A REVIEW OF THE PHYSIOLOGICAL SIGNIFICANCE OF HYPERTONIC BODY FLUIDS ON FEED INTAKE AND RUMINAL FUNCTION: SALIVATION, MOTILITY AND MICROBES[1]

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ABSTRACT

Mechanisms exist in the ruminant to detect changes in osmolality and volume of plasma during feeding to maintain fluid and electrolyte homeostasis. Feed intake during a meal can be limited by the rise in osmolality of ruminal fluid, which is sensed in the wall of the rumino-reticulum. Ruminal microbes appear to be resilient to the short-term changes in ruminal fluid tonicity, but in vitro growth rates are inhibited when the tonicity of the culture medium is increased beyond physiological levels. Although mixing contractions of the rumen are not inhibited by the normal increases in tonicity of ruminal fluid, time to first rumination is increased. This aspect of motility requires further research. The tonicity of plasma increases toward the end of a large meal as a consequence primarily of absorption of VFA and Na^+ from the rumen and fluid shifts into the gut. This hypertonicity is sensed centrally to inhibit parotid secretion by a reduction in the parasympathetic stimulation to the gland. Increases in animal production may result from future research directed toward developing ways of counteracting these negative effects of hypertonicity in body fluids on feed intake and ruminal function.

(Key Words: Tonicity, Feed Intake, Salivation, Motility, Microorganisms.)

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Introduction

Feed intake and salivation are important aspects of ruminal function and production. Ruminal function involves fermentative digestion, absorption and the movement of digesta from the ruminoreticulum to the lower gut. Therefore, ruminal function includes motility to mix the contents in the rumen, rumination, eructation, microbiology as well as epithelial blood flow, integrity and development. Ruminal function was considered to be dynamic and highly interactive by Beever et al. (1986).

Saliva provides fluid for the ruminal environment so that ruminoreticular contractions associated with motility can transport particles of fibrous feed to the lower gut and move VFA from the site of production to the epithelium for absorption. Ruminal motility mixes contents and ensures contact of saliva with feed particles and the microbes. Buffers in saliva help maintain pH in the local environment of the microbes that is conducive to fermentation. Furthermore, maintenance of pH is important, because excessive acidity is detrimental to ruminal motility (Crichlow, 1988) and epithelial integrity (Dirkson, 1970). Saliva also dilutes the contents of the rumen, which become hypertonic after eating. This is beneficial because there is a limit to which the ruminal microbes can tolerate increases in tonicity. Rumination is particularly important with forage diets because it stimulates saliva production and reduces the size of particles in the rumen so that they will pass through the reticulo-omasal orifice (Chai et al., 1988). Chewing during rumination also damages the protective coating on plant surfaces and

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thereby provides new sites on particles for microbial attack.

Pelleted and high-concentrate diets are not conducive to rumination, salivation, good ruminal function or, in some instances, high feed intakes (Grovum, 1988). A greater understanding of feed intake and salivation may help minimize or eliminate these detrimental effects in the ruminant.

Osmotic pressure of body fluids, and particularly hypertonicity, has important physiological significance for ruminal function and feed intake. Literature pertaining to the osmolality of body fluids has been reviewed with emphasis on ruminal fluid and plasma. Tonicity changes associated with feeding and factors contributing to these changes, along with some of the consequences of these changes, are discussed. Particular attention has been given to the manner in which increases in tonicity in body fluids inhibit salivation and feed intake.

Tonicity of Body Fluids

General

Osmotic pressure is measured in osmoles; a 1-Osmol (1,000 mOsmol) solution contains 6×10^{23} dissolved particles per liter of solution. The osmotic pressure of a solution can be determined by measuring the pressure required to prevent water from being pulled across a semipermeable membrane into the solution; osmotic pressure arises from dissolved particles attracting water. It is conventionally measured by the freezing point depression of the solution. A freezing point depression of 1.86°C is equivalent to an osmotic pressure of 1,000 mOsmol/kg (Berne and Levy, 1983a). When 1 Osmol of solute is dissolved in 1 kg of water, the solution has an osmolality of 1 Osmol/kg. When the tonicities of body fluids are reported as osmolality, it is assumed that the solutes occupy negligible space because they are so dilute. Thus, the term osmolarity often is used; this refers to the osmolar concentration expressed as osmoles per liter of solution. The quantitative difference between osmolality and osmolarity is less than 1% for the dilute solutions that are found in the body (Guyton, 1986).

