 mM glucose. It is also evident from these studies that there are two saturable transport systems. One of these is a high affinity system that predominates when glucose concentrations are <.5 mM. The other is a high capacity system that predominates at higher concentrations. Above 2 mM the diffusive pathway is most important (Stevens et al., 1984).

Data from steer membrane vesicles indicate that sodium acts as a competitive activator in the steer. That is, sodium affects Kt (half maximal transport) but not Jmax (maximum rate of transport) (Stevens et al., 1984). Other work from bovine membrane vesicles indicates that the coupling stoichiometry of Na^+:sugar in the sodium:glucose high-capacity system was 1:1, while the low capacity, high affinity system had a ratio of 3:1 (Kaunitz and Wright, 1984). This is in agreement with workers using kidney preparations (Stevens et al., 1984).

Glucose exit through the basolateral membrane has also been shown to function via a saturable facilitated diffusion system (Wright et al., 1980). This system has been shown to be inhibited by structural analogs (galactose) and inhibitors (cytochalasin β and phloretin) and

exhibits stereospecificity and saturation kinetics (Wright et al., 1980).

The capacity of the bovine intestinal tract to absorb glucose actively has been questioned. Work done by McAllen and Lewis (1985) and Pehrson et al. (1981) indicates that when glucose is presented to the small intestine it is absorbed with an efficiency > 80%, However when starch is presented to the small intestine the efficiency of absorption is reduced (51%). These authors found that while the starch was well decomposed, its end products for absorption were not utilized as well as glucose. Their conclusions were that at high levels of starch, maltase activity of the small intestine is inadequate to process all of the starch (Pehrson et al., 1981) and that starch digestion and utilization is different depending on the type of starch used (McAllan and Lewis, 1985).

Amino Acid and Peptide Absorption

Dietary proteins are hydrolyzed to amino acids and small peptides. Both of these forms of degraded protein are transported across the intestinal brush border although through different mechanisms. Ganapathy and Leibach (1982) provided a summary of the various features known about peptide transport. These include:

1) Most amino acids are absorbed faster as peptides than as free amino acids
2) There appears to be no direct competition at the mucosa between amino acids and peptides for transport
3) Sites of maximal absorption of amino acids and peptides in the small intestine are likely to be different

4) Peptide absorption may occur either by hydrolysis at the brush border by peptidases present there and entrance to the cell as a free amino acid or by special systems in the membrane that absorb peptides intact.

Other details of peptide absorption remain to be elucidated. The dependence of peptide transport on Na^+ concentrations or the sodium gradient in the membrane has been investigated but the results vary considerably (Ganapathy and Leibach, 1982; Adibi and Kim, 1981 and Alpers, 1985). In some studies Na^+ is essential while in others it is not. Studies investigating the area of the intestine with the greatest ability to transport peptides have resulted in the conclusion that the jejunum and ileum do not show differences in peptide transport disappearance rates (Adibi and Kim, 1981).

Recently Ganapathy and Leibach (1985) proposed a proton gradient energized peptide transport system. These investigators hypothesized that a Na^+/H^+ exchanger mechanism propels H^+ ions into the lumen where a coupled peptide - H^+ transporter moves the peptide-H^+ complex into the cytosol through a proton gradient. While this hypothesis may have merit and does explain some of the evidence presented in peptide absorption studies, it remains to be validated.

Still unclear is the extent to which peptides are hydrolyzed by brush border amino peptidases and absorbed as free amino acids or absorbed as peptides.

Amino acid transport has been better elucidated. Wiseman, over 25 years ago, established that amino acids were transported uphill (Munck, 1981). Later evidence established that transport of some amino acids

was related to and energized by the maintenance of low intracellular sodium concentrations and high intracellular potassium concentrations. Eventually Crane (1977) formulated the sodium gradient hypothesis that fit both glucose and amino acid transport data. Inhibition studies using cardiac glycosides further established the dependence of amino acid uptake on the Na^+/K^+-ATPase located on the basolateral membrane of the cell membrane (Csaky, 1963).

Not all of the amino acids transported across the cell are dependent on the sodium gradient (Stevens et al., 1984). The NBB (most neutral amino acids), IMINO (glycine and imino acids), PHE (phenylalanine, methionine), A (short chained polar amino acids) and ASC (3-4 carbon neutral amino acids, alanine, serine and cysteine) are sodium dependent. The y+ (lysine, arginine, cationic amino acids) and L system (leucine, branched and ringed amino acids) are not sodium dependent (Christenson, 1982; Stevens et al., 1984).

The most active site of amino acid absorption in sheep was found to be mid to lower ileum. The area of the greatest rate of absorption of lysine was found to be the jejunum (Johns and Bergen, 1973).

Monensin

Much work has gone into elucidating the mechanism of action of monensin in the rumen. Several reviews have been written on the subject (Bergen and Bates, 1984; Schelling, 1984). In summary, monensin shifts the acetate-propionate ratio toward increased propionate. Gram positive bacteria are inhibited and methane and fumarate production are reduced. The incidence of lactic acidosis and ruminal ammonia concentrations are reduced. Animal performance is

enhanced as indicated by decreased feed intake with no reduction in rate of gain (Goodrich et al., 1984).

Metabolism of Monensin

Several studies have been conducted to examine the metabolism of monensin in the steer, chicken, horse, pig and rat (Herberg et al., 1978; Donoho et al. 1978; Davison, 1984; Donoho, 1984). In a study with [^{14}C] monensin, 94% of the radioactivity from the dose of monensin was recovered in the feces (Herberg et al., 1978). Further investigations by Donoho et al. (1978) resulted in the identification of 4 metabolites in steer feces. Ninety percent of the 57% of the recovered radioactivity was identified by mass spectroscopy to be monensin. The remaining 10% was identified to be 4 O-demethylated fragments of monensin. Labelled monensin was also incubated in rumen fluid (12 hr), abomasal fluid (5 hr) and cattle feces (2 days) to examine alimentary degradation. In all cases at least 90% of the labelled compound was recovered.

Biliary metabolism of monensin has also been examined (Davison, 1984; Donoho, 1984). Thirty to forty percent of a labelled monensin dose was absorbed in calves. Thirty-four to thirty-seven percent of the radioactivity was recovered in the bile, 50 to 64% in the feces and trace amounts in gallbladder, GI tract, liver, kidneys and carcass (Davison, 1984). These data agree with Donoho et al. (1978) who reported absorption of approximately 50-60% of labelled monensin in steers. Of this label only 3% was found to be monensin. The rest was in the form of O-demethylated derivatives of monensin. The biological activity of these derivatives was also examined. In several systems

evaluating antimicrobial activity, anticoccidal activity, ionophoric activity and cytotoxic activity the metabolites were found to be 20 times less active than the parent compound (Donoho, 1984).

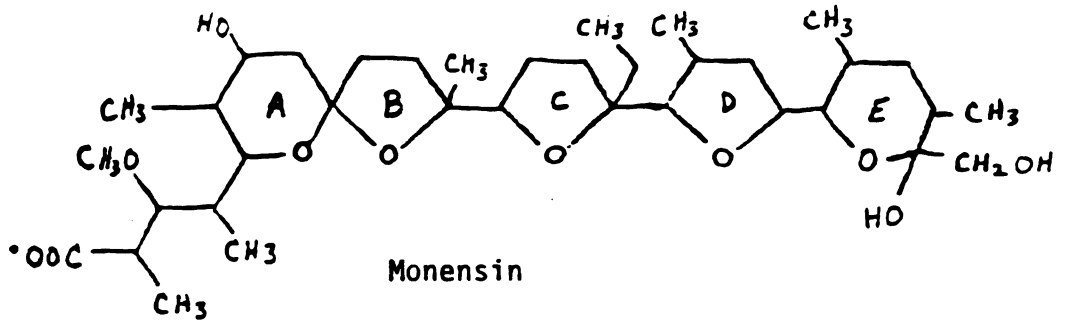
These data would indicate that ruminant animals absorb approximately one half the dose of monensin and that animal metabolism, not gut bacterial action, is responsible for monensin metabolism.

Monensin's Effect on the Na^+/K^+-ATPase

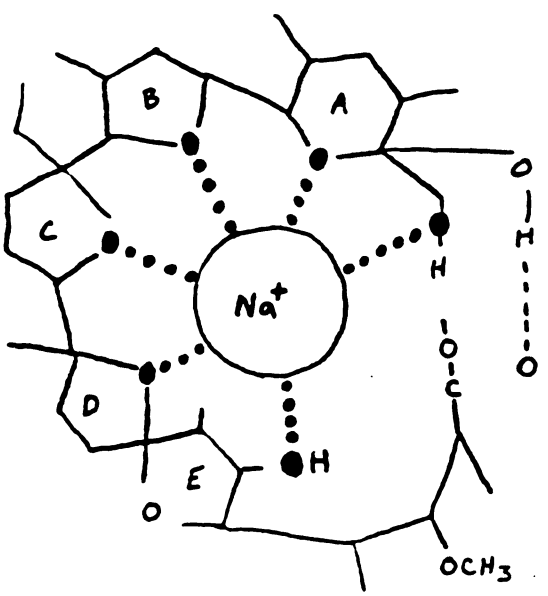
Monensin, a carboxylic ionophore (see Figure 4), has been used in many studies and with many cells and tissues as a cellular perturbant. By virtue of its ability to collapse proton and sodium gradients across cell membranes, monensin is a valuable tool in examining the role of membrane gradients on various cellular functions. Table 1 is a compilation of many of these studies. The cellular effects of monensin range from reduced secretion of products from the Golgi complex to increased secretion of catecholamines from chromaffin cells. Other effects include disruption of post-translational modification of membrane and secretory proteins, inhibition of various phases of endocytosis, intracellular pH alterations and undersulfation of chondrocytes and chondrosarcomas (Ledger and Tanzer, 1984).

The majority of these studies summarized in Table 1 use the ionophore, monensin, as a means to investigate sodium-dependent membrane processes. The mechanism by which monensin disrupts ion transport is described by Pressman (1976) and Bergen and Bates (1984) and is illustrated in Figure 5. Monensin in a protonated form diffuses to one membrane interface and releases its proton. This increases the polarity of the compound and traps it at the polar interface of the

Figure 4. Schematic Views of the Structure of Monensin and Monensin Sodium (Pressman, 1976)



Monensin



Monensin sodium

Table 1. A Summary of Studies Involving Monensin as a Cellular Perturbant

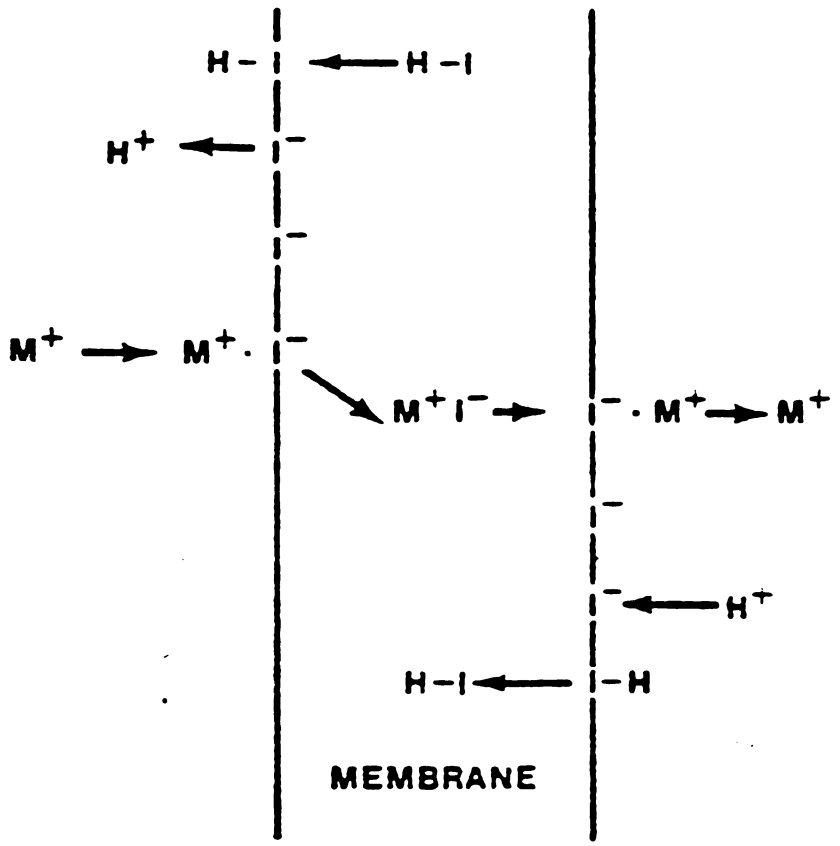
Cell Type	Substrate	Action	Reference
3T3	$^{22}Na^{+}$ $^{86}Rb^{+}$	Activate NA^{+}/K^{+} pump	Smith and Rozengurt, 1978
3T3	AIB	Activate Na/K^{+} pump	Smith and Austic, 1980
Rat pituitary	pro-opiomelanocortin	Inhibits proteolytic processing	Crine and Dufour, 1982
Rat pituitary	pro-opiomelanocortin	Inhibits protein synthesis	Devault et al., 1984
Guinea pig taenia coli	ATP	Relaxes contraction	Kishimoto et al., 1982
Chick chondrocytes	[^{3}H] serine, [14C]glycosamine	Accumulation of β-D oxyloside glycosaminoglycans	Kajiwara and Tanzer, 1982
Mouse peritoneal macrophages	β-glucuronidase β-glucosidase	Release of lysosomal and nonlysosomal enzymes	Takano et al., 1984
Plant Golgi apparatus	--	Swollen cisternae	Boss et al., 1984
Maize root rhizodermis cells	--	Decreased cytosolic pH	Brummer et al., 1984
Mouse Thymocytes	2-deoxyglucose 3-O-methylglucose	Increased glucose uptake	Nordenberg et al., 1984
Avian Erythrocytes	3-O-methylglucose	Stimulates sugar transport	Bihler et al., 1985a
Mouse diaphragm	3-O-methylglucose	Stimulates sugar transport	Bihler et al., 1985b
Rat Liver Lysosomes	N-acetyl-β-D-glucosaminmidase	Inhibited protein degradation	Fink et al., 1985

cell membrane. When a complexable cation interacts with the anionic ionophore, a complex is formed and the zwitterion complex is able to move away from the membrane and diffuses to the opposite interface. Here the cation is released and the anionic ionophore is free to become protonated and then to continue the proton-cation exchange cycle. Carboxylic ionophores act as exchange diffusion carriers because their final equilibrium is independent of the membrane potential but is sensitive to pH (Pressman, 1976). Monensin has the greatest affinity for sodium ions with decreasing affinity for potassium, rubidium, lithium and cesium (Pressman, 1976).

Smith and Rozengurt (1978) used monensin as a sodium perturbant in a study investigating the activation of quiescent fibroblasts (3T3 cells). These data indicate that the Na^+/K^+-ATPase present in those cells is limited by internal sodium concentrations and extremely sensitive to small changes in internal sodium. Monensin (at 4 µg/ml media) increased internal sodium concentrations three fold in 30 minutes. Monensin at all concentrations stimulated Na^+/K^+-ATPase activity, although 3 µg/ml media produced maximal stimulation. Ouabain (at 1mM) inhibited monensin's stimulation of the pump. Low concentrations of monensin were shown to stimulate a slight increase in cell potassium. Higher levels of monensin caused a net efflux of K^+.

Further work by this group (Smith and Austic, 1980) demonstrated an increased α-aminoisobutryic acid (AIB) uptake with a monensin induced stimulation of the Na^+/K^+-ATPase pump in the 3T3 cells. AIB is the non-metabolizable substrate for the Na-dependent A system

Figure 5. A Schematic View of the Mechanism of Monensin's Action in the Cell Membrane



Carboxylic ionophore mediated cation transfer across a bimolecular lipid membrane. M^+ = metal cation; I = ionophore; H^+ = proton, H-I = protonated ionophore; M^+I^- = zwitterion of metal cation and anionic form of ionophore

Pressman, 1976

absorption (Kilberg, 1986). Monensin, at 15 μM, increased the initial rate of AIB uptake as well as the steady state level of AIB at least 3-fold. Ouabain (1mM) which had no effect on AIB uptake without monensin, inhibited monensin stimulation of AIB uptake. Again monensin increased sodium concentration in the cell but there was no increased potassium.

Nordenberg et al. (1984) and Bihler et al. (1985a) have demonstrated that monensin affects glucose uptake in mouse thymocytes and mouse skeletal muscle. In the work of Nordenberg et al. (1985) monensin (7.2 μM) significantly ($P<.001$) stimulated 2-deoxyglucose uptake and 3-O-methylglucose transport in mouse thymocytes. Ouabain did not inhibit monensin's stimulation of glucose uptake, but low concentrations of sodium and cytochalasin β (an inhibitor of glucose uptake that binds to the membrane receptor) did. Bihler et al. (1985) found an increase in internal sodium, calcium and 3-O-methyl-D-glucose concentrations and a stimulation of the Na^+/K^+-ATPase with monensin addition (12 μl monensin) to mouse diaphragm. Mitochondria isolated from rat hind limbs indicated the monensin increased mitochondrial calcium when calcium was present in the media. This is likely to be an effect on mitochondrial Na^+/Ca^+-ATPase.

In avian erythrocytes (Bihler et al., 1985b), monensin-stimulated sodium and 3-O-methyl-D-glucose uptake was dependent on increased sodium influx. Monensin was also shown to decrease cellular ATP concentrations reflecting increased Na^+ pumping in the plasma membrane.

Blood Flow Technique

Growth, lactation and pregnancy are demanding physiological states for the ruminant animal. Much work has been done to examine the metabolic processes occurring in the rumen and lower digestive tract but less information is available about the amount of absorbed metabolites reaching the liver. The splanchnic bed, the gut, and the liver play key roles in regulation of nutrient availability. Several techniques have been used to evaluate the amount of absorption through the gut and hepatic metabolism. Two techniques have been applied to the whole animal. These are based on venoarterial (VA) concentration differences coupled with measurements of blood flow or isotope dilution methods designed to measure entry rates in the whole body (Annison, 1965).

Techniques that evaluate V-A differences and turnover rates depend on animals with many catheters. Catheters may be placed in the portal vein, hepatic vein and an artery for sampling and mesenteric or peripheral veins for infusions.

The techniques that utilize V-A differences to assess absorption or metabolism rely on the direct measurement of blood flow rates or assumption of blood flow. Blood flow may be measured by several means. These include: thermodilution (Bensadoun et al., 1962, Webster et al., 1975); radioactive microspheres (Shephard et al., 1984, Dinda et al., 1983); Laser-Doppler, fluorescein or electromagnetic flowmetry (Perbeck et al., 1985a,b; Shepherd et al., 1984); hydrogen gas clearance (Lundgren, 1980); iodoantipyrene clearance (Dugas and Wechsler, 1982); dye dilution (Katz and Bergman, 1969; Huntington, 1982). Dyes such as

Evan's blue, indocyanine green, sulfobromophthalein (BSP) and p-aminohippuric acid (PAH) are used most commonly and their use is dependent upon the system to be investigated.

The most important consideration in blood flow studies is the same as in any marker study, complete mixing (Katz and Bergman, 1969). Blood samples are withdrawn very slowly over several minutes to insure mixing of metabolites and dye (Katz and Bergman, 1969). Another consideration important in blood flow techniques is insuring that the dye is at steady state. This is done via a bolus infusion of dye in the mesenteric vein for 15 minutes in sheep (Katz and Bergman, 1969) and 10 minutes in cattle (Huntington, 1982) followed by continuous infusion of dye for another 20 minutes before sampling (Huntington, 1982).

p-Aminohippuric acid is the dye used most commonly in blood flow studies in cattle and sheep. Data presented by Huntington (1982, 1983) and Huntington et al. (1981, 1983) have examined portal blood flow and net nutrient appearance in the portal vein under various experimental experimental conditions. These studies indicate that dietary differences may affect portal blood flow estimates (Huntington, 1983), net D-lactate absorption estimates (Huntington et al., 1981), ammonia-nitrogen absorption estimates (Huntington, 1982), plasma urea nitrogen absorption estimates and some amino acid absorption rates (Huntington et al., 1981).