The tonicity of blood and interstitial fluid is maintained normally at approximately 300 mOsmol/kg. However, there are significant deviations from this value in the fluid in the gastrointestinal tract. Ruminal osmolality is mainly a function of diet and its fermentation. Prior to feeding, the rumino-reticular fluid is hypotonic to plasma, being 247 ± 18 mOsmol/kg in sheep (Engelhardt and Hauffe, 1975). Data on the range of tonicity values distal to the rumen are limited, although tonicity was reported to be 250 to 350 mOsmol/liter for abomasal and ileal fluid (Maloiy et al., 1979). In the goat, mean tonicity values for the reticulum, omasum and abomasum were 268 ± 11, 256 ± 9 and 282 ± 6 mOsmol/kg, respectively (Engelhardt and Hauffe, 1975). Values reported for the cow were 270 to 300 mOsmol/liter for the abomasum, 410 to 570, 365 to 480 and 320 to 375 for the proximal, middle and distal small intestine, respectively, and 310 to 350, 270 to 300, 240 to 260 and 210 to 235 for the cecum and the start, middle and end of the large intestine, respectively (Brouwer, 1961).

Osmoregulation is achieved by diffusion, active transport and hormone-regulated secretion (Maloiy et al., 1979). Thirst centers of the central nervous system regulate endocrine control (antidiuretic hormone and aldosterone) of renal function to maintain osmolality of body fluids and extracellular fluid volume. The threshold increase in tonicity of plasma eliciting thirst is reported to be greater than that required to stimulate the release of vasopressin resulting in antidiuresis (Stricker and Verbalis, 1988). Thus, physiological mechanisms respond to an increase in plasma osmolality before the urge to drink arises. Regulatory mechanisms operate during the normal feeding patterns in ruminants and require receptors that detect changes in osmolality and plasma volume. The demands on water and sodium for saliva during feeding have been associated with the rapid onset of antidiuresis and increased sodium retention in sheep (Stacy and Brook, 1964). This antidiuresis probably is mediated by ADH release due to an increase in plasma osmolality. This is sensed by osmoreceptors thought to be located in the supraoptic and paraventricular nuclei in the optic chiasm and pituitary region at the base of the brain (Berne and Levy, 1983b), although the exact location of the osmoreceptors may vary among species (McKinley et al., 1978).

Tonicity Changes Associated with Feeding

Ruminal Fluid

Osmolality of ruminal fluid exhibits a postprandial rise. After the consumption of alfalfa chaff mixed with wheaten chaff or oats (water

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withheld), ruminal fluid tonicities approached 500 mOsmol/kg (Warner and Stacy, 1968). More generally reported maximal values for ruminal fluid tonicities are in the range of 350 to 400 mOsmol/kg for roughage- or silage-based diets (Warner and Stacy, 1965; Bergen, 1972; Bennink et al., 1978). However, the rate and extent of the rise in ruminal fluid tonicity will depend on the diet (Bennink et al., 1978), the amount consumed in a given time (Warner and Stacy, 1968), the activity of the ruminal microbiota (Schwartz and Gilchrist, 1975) and water intake.

The fiber component of a diet contributes to the buffering capacity in ruminal fluid through its hydration and cation exchange capacities. At exchangeable surfaces of plant fiber, reactions with di- and trivalent cations occur to form stable chelates. Hydrogen ions are liberated that react with bicarbonate ions in ruminal fluid (Van Soest et al., 1984). This process will limit the post-prandial rise in tonicity of ruminal fluid. Cation exchange and hydration capacity are proportional to forage quality (Allen et al., 1985; McBurney et al., 1986) and have been measured in various feedstuffs (McBurney et al., 1981; Van Soest et al., 1984).

The dissolution of minerals from ingested feed and the production of VFA from microbial fermentation are the main determinants of the rise in osmolality, but the relative contribution of minerals and VFA varies according to diet (Bennink et al., 1978). Ammonium ions also can make a small contribution to tonicity (Warner and Stacy, 1965). Within 20 min of eating alfalfa hay, tonicity increased by 37 mOsmol, whereas after 40 min of eating alfalfa pellets, it was increased by 60 mOsmol (Bennink et al., 1978). Maximal values were 421, 363 and 369 mOsmol after feeding alfalfa pellets (2 h), milo (2 h) and a concentrate-silage-hay diet (4 h), respectively (Bennink et al., 1978). The tonicity of ruminal fluid also increased from 280 mOsmol/kg to approximately 400 mOsmol/kg after 1 h in sheep consuming an alfalfa-wheaten chaff mix when water was withheld (Warner and Stacy, 1965).