In Vitro Measurements of Na^+/K^+-ATPase Activity in Biopsies of Intestinal Mucosa Taken From Duodenally Cannulated Beef Steers and Heifers

Introduction

After examination of the available literature on monensin made of action in ruminant animals, data on monensins fate in the animal and data in which monensin is used as a cellular perturbant, it becomes apparent that there is a possibility, in fact a likelyhood, that monensins action goes beyond those seen in the rumen. Data of Davison (1984, Donoho (1984) and Herberg et al. (1978) indicate that at least 50% of monensin fed is absorbed through the small intestine tract before fecal excretion. This observation coupled with numerous in vitro studies in which monensin stimulates the Na^+/K^+-ATPase pump in eucaryotic cells poses an interesting question. What is the effect of monensin feeding upon the mucosal Na^+/K^+=ATPase in the gastrointestinal tractπ

In order to answer this question two studies were designed and carried out. The first of these involved in vitro incubation of mucosal tissue obtained with a suction biopsy device to examine the Na^+/K^+-ATPase activity with graded quantities of monensin. The second involved feeding monensin to cannulated steers and heifers with biopsies removed to measure Na^+/K^+-ATPase activity in vitro.

Materials and Methods

Biopsy Technique

Biopsies were obtained, from steers and heifers, using a suction biopsy device (Quinton Instrument Co., Seattle, Washington). The biopsy tube was passed through a duodenal cannula and inserted into the descending duodenum. Either one or two biopsies were removed per cut,

washed in warm, air-saturated buffer and transferred to another vial of buffer until oxygen consumption could be measured. Best results were obtained with the biopsy instrument after the duodenal contents had been allowed to drain out of the cannula for about 10 minutes. Infusing air through the instrument with a syringe several times also helped biopsy removal. This seemed to clear the duodenum and permitted the instrument to move against the duodenal wall which allowed the suction to be effective. It might also be noted that there seemed to be a relationship between the size of animal and effectiveness of the instrument. Small animals, 230-400 kg vs 725 kg, enabled more rapid sampling. This might be due to the size of the duodenum with the lighter animals having a smaller duodenal circumference than larger animals.

Up to six individual biopsies per animal were removed at each sampling time. Animals were sampled on two consecutive days and then allowed to recover for at least four days. There was never any evidence that taking biopsies altered the animals behavior, eating patterns or health.

Handling of Biopsies

As stated previously, as soon as the biopsy was removed from the instrument capsule or wire, it was washed in buffer and then 1 transferred to another buffer solution. The buffer used was Krebs-Henseleit buffer (KH) (Table 2) containing 10 mM D-glucose and 20 mM Hepes. This buffer was kept at 37°C and was air saturated (700 mm Hg, 180 nmol O_2/ml; Umbreit et al 1964). Biopsies were kept in this buffer until oxygen consumption measurments could be initiated.

Oxygen Consumption Measurements

Excised biopsies were transferred to oxygen (O_2) electrode chambers containing 4 ml of Krebs-Henseleit buffer (air saturated, 37°C). O_2 consumption was measured polarographically using a YSI (model 53) Biological Oxygen monitor. Initial oxygen consumption was measured for 15 minutes (McBride, 1984). At this time biopsies were transferred to chambers containing KH buffer and $2x10^{-3}$ M ouabain. Further oxygen consumption measurements were made for 40-45 minutes in order to measure ouabain-insensitive respiration. Biopsies were then dried at 90°C for 12 hours to determine dry matter content.

The principles behind the operation of the oxygen monitor is that the oxygen sensor (probe) measures oxygen pressure. The membrane placed on the sensor is oxygen (gas) permeable. Oxygen in solution diffuses through the membrane and is consumed by the sensor; $O_2+2H_2O+4e^-=4OH^-$. The consumption of oxygen generates a current which is proportional to the amount of oxygen to which the sensor is exposed (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio).

Calculations

The difference between oxygen consumption measured initially and that measured after ouabain addition was called ouabain-sensitive respiration. This is the respiration attributable to the Na^+/K^+-ATPase activity in the cell. All respiration values are expressed as nmoles O_2 consumed/mg biopsy DM/min.

Table 2. Modified Krebs-Henseleit Buffer[1]

Chemicals	Parts By Volume
Sodium Chloride (0.154 M)	100
Potassium Chloride (0.154 M)	4
Calcium Chloride (0.11 M)	3
Potassium Phosphate (0.154 M)	1
Magnesium Sulfate (0.154 M)	1
Sodium Bicarbonate[2] (.151 M)	21
D-Glucose 10 mM	
Hepes 20 mM	

[1]Dawson et al, 1969; pH = 7.4.

[2]Gassed with 100% CO_2 for 1 hour before mixing.

Preliminary Experiments

The techniques used to measure oxygen consumption and ouabain-sensitive respiration from duodenal biopsies of sheep and cattle were developed by McBride and Milligan (1984). In order to revalidate some of their results and to insure that the procedures would work in our hands, several preliminary studies were conducted.

Two Angus heifers (225 kg and 250 kg) were fitted with duodenal cannulas. These cannulas were made of silastic tubing (Weber and Rumpler, 1983) and inserted approximately 10 cm from the pylorus. After a week of healing, the heifers were examined endoscopically and then a biopsy was excised using the suction biopsy device available to us courtesy of Dr. Thomas Herdt, M.S.U. Veterinary Clinic. The biopsies excised were of adequate size to measure in the oxygen monitor and were reasonably easy to obtain. At this time we attempted to estimate the degree of damage to the duodenum at the biopsy site but there was too much bleeding to see the site. Several days later the site was unidentifiable and there was no evidence of damage to the duodenum. There appeared to be no evidence of any discomfort to the animal during the procedure and no subsequent alterations in feed intake or behavior.

The heifers were placed on a high moisture corn:ground hay diet (Table 3) and were accustomed to the metabolism room. Biopsies were removed, and a dose response curve was constructed. Maximal inhibition of respiration was achieved at $1x10^{-3}M$ ouabain. This is similar to McBride's data for cow duodenal biopsies, although in his data, maximal inhibition was achieved at $1x10^{-5}M$ as well as $1x10^{-3}M$ ouabain. This data are in agreement with those of Liberman et al. (1979) for rat

jejunal mucosa. All further studies used concentrations of $2x10^{-3}M$ ouabain to inhibit respiration. It should be noted that actual inhibition of Na^+/K^+-ATPase-dependent respiration was not directly measured so these data represent lesser estimates than those that would have been observed to all enzyme units were exposed to ouabain.

The length of time that the biopsies remained viable (i.e., steady oxygen consumption traces) was also examined. It was found that the biopsies remained viable for approximately 70 minutes in ouabain-free media. This is in agreement with McBride and Milligan (1984) who found duodenal biopsies from cows viable for 70-80 minutes in ouabain-free media. Therefore, when a biopsy was removed, the time at which it was placed in buffer was recorded and any biopsy older than 20 minutes was not used. This occasionally occurred in transit from the Beef Cattle Research Center to the laboratory (especially if there was a train), or in situations when there was difficulty in obtaining another biopsy.

Study 1: In Vitro Addition of Monensin to Duodenal Mucosal Biopsies

This study was designed to answer the question, does addition of monensin to duodenal biopsies taken from beef cattle affect ouabain-sensitive respiration (Na^+/K^+-ATPase). The hypothesis was that measured respiration rates with monensin additions of 1, 5 and 10 ppm would not differ from biopsies with no added monensin.

Animals

Two sets of beef cattle were used in a study designed to investigate the effect of monensin addition in vitro. One set was two Angus heifers (225 kg and 250 kg) and the other was two Simmental crossbred steers (300 kg and 318 kg). All animals were fitted with duodenal cannulas inserted approximately 10 cm from the pylorus. These cannulas were made of silastic tubing (Weber, 1983) and were exteriorized just distal to the last rib in both heifers and 1 steer and between the 12th and 13th rib in the other steer. All animals had been cannulated at least 2 months prior to the beginning of this study and were well adjusted to their diet. Animals were housed at the Beef Cattle Research Center in the metabolism room and had ad libitum access to feed and water. Feeding was done twice daily at 8:00 AM and 6:00 PM. Lighting was continuous and daily exercise in a holding pen was permitted.

Diets

The diet fed to the heifers is presented in Table 3. The diet fed to the steers is shown in Table 4.

Sampling

Biopsies were obtained as previously described. Each animal was sampled twice for each concentration of monensin examined.

Media

Krebs-Henseleit buffer (Table 2) as described previously was used. Monensin concentrations examined were 0, 1, 5 and 10 ppm which were equivalent to 0, 1.49 µM, 7.46 µM and 14.9 µM. All biopsies were preincubated 10 minutes in buffer containing appropriate monensin concentrations before oxygen consumption measurements were taken. Monensin was provided courtesy of Dr W.G. Bergen.

Analysis

Data were analyzed using a one way analysis of variance. Contrasts were made using a T test. The contrasts examined were: Control versus monensin supplementation, 1 ppm monensin versus 5 and 10 ppm monensin and 5 ppm versus 10 ppm monensin.

Table 3. Composition of Heifer Rations

Feed	kg DM/day
High Moisture Ground Ear Corn	4.44
Ground Hay	1.60
Supplement[1]	.65
	6.69

[1]Supplement contained soybean meal (94.6%), trace mineral salt (2%), limestone (1%), selenium 90 and Vitamins A, D and E.

Table 4. Composition of Steer Rations

Ingredient	kg DM/day
High Moisture Ground Ear Corn	6.24
Ground Hay	2.11
Supplement[1]	.68
	9.0

[1]Supplement contained soybean meal (94.6%), trace mineral salt (2%), limestone (1%), selenium 90, Vitamins A, D and E.

Study 2. The Effect of Monensin Supplementation On Duodenal Mucosal Na^+/K^+-ATPase Activity

The purpose of this study was to determine whether supplementation of monensin (Rumensin™) to beef cattle diets altered Na^+/K^+-ATPase activity in the duodenal mucosa of heifers and steers. The hypothesis was that monensin addition (300 mg/hd/day) had no effect on Na^+/K^+-ATPase activity as measured by in vitro oxygen consumption.

Animals

The animals used were two Angus heifers (225 kg and 250 kg) and two Simmental crossbred steers (300 kg and 318 kg). All animals had been previously fitted with duodenal cannulas approximately 10 cm from the pylorus. Cannulas were made from silastic tubing according to Weber (1983). The cattle were housed in the metabolism room at the Beef Cattle Research Center. Water was available ad libitum; and feed was provided twice daily. Heifers and steers were allowed daily exercise and lighting was continuous.

Design

The design of this study was a switchback with each animal serving as its own control. Treatments were no added monensin or monensin (Rumensin™) fed at 300 mg/hd/day. The trial was run in two replicates. The first was with the heifers and the second followed approximately 6 months later with the steers. Each animal was sampled twice (8 total biopsies) per treatment. The cattle received Rumensin™ at full dosage for at least 10 days prior to sampling and were removed from Rumensin™ at least 15 days prior to sampling for control observations.

Diets

Diets are given in Tables 3 and 4. The only difference was the added Rumensin™ to the supplement.

Sampling

All sampling was done 4 hours post-feeding and samples were handled as discussed previously.

Analysis

Data were analyzed using a paired T-test. Observations from the no-monensin treatment was compared to monensin-supplemented observations and mean differences were tested. There were no significant differences due to sex so all observations were pooled.

Results

Study 1 In Vitro Addition of Monensin to Duodenal Mucosal Biopsies

Monensin addition to incubated biopsies resulted in increased total oxygen consumption and increased ouabain-sensitive respiration (Na^+/K^+-ATPase). There was no effect on Na^+/K^+-ATPase-independent respiration. Means and significance levels are presented in Table 5.

Total O_2 consumption of the duodenal mucosal biopsies without monensin supplementation (control) was 5.59 nmol O_2/mg DM/min. O_2 consumption increased with increasing monensin concentration. Control O_2 consumption rates differed significantly from all monensin concentrations examined ($P<.01$). One ppm monensin increased O_2 consumption to 6.40 nmol O_2/mg DM/min, 5 ppm monensin increased consumption to 6.79 nmol O_2/mg DM/min, and 10 ppm monensin increased consumption to 8.18 nmol O_2/mg DM/min. When 1 ppm monensin was compared to 5 and 10 ppm monensin, O_2 consumption was found to be significantly different ($P<.05$). The response in total O_2 consumption determined at 5 ppm also differed significantly from that seen at 10 ppm monensin.

Ouabain-sensitive, Na^+/K^+-ATPase-dependent respiration rates increased significantly ($P<.01$) with increasing concentrations of monensin in incubation media. Control O_2 consumption rates were found to be 2.76 nmol O_2/mg DM/min. This rate increased to 3.77 nmol O_2/mg DM/min with addition of 1 ppm monensin, 4.15 nmol O_2/mg DM/min with 5 ppm monensin and 4.9 nmol O_2/mg DM/min with 10 ppm monensin. One ppm monensin differed significantly from 5 and 10 ppm

Table 5. Mean mucosal O_2 consumption, Na^+/K^+ATPase dependent and independent respiration of duodenal mucosa with different concentration of monensin added to the media (Means plus standard errors)[1,2]

			Na^+/K^+-ATPase			
	Total O_2 consumption (nmol O_2/mg/min)		-dependent respiration (nmol O_2/mg/min)		-independent respiration (nmol O_2/mg/min)	
Treatment	Mean	SE	Mean	SE	Mean	SE
Control	5.59[a]	0.29	2.76[a]	0.17	2.83	0.23
Monensin (ppm)						
1	6.40[bc]	0.29	3.77[bc]	0.17	2.64	0.23
5	6.79[bd]	0.29	4.15[bfc]	0.17	2.87	0.23
10	8.18[bdef]	0.29	4.91[bfd]	0.17	3.36	0.23

[1]All respiration results are expressed on a mg dry weight basis.

[2]Means represent 64 biopsies.

[a,b]Means within a column different P<.01.

[c,d]Means within a column different P<.05.

[e,f]Means within a column different P<.10.

monensin ($P<.05$) and 5 ppm was significantly lower than 10 ppm ($P<.10$).

Monensin addition did not affect Na^+/K^+-ATPase-independent respiration regardless of concentration. Control respiration rates were 2.83 nmol O_2/mg DM/min, while respiration rates from monensin treated biopsies ranged from 2.64 nmol O_2/mg DM/min to 3.36 nmol O_2/mg DM/min.

Study 2: The Effect of Monensin Supplementation On Duodenal Mucosal Na^+/K^+-ATPase Activity

Monensin addition to the diets of beef cattle significantly increased total O_2 consumption ($P<.01$) and Na^+/K^+-ATPase-dependent respiration ($P<.01$). There was no effect on Na^+/K^+-ATPase-independent respiration although there was a numerical increase (2.68 nmol O_2/mg DM/min versus 2.85 nmol O_2/mg DM/min). Means are presented in Table 6.

Control total O_2 consumption was 5.85 nmol O_2/mg DM/min and monensin supplementation increased this to 7.03 nmol O_2/mg DM/min. Ouabain-sensitive respiration increased from 3.14 nmol O_2/mg DM/min to 4.15 nmol O_2/mg DM/min, an increase of 32%. The percent inhibition by ouabain increased from 53.6% to 58%.

There was no significant differences between heifers and steers in either their control O_2 consumption rates or monensin supplemented rates.

Table 6. Mean mucosal O_2 consumption, Na^+/K^+ATPase dependent and independent respiration of duodenal biopsies taken from control and monensin supplemented beef cattle[1,2] (Means plus standard errors)

	Total O_2 consumption (nmol O_2/mg/min)		Na^+/K^+-ATPase -dependent respiration (nmol O_2/mg/min)		Na^+/K^+-ATPase -independent respiration (nmol O_2/mg/min)	
Treatment	Mean	SE	Mean	SE	Mean	SE
Control	5.85[a]	0.32	3.14[a]	0.27	2.68	0.21
Monensin Supplemented	7.03[b]	0.32	4.15[b]	0.27	2.85	0.21

[1]All respiration results are expressed on a mg dry weight basis.

[2]Means represent 64 biopsies.

[a,b]Means within a column with different superscripts are different $P<.01$.

Discussion

Total oxygen consumption measurements for the control treatment in both studies (5.59 and 5.85 nmol O_2/mg/min) are higher than those reported by McBride (1984) for lactating and dry, pregnant dairy cattle. His values ranged from 3.89 $\pm$ .41 nmol O_2/mg DM/min for cows in week 5 of lactation to a high of 4.92 $\pm$ .17 nmol O_2/mg DM/min at week 22-25. They are also higher than the 4.65 nmol O_2/mg DM/min reported by Webster and White (1973) for the entire gastrointestinal tract of the sheep. However, these data are in line with that reported by McBride (1984) for sheep feed different levels of digestible energy. In this study his mucosal O_2 consumption (nmol O_2/mg DM/min) values ranged from 5.21 for starved sheep to 6.07 for sheep fed a higher digestible energy diet. Estimates of ouabain-sensitive respiration (Na^+/K^+-ATPase-dependent respiration) and ouabain-insensitive respiration (Na^+/K^+-ATPase independent) also agree with McBride (1984). This is likely to be related to energy intake. Like the sheep in his energy intake study, the cattle in this study were fed a high energy diet.

When monensin was added to the media incubating biopsies the rate of oxygen consumption, both total and Na^+/K^+-ATPase-dependent respiration increased significantly. Total mucosal oxygen consumption increased linearly (R^2=.94) with increasing monensin concentration and Na^+/K^+-ATPase-dependent respiration increased linearly (R^2=.85). The increase in total O_2 consumption is due primarily to a stimulation of the Na^+/K^+-ATPase, since Na^+/K^+-ATPase-independent respiration rates did not change (although at 10 ppm

monensin, 3.36 nmol O_2/mg DM/min was consumed which is a 15% increase over that found in control incubations).

The significant increase in Na^+/K^+-ATPase-dependent respiration is likely to be due to the action of monensin on the flux of sodium ions into the enterocyte. Monensin has been shown to increase sodium concentrations intracellularly in 3T3 cells (Smith and Rozengurt, 1978; Austic and Smith, 1980). Both studies with 3T3 cells used concentrations of monensin similar to those used in this study. One, 5 and 10 ppm (1, 5 and 10 µg/ml) were used in these studies and they used 1-20 µg/ml monensin.

The magnitude of the increase in pump activity seen with monensin is different between studies. In the 3T3 cells approximately 3 µg/ml monensin provided maximal stimulation. This stimulation was a 6 fold increase in activity. The present data indicate a 1.7 fold increase in Na^+/K^+-ATPase-dependent respiration from 0 to 10 µg/ml. This is probably due to the difference in tissue type but may also reflect a difference in the amount of monensin reaching the cell. Biopsies would not be as likely to have a uniform distribution of cells like a 3T3 cell culture experiment.

Addition of monensin to steers and heifers on feedlot diets significantly increased total mucosal O_2 consumption ($P<.01$) and Na^+/K^+-ATPase-dependent respiration rates ($P<.01$). Total mucosal O_2 consumption increased from 5.85 nmol O_2/mg DM/min in duodenal biopsies from steers not fed monensin to 7.03 nmol O_2/mg DM/min in duodenal biopsies from steers supplemented with monensin. Again the increase in total mucosal O_2 consumption is likely to be due to the

significant increase in Na^+/K^+-ATPase-dependent respiration. The activity of this pump, as measured by oxygen consumption, increased 1.32 fold from 3.14 nmol O_2/mg DM/min to 4.15 nmol O_2/mg DM/min.

The proportion of respiration that was inhibited by ouabain increased from 53.6% to 59% reflecting an increase in Na^+/K^+-ATPase activity. This is also in keeping with data of other workers who indicate that monensin stimulates sodium uptake into the cytosol of the cell (Smith and Rosengurt, 1978, Austic and Smith, 1980). This stimulation is independent of membrane potential and doesn't interfere with the electrochemical gradient (Smith and Austic, 1980; Bihler et al, 1985). The increased intracellular sodium stimulates the Na^+/K^+-ATPase pump. Bihler et al. (1985) also noted that monensin decreased intracellular ATP concentrations in avian erythrocytes.