Plasma

Plasma tonicity also increases after feeding in sheep (Ternouth, 1967). The tonicity of jugular plasma rose by 2 to 4 mOsmol/kg within 5 min after sheep started a 1-kg meal of alfalfa chaff following a fast of 15 h (Carr and Titchen, 1978). Plasma tonicity continued to increase by 18 mOsmol/kg after 3 h, then declined over the next 2 to 3 h, but remained above prefeeding levels for 10 h after feed was offered. Similar changes were observed when fresh feed was provided to two sheep with ad libitum access to feed, although the changes were not as great as those above (Carr and Titchen, 1978). The tonicity of jugular plasma continued to rise over a 3-h period during and after feeding even though water was provided (Warner and Stacy, 1965). Post-prandially, plasma protein concentration increased (Stacy and Warner, 1966) and plasma volume decreased (Christopherson and Webster, 1972). Blair-West and Brook (1969) reported a decrease in plasma volume after only 5 min of eating, with a maximal decrease at 20 to 60 min. These effects could be attributed to the elevated salivary secretion during a meal, to diffusion of water into the rumino-reticulum and to increased gastric (Ash, 1961; McLeay and Titchen, 1970), pancreatic (Taylor, 1962) and biliary (Harrison, 1962) secretions.

Carr and Titchen (1978) compared the increase in plasma tonicity from jugular versus portal blood during a meal of alfalfa chaff after the sheep had been fasted for 15 h. Portal tonicity was 4 mOsmol/kg higher than jugular tonicity prior to feeding. After 30 min, the increases in portal and jugular tonicity were 9 and 8 mOsmol/kg, and after 60 min they were 14 and 11 mOsmol/kg, respectively. Thus, the increases in tonicity of both portal and jugular plasma were similar, but in the first 60 min of the feeding period, feed and water intakes were 295 g and 1,100 ml, respectively. Hence, plasma tonicity increased despite substantial water consumption. The increase in the tonicity of portal plasma is due presumably to the absorption of osmotically active particles from the rumen and to increased secretions from digestive glands. The increase in the tonicity of jugular plasma may be due largely to the loss of water into saliva and other secretions, but also to the presence of VFA in the blood (mainly acetate). The appearance of VFA and energy-yielding metabolites in plasma has been shown to depend on the type of diet and the amount consumed. Bines (1968) reported a relatively small change in energy-yielding metabolites in jugular plasma when cows consumed an all-roughage diet compared with an all-concentrate diet. Small, spontaneous

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meals in goats fed ad libitum had no effect on the peripheral or portal VFA concentration, although larger spontaneous meals were associated with increases in blood VFA concentration (de Jong, 1981). Ruminal vein concentrations of the VFA exceed those of jugular plasma partly because of less dilution, but also because propionate and butyrate are removed by the liver (Theurer and Wanderley, 1987). Barnes et al. (1983) reported no immediate post-prandial increase in portal blood flow in sheep, although after 2 h, flow had increased by 19% compared with that before feeding. Part of this increase was due to blood flow to the ventral sac epithelium, which increased from 91 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ tissue at 20 min before feeding to 332 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ tissue after 2 h. It increased to 130 $ml \cdot min^{-1} \cdot 100\ g^{-1}$ tissue after only 4 min of eating, but this was not reflected in the portal flow measurements. Portal blood flows in sheep also were shown to increase from 1.8 liters/min over a 24-h period in the starved state to 2.4 liters/min and 4.0 liters/min when fed to maintenance and 2.5 times maintenance, respectively (Webster et al., 1975). Dobson et al. (1976) concluded that hypotonic and hypertonic ruminal fluid increased mucosal blood flow. Hence, tonicity changes in portal blood associated with feeding are accompanied by increases in blood flow to the ruminal epithelium and in the portal vein. Increased portal blood flow would tend to limit the degree of hypertonicity attained in portal blood.

Saliva

Mixed saliva (i.e., the combined flow from all glands) is thought to be isotonic to plasma and resemble the composition of parotid saliva (Kay, 1966). However, the osmolality of mixed saliva collected from esophageal fistulas in steers was 259 mOsmol/kg (Froetschel et al., 1986). Furthermore, the tonicity of parotid saliva, which represents approximately 50 to 60% of total salivary secretion (Kay, 1966), can change depending on the flow rate (concomitant with changing composition) and the tonicity of blood. Hence, the tonicity of parotid saliva can be hypotonic, isotonic or hypertonic. For example, Carr and Titchen (1978) reported that the tonicity of parotid saliva in conscious sheep usually was 5 to 20 mOsmol/kg less than that of jugular plasma. In anesthetized sheep, however, the tonicity of parotid saliva in sodium-replete animals was similar to that of plasma, but the tonicity increased from 281 to 295 mOsmol/kg upon electrical stimulation of the parotid nerve or close-arterial infusion of acetylcholine, which increased the flow rate from .3 (unstimulated) to 1.9 ml/min (Compton et al., 1980). The plateau in parotid saliva tonicity was seen after a secretion rate of only about 1.2 ml/min. This increase in tonicity is the result of insufficient time for reabsorption of electrolytes during passage through the ducts draining the acini, with sodium and bicarbonate concentrations increasing rapidly to a plateau as secretion rate increases (Young and Schneyer, 1981). Argenzio (1984) depicted that Na^+ concentration in ruminant saliva decreased with increasing flow rate, but this appears to be incorrect based on the results of Coats and Wright (1957), Blair-West et al. (1967), Compton et al. (1980) and Froetschel et al. (1986). During the course of a meal, parotid saliva tonicity continued to rise along with plasma tonicity over a period of hours when the flow rate ranged from 5.37 ml/min at the start of feeding to .84 ml/min after 90 min (Carr and Titchen, 1978). The initial rise in tonicity of saliva probably is explained by insufficient time for reabsorption of electrolytes at the high flow rate. However, a continued rise could reflect an increase in plasma tonicity.