The amount of O_2 utilized/mg of dry intestinal tissue was 7.03 nmol O_2/mg/min. This value is a 10% increase over that seen by McBride (1984) with sheep fed high energy diets and is within the range (6.55-7.18 nmol O_2/mg/min) reported by Levin and Syme (1975) and Liberman et al. (1979). To what degree a 20% increase in O_2 utilization by the gastrointestinal tract would effect an animal's maintenance requirements would depend upon the degree to which other monensin effects (e.g. increased propionate production) contribute to that requirement.

The increase in Na^+/K^+-ATPase activity may also have other implications in the gastrointestinal tract. The Na^+/K^+-ATPase pump has been shown to be associated with coupled transport of glucose, amino acids, sodium and chloride. An alteration in Na^+/K^+-ATPase activity by 32% might significantly affect transport of these nutrients.

In Vivo: Net Nutrient Absorption by the Gastrointestinal Tract of Growing Beef Steers

Introduction

The previously reported in vitro studies indicated a 20% increase in total mucosal oxygen consumption with monensin feeding and a 32% increase in the activity of the Na^+/K^+-ATPase pump in mucosal biopsies. Since the Na^+/K^+-ATPase pump was stimulated in beef steers and heifers fed monensin as Rumensin^{TM}, and since the Na^+/K^+-ATPase pump is an important constituent of glucose (Crane, 1961), amino acid (Crane, 1977), and mineral (Bihler and Cybulsky, 1973) absorption in the small intestine a study was conducted to evaluate whether monensin supplementation altered net absorption of nutrients across the intestinal tract.

The hypothesis of this study was that monensin had no effect upon glucose, alpha amino nitrogen, lactate and mineral absorption across the intestinal tract as measured by appearance in the portal vein.

Materials and Methods

Animals

Four steers averaging approximately 363 kg in weight were used in a switchback design to evaluate the effect of monensin feeding upon net nutrient absorption from the gastrointestinal tract. All of the steers were Simmental crossbreeds. The steers were housed in the metabolism room in individual pens measuring 1.8 x 2.4 m. They had free access to water and there was no bedding as the pens have slotted floors. Steers were either haltered or held in stanchions when cannulated to prevent licking at catheters.

Diet

Diet composition is given in Table 7. On a dry matter basis it

Table 7. Diet Composition for Net Absorption Studies

Ingredient	% DM
High Moisture Ground Ear Corn	75
Ground Hay	21
Supplement[1]	4

[1]Supplement contained soybean meal (95%), trace mineral salt (2%), limestone (1%), selenium 90 (2.4%) and Vitamins A, D and E. Rumensin™ was added at 200 mg/lb.

consisted of 75% high moisture whole shelled corn, 21% ground hay and 4% supplement. Monensin was added as Rumensin™ in the supplement at a level of 300 mg/head/day when the animals received the monensin treatment. The daily ration was fed 12 times/day via automatic feeders positioned over the pens. The diet was formulated to meet NRC (1984) requirements for steers gaining 0.68 kg/day.

Catheterization

Catheters were placed in the portal vein, mesenteric vein (2), [two catheters were placed in mesenteric veins to allow infusion of dye in case one catheter closed] and iliac artery using methods developed by McGillard and Thorp (1971) and Huntington (1982). Portal vein catheters were made of teflon (Essex Group, Magnet Wire and Insulation Division, Ft. Wayne, Indiana) and were encased in silastic tubing. Mesenteric vein and iliac artery catheters were made of tygon tubing and were treated repeatedly with TDMAC-heparin (Polysciences Inc., Warrington, PA), to help prevent clotting, before sterilization. All catheters were washed and gas sterilized prior to surgery. Exteriorization of portal and mesenteric catheters was done along the spine. Iliac artery catheters were exteriorized above the incision site. Catheters were then placed in patches which were cemented to the hair with tag cement. All catheters were filled with physiological sterile saline containing 200 units of heparin/ml and 1% penicillin capped and left alone until collection or until an animal had disturbed them.

Protocol

Prior to surgery each steer was adjusted to the experimental diet

and the metabolism room for several days. After surgery animals were returned to the metabolism room and brought back up on full feed gradually. At this time the animals which were to receive monensin were supplemented with Rumensin™ and adjusted to 300 mg/hd/day. Animals were treated with antibiotics for the first 7 days postsurgery. Generally it was 10-15 days before steers were on full feed. At this time a blood flow measurement was made to validate correct placement of the portal catheter. This resulted in 3 steers being removed from the study. Two of the three had nonphysiological blood flows (2940 L/hr, 1511.25 L/hr), i.e., too rapid, suggesting incorrect placement of the portal catheter. The other had extremely low blood flows (189.7 L/hr) again suggesting improper catheter placement. These steers were replaced with newly cannulated steers whose blood flows were physiological.

After the steers had been on full feed for at least 5 consecutive days blood collections were initiated.

Blood Flow Measurements

Blood flow through the portal vein was measured by dye dilution techniques using p-aminohippuric acid (PAH) originally used by Roe et al (1966) and modified by Katz and Bergman (1969) and Huntington (1982). Using a Harvard infusion pump, a bolus of PAH was infused for 15 minutes. This bolus was a solution of PAH containing 132.6 mg/ml infused at a rate of 1.5 ml/min. After the 15 minutes of infusion the pump was turned down to deliver at a rate of .754 ml/min. This is equivalent to an infusion rate of 6 gm PAH/hr. Blood samples from the iliac artery and portal vein catheters were withdrawn simultaneously

over a 2 minute period. The slow withdrawal period was to help insure adequate mixing of dye. Each animal was sampled in 4 hour blocks for a 24 hour period of time. This was done over several days. There was a span of at least 8 hours between infusions.

Sample handling

Blood samples were collected in Sarstedt tubes containing lithium heparin as an anticoagulant and were used for determination of plasma minerals, total lipid, α-amino nitrogen and blood urea nitrogen. Blood samples that were to be analyzed for glucose were collected in tubes containing sodium fluoride. Blood samples collected in both types of tubes were placed on ice immediately and centrifuged as soon as possible (about 30 min.). An initial 0.5 ml volume of whole blood was placed in 5.5 ml ice cold 10% trichloroacetic acid (TCA), vortexed, centrifuged and placed in a cooler (4°C) for subsequent analysis for PAH. A blood sample was also taken for packed cell volume analysis for each infusion period. Twenty µl of whole blood was added to a hemolysis tube with .99 ml of a sodium azide solution (1 gm/L) and frozen immediately for later lactate analysis. Blood samples for glucose and lactate analysis were taken every hour and for PAH every half hour. After centrifuging, plasma samples were frozen for later analyses.

Assays

L^+-lactate

L^+-lactate was analyzed using a Kontron 640 Lactate Analyzer (courtesy Dr. Wayne Van Huss of the Human Performance Lab). One hundred µl of hemolyzed whole blood in sodium azide was injected into

the analyzer and mmol/L lactate was read off the screen. The measurement of lactate is achieved by monitoring the concentration of hexacyanoferrate II formed during the irreversible oxidation of lactate to pyruvate in the presence of cytochrome b_2. For each lactate molecule oxidized, two electrons are collected on the platinum electrode creating a measurable current. The reactions are:

i. Lactate + $2[Fe(CN)6]^{-3} \xrightarrow{\text{cyt b2}}$ pyruvate + $2\ [Fe(CN)6]^{-4}$

ii. $2[Fe(CN)_6]^{-4} \xrightarrow{Pt} 2\ [Fe(CN)^6]^{-3} + 2e^-$

This procedure is specific for L^+-lactate.

Glucose

Plasma glucose concentrations were measured using the glucose (Trinder) kit (Sigma Diagnostics #315). The reactions involved are the oxidation of glucose to gluconic acid and hydrogen peroxide and the subsequent reaction of the hydrogen peroxide with 4-amino antipyrene and p-hydroxybenzone sulfonate. This forms a quinoneimine dye which is read spectrophotometrically at 505 nm. Absorbance readings are then regressed along a standard curve of glucose concentrations for quantitation.

Blood Urea Nitrogen

Blood urea nitrogen (BUN) concentration was measured using a Sigma Diagnostics kit (535). In this procedure urea reacts with diacetyl monoxime to form a pink chromogen. The concentration of this color production is read spectrophotometrically at 535 nm and BUN concentration is determined from a urea nitrogen standard curve.

Alpha amino nitrogen

Alpha amino nitrogen (AAN) was analyzed by using an adaptation of a procedure by Palmer and Peters (1967). Samples were deproteinized with 10% trichloroacetic acid and 0.2 ml of filtrate used for analysis. To the protein free filtrate 1.6 ml of .05M sodium borate and 0.2 ml of 0.25% trinitrobenzene sulfonic acid (TNBS) was added. The mixture was incubated at 37°C for 20 minutes. 2 ml of 1N HCl were added and the tube vortexed. The sample was read on a spectrophotometer at 420 nm. Citrulline was used to make a standard curve which was then used for quantitation of AAN.

The basis for this procedure is the production of a TNBS-amino acid complex at pH 9.2. This complex is unstable and upon acidification (1N HCl) forms a stable yellow trinitrophenol-amino acid derivative.

Total Lipid

Total lipid was measured by using an adaptation of Hara and Radins (1978) procedure. Two ml of plasma were added to a 20 x 150 mm screw cap tube with a teflon lined cap. Nine ml of a 3:2 hexane:isopropanol mixture were added and the tube vortexed 30 seconds. The tubes were then allowed to separate and 7 ml of a 6.7% $NaSO_4$ solution was added. The tubes were vortexed 30 seconds again and allowed to separate. The supernatant was pipetted to a 20 ml test tube and the meniscus washed with 4 ml of a 7:2 mixture of hexane:isopropanol. The solvent was then evaporated in a heated sand bath under nitrogen until 1-2 ml remained. This was transferred to a weighed 12 x 75 mm test tube and the 20 ml tube was washed with 2 ml of a 7:2 hexane:isopropanol mixture. The

solvent was then evaporated completely from the 12 x 75 mm test tube in a heated sandbath under nitrogen. The tube was then reweighed and total lipid was calculated from the difference in weights.

p-Aminohippuric acid

p-Aminohippuric acid concentrations in the whole blood of steers were analyzed using the procedure outlined by Katz and Bergman (1969) and Huntington (1982). A protein free supernatant was prepared immediately after blood collection (see Blood Flow Measurements). One ml of this supernatant was added to a disposable 13 x 100 mm Kimax tube. To this tube 0.2 ml of 1.2 N HCl, 0.1 ml of 100 mg/dl $NaNO_2$, 0.1 ml of 500 mg % ammonium sulfamate, 0.1 ml of 100 mg/dl (N-(1-napthyl) ethylenediamine dihydrochloride were added. After 10-15 minutes the absorbance was read at 540 nm on using a spectrophotometer. Standard curves were made using the infusate and were used for quantitation of PAH concentrations.

Mineral Analysis

Chloride

Plasma chloride was analyzed using a Digital Chloridometer (Buchler model 4-2500). The principle of analysis is the generation of silver ions at a silver anode. These ions are complexed with chloride ions present in the sample and are precipitated. When all available chloride in the sample has reacted, the free silver ion generates an increase in current which is sensed at the indicator electrodes. At this point the action stops and a reading in mEq/L is generated.

Phosphorus

Phosphorus was analyzed using the Gomori modification of the Fiske-SubbaRow (1946) inorganic phosphate determination.

Selenium

The Whetter and Ullrey (1978) variation of the AOAC procedure for the spectrofluorometric determination of selenium was used to determine plasma selenium concentrations. The principle is the formation of a selenium-diamino-napthalene complex that is light sensitive, soluble in cyclohexane and fluorescent. The fluorecence is regressed against a standard curve for quantitation of plasma selenium.

Magnesium, Calcium, Copper and Zinc

Plasma magnesium, calcium, copper and zinc concentrations were determined using a model IL 951 (Wilmington, MA) atomic absorption (AA) spectrophotometer.

Plasma samples were deproteinized in 12.5% trichloroacetic acid and then diluted to appropriate concentrations and read. Wavelengths (nm) used were magnesium, 285.2; calcium, 427.7; copper, 342.7; and zinc, 313.9. The AA regresses readings against a standard curve and gives actual concentration readings.

Potassium and Sodium

Potassium and sodium concentrations in plasma was determined using a Model IL 951 atomic emission spectrophotometer (Wilmington, MA). This procedure differs from atomic absorption in that only the flame and no lamps are used. Concentrations are calculated from a standard curve. Wave lengths (nm) used were: sodium, 589 and potassium, 766.5

Statistical Analysis

The original design was a switchback with each animal serving as its own control. Because of problems with intake, heat, possible

infections and eventual catheter malfunction. The original design of this study could not be carried out. Measurements with and without monensin supplementation were to have been made for each animal, however. The eventual data set included 2 steers on each treatment. This became a problem to analyze and detect differences. Ultimately I settled for little power and analyzed the study using a split-plot analysis with one degree of freedom for treatment. In the subplot, time within treatment was never significantly different so it was dropped and its degrees of freedom added to residual. Time was also not different. This is not surprising because everything was done to minimize time effects. Feeding 12 times a day reduces some of the variation, if not all, in digesta flow to the small intestine (Weber and Rumpler, personal communication). Sampling every half hour also minimized any time effects in blood flow.

The general linear models (GLM) procedure of the SAS (SAS Institute, N.C.) computer package was used to generate least squares means and test for treatment differences.

Calculations

Blood flow was calculated using the following equation developed by Katz and Bergman (1969):

$$F_{PV} = \frac{I}{CPV-CA}$$

Where: I = infusion rate of PAH (mg/min)

CPV = concentration of PAH in the portal blood (mg/ml)

CA = concentration of PAH in arterial blood (mg/ml)

F_{PV} = flow through the portal vein (ml/min or L/hr)

Net absorption was calculated using the equations of Bergman et al (1974).

Net portal absorption (NPA) = $F_p(CP-CA)$

Where: F_p = portal plasma flow (L/hr)

CP = metabolite concentration in portal blood (units/ml)

CA = metabolite concentration in arterial blood (units/ml).

Results

In this study, there was no effect of monensin on dry matter intake. Control steers consumed approximately 7.86 kg of DM/day while monensin fed steers consumed 7.89 kg DM/day. There was also no effect of monensin on animal performance. All steers averaged 0.70 kg gain/head/day while cannulated.

Arterial and Portal Blood Concentration Differences

Minerals

Mean arterial and portal blood concentrations of calcium and phosphorus are given in Table 8. Monensin supplementation significantly increased arterial calcium concentrations ($P<.08$) from 10.48 mg/dl in control animals to 11.28 mg/dl in monensin supplemented steers. Portal blood calcium concentrations were numerically greater for monensin-fed steers (11.35 mg/dl) than for control animals (10.85 mg/dl) but this was not significantly different ($P>.10$). Arterial blood phosphorus concentrations tended to be lower for cattle fed monensin (6.37 mg/dl) than for steers fed the control diet (7.43 mg/dl). This was not found to be significantly different ($P>.10$).

Portal phosphorus concentrations were significantly different, however ($P<.01$). Control portal phosphorus concentrations (7.72 mg/dl) were higher than those determined in monensin fed cattle (6.70 mg/dl).

There were no significant differences ($P>.10$) in mean arterial or mean portal concentrations of magnesium, copper, zinc or selenium (Table 9). Copper and zinc concentrations did tend to be higher for monensin supplemented steers but the large amount of variation,

Table 8. Mean Arterial and Portal Blood Concentrations of Calcium and Phosphorus from Control and Monensin Supplemented Steers[1]

	Control		Monensin	
	A	P	A	P
Ca (mg/dl)	10.48[2]	10.85	11.28[2]	11.35
	±.30	±.34	±.30	±.34
P (mg/dl)	7.43	7.72[3]	6.37	6.70[3]
	±.39	±.129	±.39	±.129

[1]Means are 24 samples analyzed in duplicate.

[2]Means with the same superscript differ P<.08.

[3]Means with the same superscript differ P<.01.

Table 9. Mean Arterial and Portal Blood Concentrations of Magnesium, Copper, Zinc and Selenium from Control and Monensin Supplemented Steers[1]

	Control		Monensin	
	A	P	A	P
Mg (mg/dl)	2.41	2.34	2.32	2.78
	±.22	±.21	±.22	±.21
Cu (µg/dl)	121.26	123.87	145.45	140.83
	±27.08	±23.31	±27.08	±23.31
Zn (µg/ml)	121.74	113.17	138.47	125.72
	±10.59	±41.82	±10.59	±41.82
Se (µg/ml)	.091	.091	.086	.089
	±.007	±.006	±.007	±.006

[1]Means represent 24 observations analyzed in duplicate.

approximately 18% for copper and 8% for zinc, prevented the detection of any possible differences.

Arterial concentrations of sodium, potassium and chloride showed no significant differences (P>.10) when comparing control and monensin supplemented animals (Table 10). Sodium concentrations tended to increase when monensin was supplemented (218.41 mg/dl vs. 223.95 mg/dl). Potassium concentrations also tended to increase with monensin supplementation. Portal blood concentrations of both sodium and potassium were significantly increased (P<.10) in monensin fed animals. Mean sodium concentrations in portal blood for control animals were 220.46 mg/dl and 232.79 mg/dl in the supplemented steers. Potassium concentrations increased from 17.36 mg/dl to 18.49 mg/dl with monensin supplementation. Portal chloride concentrations were not different. It should be noted that all of the sodium values appear to be slightly lower than reported normal values. Normal sodium levels in bovine whole blood range from 260-280 mg/dl which is equivalent to 113-121 mEq/L.

Lactate, Glucose and Total Lipid

L-lactate, glucose and total lipid concentrations in the arterial and portal blood were not found to be significantly different between the control and monensin treated animals. Lactate concentrations in both arterial and portal blood tended to be lower in monensin supplemented steers than control steers (Table 11). In contrast, glucose concentrations showed the opposite trend. Both arterial and portal glucose concentrations appeared to increase in monensin fed steers.

Table 10. Mean Arterial and Portal Blood Concentration of Sodium, Potassium and Chloride from Control and Monensin-Supplemented Steers[1,2,3] (mg/dl)

	Control				Monensin			
	A		P		A		P	
Na	218.41	(95.0)	220.46[2]	(95.9)	223.95	(97.42)	232.79[2]	(101.26)
	±13.06	(5.68)	±4.24	(1.84)	±13.06	(5.68)	±4.24	(1.84)
K	16.98	(4.35)	17.36[2]	(4.44)	17.43	(4.46)	18.49[2]	(4.73)
	±1.32	(.34)	±1.02	(.26)	±1.32	(.34)	±1.02	(.26)
Cl	339.32	(96.95)	330.47	(96.42)	338.77	(96.79)	328.65	(93.90)
	±2.48	(.71)	±2.56	(.73)	±2.48	(.71)	±2.56	(.73)

[1]Means represent 24 observations.

[2]Means in a row with similar superscripts differ P<.10.

[3]Values in parentheses are mEq/L.

Table 11. Mean Arterial and Portal Blood Concentrations of L-Lactate, Glucose, and Total Lipids in Control and Monensin Supplemented Steers

	Control		Monensin	
	A	P	A	P
L-Lactate (mM)[1]	.936	.961	.906	.917
	±.11	±.15	±.11	±.15
Glucose (mM)[1]	4.02	3.98	4.34	4.28
	±.14	±.24	±.14	±.24
Total Lipid (mg/dl)[2]	233.61	241.67	197.0	212.43
	±34.4	±30.81	±34.4	±30.81

[1]Means represent 48 observations analyzed in duplicate.

[2]Means represent 24 observations analyzed in duplicate.

Alpha Amino Nitrogen and Blood Urea Nitrogen

Supplementation of monensin did not significantly alter α-amino nitrogen and blood urea nitrogen concentrations in either arterial or portal blood. In both arterial and portal blood, α-amino nitrogen concentrations tended to increase in steers supplemented with monensin while blood urea nitrogen concentrations appeared to decline when monensin was fed (Table 12).

Blood Flow Estimates

Estimates of portal blood flow rates were not significantly different with addition of monensin (Table 13). Control steers averaged 623.93 L/hr while those steers supplemented with monensin had blood flow rates of 607.18 L/hr. The standard error of the differences for these estimates is 12-13% of flow values which is a reasonably low error percentage for studies of this type.

Portal-Arterial and Net Absorption Differences

Portal-arterial differences can be obtained by two methods. One method, and the way that most workers use, is to take the mean arterial concentration and subtract it from the mean portal concentration of the metabolite of interest. Using this method a representative difference for a time period is calculated. This number is then multiplied by a mean blood flow value and net absorption rates may be calculated. Other workers in the field use a mean P-A difference and a mean net absorption value calculated from a concentration difference and its blood flow rate. By this method statistics may be run and comparisons can be made between treatments. Using either method the values obtained are usually similar although not identical. In this study mean P-A differences were generated and examined statistically.

Table 12. Mean Arterial and Portal Blood Concentrations of Alpha Amino Nitrogen (AAN) and Blood Urea Nitrogen (BUN) from Control and Monensin Supplemented Steers[1]

	Control		Monensin	
	A	P	A	P
A-AN (mM)	.783	1.083	.803	1.175
	±.015	±.02	±.015	±.02
BUN (mM)	3.30	3.29	3.14	2.92
	±.04	±.01	±.04	±.01

[1]Means represent 48 observations in duplicate.

Table 13. Blood Flow Estimates (L/hr) from Control and Monensin Supplemented Steers[1]

Control	Monensin	SED
623.93	607.18	78.70

[1]Means represent 96 observations.

Net absorption rates were calculated using a mean blood flow estimate multiplied by a mean P-A difference. This means that a true standard error of the net absorption estimate cannot be calculated.

Portal-arterial calcium concentration differences were significantly different with monensin supplementation (Table 14). The difference between portal and arterial concentrations was smaller with monensin reflecting the increased arterial calcium concentrations reported earlier (Table 8). When putting the differences on a blood flow basis, net absorption rates were 2.3 g calcium/hr for control and .425 g calcium/hr for monensin supplemented cattle.

There were no differences ($P>.10$) in P-A differences for phosphorus despite the significant decrease in portal phosphorus concentrations (Table 8). Net absorption rates were 1.81 g/hr for control steers and 2.00 mg/hr for monensin supplemented steers.

Table 15 shows P-A differences and net absorption rates for magnesium, copper, zinc and selenium. Magnesium P-A differences were significantly different ($P<.02$). This indicates net absorption of magnesium through the gastrointestinal tract into the portal blood with monensin treatment. Net absorption rates were negative for control steers indicating no net output of magnesium into the portal blood. Rates were -.392 g/hr for control and .243 g/hr for monensin supplemented animals. Copper P-A differences were not different ($P>.10$) nor were zinc and selenium P-A differences found to be different ($P>.10$).

Portal-arterial differences were not different ($P>.10$) between treatments for sodium, potassium or chloride (Table 16). Net absorption rates are also given. There was a tendency to show

Table 14. Mean Portal-Arterial (P-A) Differences and Net Absorption Rates (NA) in Control and Monensin Supplemented Steers: Calcium and Phosphorus

	Control	Monensin	SED
Calcium			
P-A (mg/dl)	.37[1]	.07[1]	.171
NA (g/hr)	2.3	.425	
Phosphorus			
P-A (mg/dl)	.29	.33	1.04
NA (g/hr)	1.81	2.00	

[1]Means with the same superscript are different P<.08.

Table 15. Mean Portal-Arterial (P-A) Differences and Net Absorption Rates (NA) in Control and Monensin Supplemented Steers: Magnesium, Copper Zinc and Selenium

	Control	Monensin	SED
Magnesium			
P-A (mg/dl)	-.063[1]	.04[1]	.0237
NA (g/hr)	-.392	.243	
Copper			
P-A (µg/dl)	2.61	-4.62	3.83
NA (mg/hr)	16.28	-28.05	
Zinc			
P-A (µg/dl)	-8.58	35.18	34.94
NA (mg/hr)	-53.53	213.61	
Selenium			
P-A (µg/dl)	-.001	.003	.0013
NA (µg/hr)	-6.23	18.21	

[1]Means with the same superscript are significantly different P<.02.

Table 16. Mean Portal - Arterial (PA) Differences and Net Absorption Rates (NA) in Control and Monensin Supplemented Steers: Sodium, Potassium and Chloride

	Control	Monensin	SED
Sodium			
P-A (mg/dl)	2.05	8.84	14.28
NA (g/hr)	12.79	53.67	
Potassium			
P-A (mg/dl)	.38	1.06	1.905
NA (g/hr)	2.37	6.44	
Chloride			
P-A (mg/dl)	- 8.93	-10.12	1.25
NA (g/hr)	-55.72	-61.45	

increased sodium and potassium absorption into the portal blood for monensin treated steers (Table 16). Control steers had net absorption rates of 12.79 g Na/hr and 2.37 g K/hr, while monensin supplemented steers had absorption rates of 53.567 g Na/hr and 6.44 g K/hr. Chloride absorption values were similar for both treatments (P>.10).

P-A differences in lactate reflect the tendency for decreased lactate concentrations in arterial and portal blood with monensin supplementation (Table 17). Control steers had net absorption rates of 15.60 m/hr while monensin supplemented animals had net absorption rates of 6.68 m/hr. Glucose P-A differences and net absorption rates were lower for animals supplemented with monensin. These differences were not significantly different (P>.10). Total lipid P-A differences were also not different (P>.10).

There were no significant differences (P>.10) in P-A differences in alpha amino nitrogen or blood urea nitrogen concentrations. Numerically there appears to be a stimulation of alpha amino nitrogen transport into the portal vein. Absorption rates were 187.18 m/hr for control steers and 226.48 m/hr for monensin supplemented animals. Blood urea nitrogen showed the opposite trend. In this case there was a tendency for blood urea nitrogen absorption rates to be lower in those animals receiving monensin (-133.57 m/hr vs. -6.24 m/hr for control).

Table 17. Mean Portal-Arterial (P-A) Differences and Net Absorption Rates (NA) Rates in Control and Monensin Supplemented Steers: Lactate, Glucose and Total Lipids

	Control	Monensin	SEU
Lactate			
P-A (mM)	.025	.001	.074
NA (mM/hr)	15.60	6.68	
Glucose			
P-A (mM)	-.04	-.06	.114
NA (mM/hr)	-24.95	-36.43	
Total Lipid			
P-A (mg/dl)	18.06	15.43	16.87
NA (g/hr)	112.68	93.69	

Table 18. Mean Portal-Arterial (P-A) Differences and Net Absorption (NA) in Control and Monensin Supplemented Steers: Alpha Amino Nitrogen (AAN) and Blood Urea Nitrogen (BUN).

	Control	Monensin	SED
A-AN			
P-A (mM)	.30	.37	.235
NA (mM/hr)	187.18	226.48	
BUN			
P-A (mM)	-.01	-.22	.963
NA (mM/hr)	-6.24	-133.57	

Discussion

It is truly an understatement that this study was without any difficulty. It was originally planned as a disappearance (of digesta) appearance (of metabolites in the portal blood) study but this became impossible. Early attempts at catheterization of the portal vein were not successful in maintaining the catheters for periods longer than 30 days. The problem seemed to be the inability to suture the catheter to the vein to hold it in position. Communications with Dr. G. Huntington (U.S.D.A., Beltsville, MD) indicated that he could not pull a portal catheter out easily, I could. With such a short catheter patency, it became essential that the steers be placed on feed as quickly as possible. The cannulation of the duodenum, although always successful, appeared to slow the recovery of appetite. These animals would come back on feed as usual but then back off. It was then decided to forgo disappearance and concentrate on measuring net portal appearance.

Initially the right cartid artery was exteriorized for later catherization for blood flow studies. Although this was successful, healing was not rapid (probably because of stanchions). However after a carotid artery burst, I opted to switch to catheterization of the iliac artery. A catheter was placed in the iliac artery during surgery. These preparations healed quickly and did not cause any further problems.

A total of 8 steers were catheterized. Three of the steers which were not used, were found to have non-physiological blood flow estimates indicating incorrect catheter placement and another steer died after uncontrollable carotid bleeding. Four steers were

subsequently used in the study.

Monensin supplementation at 300 mg/head/day had several effects on mineral composition of blood plasma and net absorption. Arterial calcium concentrations significantly increased ($P<.08$) from 10.48 mg/dl to 11.28 mg/dl. It did not significantly ($P>.10$) increase portal concentrations although they did increase numerically. Despite an increase in arterial calcium concentration, net absorption of calcium was not increased (Table 14). Instead it was decreased from 2.3 g/hr to .425 mg/hr.

It is important to view these findings in their entirety. Arterial and venous concentrations and their differences may be interesting and significant, but the most important criteria must be net absorption. For example there was no difference of monensin on total blood lipid concentrations (Table 17) with added monensin. There was also no significant differences in the P-A difference of lipids, however when blood flow rates are applied to the P-A difference it was determined that net absorption of lipids was over 2 kg/day. This is highly unlikely. The calcium data would indicate that net absorption from the rumen and intestine is decreased and the animal retained more calcium.

Monensin also decreased ($P<.01$) portal concentration of phosphorus from 7.72 to 6.70 mg/dl. It had no affect on arterial phosphorus concentration (7.43 mg/dl-control and 6.37 mg/dl for supplemented steers). P-A differnces of phosphorus were not different and net absorption (NA) rates were also unaffected (Table 14).

There was no significant effects of monensin supplementation on portal blood or arterial concentrations of magnesium, copper, zinc or calcium. Concentration of copper and zinc showed numerical increases with monensin supplementation. This agrees with data reported by Starnes et al. (1984) who also reported elevated copper and zinc levels in jugular blood from beef steers supplemented with monensin. Starnes et al. (1984) offered no explanation as to why serum levels of zinc and copper were higher in animals receiving ionophores. It would be attractive to speculate that the increase in plasma zinc and copper levels is an absorption effect, however, the data in this study do not support such an effect (Table 15).

Magnesium concentrations were not significantly altered by monensin supplementation but the P-A difference of magnesium was significantly elevated in monensin supplemented animals (Table 15). This resulted in net absorption estimates of -39.3 g/hr in control and 24.73 g/hr in supplemented animals. This clearly demonstrates that magnesium absorption from the rumen was altered by monensin. This magnesium effect has been speculated by Greene et al. (1985) and Starnes et al. (1984). These researchers demonstrated an apparent net increase in magnesium retention in beef cattle and sheep fed monensin. This maybe due to a stimulation by monensin of the Na^+/K^+-ATPse activity in the rumen epithelium. Martens (1983) demonstrated that magnesium uptake was inhibited with ouabain administration. This stimulation of magnesium uptake may have important implications in prevention of grass tetany.

Portal concentrations of sodium and potassium were increased with monensin supplementation (Table 10), but P-A differences were not different. Net absorption rates indicated a numeric increase in sodium (1.28 g/hr versus 5.37 g/hr) and potassium (2.37 g/hr versus 6.44 g/hr) with monensin supplementation. Elevated portal concentrations of both minerals may be a result of an increase in either ruminal absorption or an increase in intestinal absorption. Both mechanisms of absorption would be stimulated by the presence of monensin because both are sodium dependent processes (Birge et al, 1974; Wasserman, 1981). It is not surprising to find no change in arterial concentrations as sodium and potassium levels are tightly regulated at the level fo the kidney. Perhaps with more power to test (ie. different experimental design) the detection of different net absorption rates might be more likely.

Chloride was unaffected by monensin supplementation, and the data indicates that chloride is taken up by the intestine (negative net absorption rates (Table 16).

I was also unable to detect differences in either blood L-lactate, blood glucose and total blood lipid concentrations or their P-A differences with monesin. L-lactate concentrations in portal blood (Table 11) decreased from .961 mM to .917 mM and net absorption values declined from 15.60 mM/hr to 6.68 mM/hr. This is in agreement with data obtained by Harmon et al. (1986) and is likely to be a reflection of a decreased ruminal production of lactate, a well documented monensin effect on the rumen (Bergen and Bates, 1984). However, the data is contrary to other data reported by Huntington and Harmon (1986) and Harmon and Avery (1986). Neither of these studies showed any

significant differences in L-lactate absorption with monensin supplementation. Table 19 compares published data to the data of this study.

Glucose concentrations were also not different although there was a tendency for monensin supplementation to increase portal glucose concentrations. This finding is also in agreement with Huntington and Harmon (1986), Harmon and Avery (1986) and Harmon et al. (1986). The data of the other studies in Table 19 do indicate a tendency for monensin to increase glucose uptake which might be explained by an increase in Na^+/K^+-ATPase activity with monensin supplementation. However, none of these previous studies were able to detect a significant increase in glucose uptake.

Blood alpha-amino nitrogen (AAN) and blood urea nitrogen (BUN) concentrations were not significantly afffected by monensin supplementation (Table 12). AAN values were higher for monensin supplemented cattle than control cattle. BUN was lower for monensin supplemented than control steers. These data are also in agreement with previous studies (Table 12). Net absorption rates for AAN increased from 187.18 mM/hr to 226.47 mM/hr, while BUN net absorption rates decreased.

Portal blood flow rates were not signifantly altered with monensin supplementation. The portal blood flow rates observed in this study are similar to rates obtained by others (Huntington, 1983; Huntington et al, 1981) for steers of this size on a high energy diet. They are also in agreement with all studies except the Huntington and Harmon (1986) study. This may be a result of the way in which the studies

Table 19. A Summary of Recent Data Concerning Monensin Addition to Beef Cattle Diets and its Effect on Net Nutrition Absorption

Workers	Present Data		Huntington and Harmon (1986)		Harmon and Avery (1986)		Harmon et al. (1986)	
Diet	75% HMSC 21% Grd. hay		90% Hay		85% Conc.		85% Conc.	
(n/trt) Anim. wt.	(2) 363 kg		(2) 422 kg		(4) 211 kg		(4) 252 kg	
TRT	C	M	C	M	C	M	C	M
Portal Blood flow (L/h)	623.9	607.2	610	767	651.1	668.7	705.2	683.6
Lactate (mM/h)	15.6	6.68	69	98	40.1	84.2	76	68
Glucose (mM/h)	-24.9	-36.4	26	38	-22.6	-3.8	-5.7	3.6
Alpha Amino (mM/h)	187.18	226.48	69	94	136.2	131.9	NA	NA
Blood Urea Nitrogen (mM/h)	-6.24	-133.5	45	98	45.8*	-11.4*	-8.7**	-36**

*Treatment effect P<.05.
**Treatment effect P<.10.

were conducted. Huntington and Harmon (1986) utilized a switchback design to measure the effect of monensin removal. They therefore measured an acute situation, rather than a chronic situation as measured by other studies.

From these data and those reported in Table 19, it is obvious that there cannot be a clear picture of the effects of monensin supplementation in beef cattle. There seems to be some evidence of an effect in the rumen (magnesium transport) and an effect on some minerals but no clear effect on intestinal absorption processes that might be altered by known mechanisms of monensin action (i.e. sodium dependent processes). It may be that monensin is having an effect that is unmeasurable by our present techniques or that alterations do occur but the intestine buffers those alterations in a homeostatic mechanism.

These data when coupled with the significant increase in Na^+/K^+-ATPase activity in mucosal biopsies from monensin supplemented steers indicate that monensin does have an effect at the cellular level. Our inability to detect significant differences in sodium-dependent processes do not preclude them from occurring. Further studies of monensin absorption in the intestine should couple in vitro examinations of the effect of monensin on the enterocyte with infusion studies in the animal.

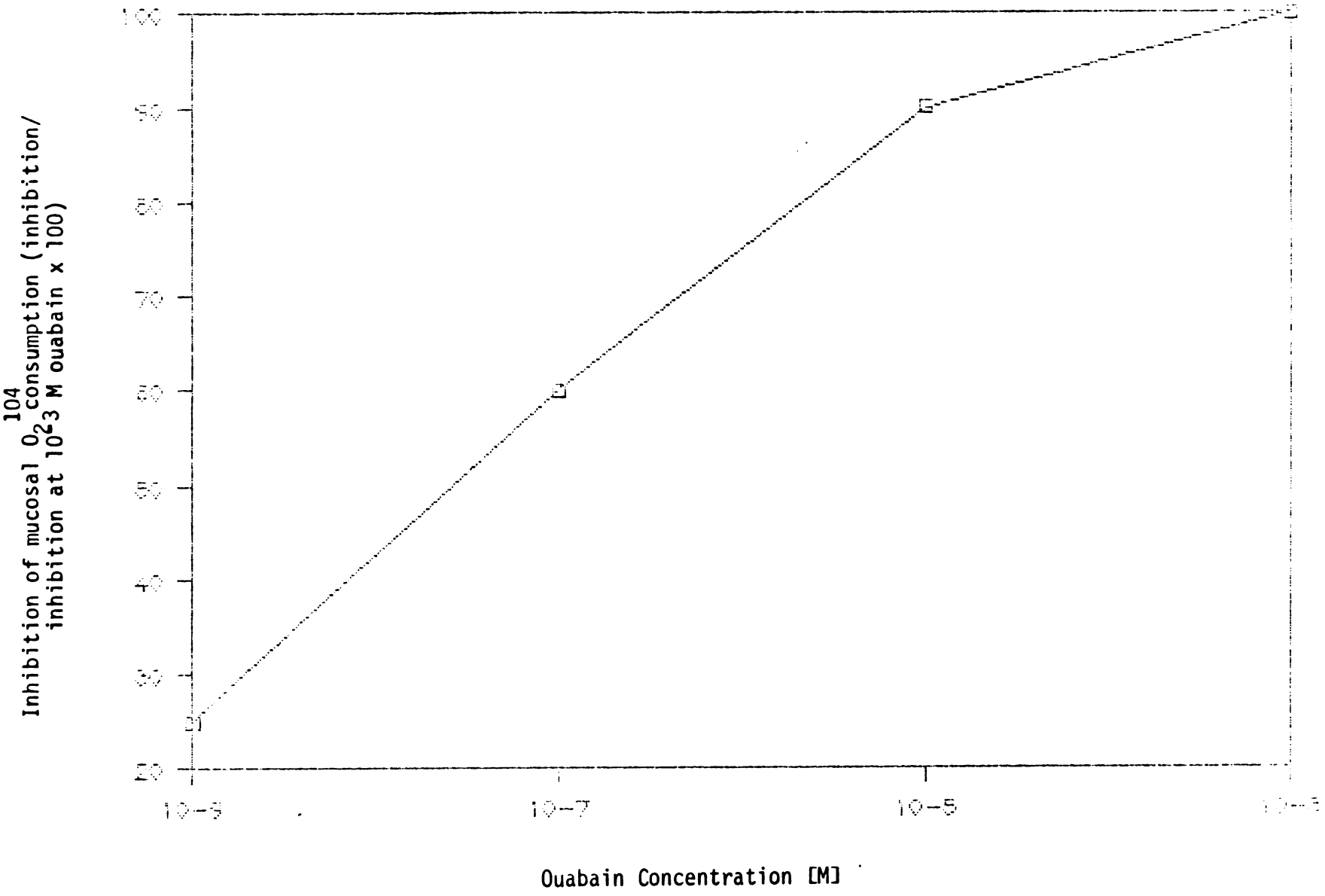
Intracellular alterations of monensin may provide more insight into metabolism of the cell. It may be that ions are altered in their sequestration patterns in the cell. Metabolic pathways and processes may also be altered. Another important consideration is the unstirred

water layer. To what concentration is the cell exposed to This is an important consideration in absorption studies and one that needs to be addressed.

Further examinations of net absorption should involve either monensin infusions into the small intestine to examine intestinal effects or, the rumen venous drainage should be cannulated along with the portal vein. This would help partition out the effect of monensin in the rumen from that in the small intestine.

APPENDIX

Figure 1. Inhibition by ouabain of respiration in duodenal mucosa.



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