VFA, Sodium and Water Movement Across the Ruminal Epithelium

VFA Absorption

The principal VFA in ruminal fluid are acetic, propionic and butyric acids. Several studies found that they were absorbed from the rumen at an increasing rate with increasing carbon chain length (Danielli et al., 1945; Pfander and Phillipson, 1953; Stevens and Stettler, 1966). Butyric acid was absorbed at a greater rate than propionic acid, which was absorbed more rapidly than acetic acid at a ruminal pH of 5.8 (Danielli et al., 1945). However, Annison et al. (1957) found the reverse order for rate of absorption despite maintaining ruminal fluid pH within the normal range. Danielli et al. (1945) concluded that the reversed order for absorption rate (i.e., acetic acid > propionic acid > butyric acid) occurred at an alkaline ruminal fluid pH. However, Oshio and Tahata (1984) reported no

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significant difference between the absorption rates of acetic, propionic and butyric acids from the rumen of sheep at a pH range of 6.8 to 8.0.

Data concerning VFA absorption have been conflicting, probably because of the different experimental methods employed (e.g., in vivo, in vitro, anesthetized or conscious animal studies). The relative merits of in vivo and in vitro VFA absorption studies were discussed by Stevens (1970); he concluded that in vitro experiments were more useful in elucidating the mechanisms of absorption. This conclusion supports the studies that showed a greater absorption rate with increasing chain length, which may be the result of increased lipid solubility.

Absorption of VFA from the rumen has been reviewed recently (Argenzio, 1988). Substantial dissociation of VFA will occur at pHs that are normal for ruminal fluid (pH 6 to 7). The bicarbonate and phosphate ions secreted in saliva can neutralize about one-third of the VFA produced in the rumen (Kay, 1966) with absorption acting as a physiological buffer for the remainder. Ash and Dobson (1963) concluded that acetate was absorbed down its concentration gradient, and that approximately equal amounts of acetate were absorbed in the acid vs the anion form. This occurred in spite of acetate being about 99.5% anion at pH 7. Hence, they suggested that there must be a membrane separating ruminal fluid and blood that is relatively impermeable to the acetate anion. This also would imply that the membranes are readily permeable to acetic acid. In their experiments, acetate absorption was accompanied by an increase in ruminal HCO_3^- concentration and a decrease in CO_2 concentration. According to their model (Figure 1a), the ionized form of acetate combined with H^+, which was supplied by the breakdown of H_2CO_3. These reactions would cause HCO_3^- concentration to increase and CO_2 levels to decrease. Ash and Dobson (1963) considered that this could occur in the ruminal fluid or somewhere in the ruminal epithelium, but they showed it occurring in the ruminal fluid in their diagram. However, there was an inequality in the amount of acetate removed from the rumen and the amount of HCO_3^- appearing in ruminal fluid. These workers considered that acetate disappearance exceeded bicarbonate appearance because of absorption of the acetate anion, which appears to be accompanied by the absorption of Na^+ (Dobson, 1959). In an alternative explanation, Argenzio (1988) proposed a Na^+/H^+ exchange system at the luminal membrane. This would result in a net secretion of H^+ into the rumen that would neutralize an equivalent amount of HCO_3^- and thus account for the discrepancy between HCO_3^- appearance and acetate absorption. According to Argenzio's model (Figure 1b), the H^+ secreted into the ruminal fluid also could combine with acetate anion to form acetic acid, which then would be absorbed. Although Argenzio did not dismiss paracellular permeability to ionized VFA, he suggested that it required further investigation.

A model similar to that of Ash and Dobson (1963) was hypothesized by Stevens (1970); he proposed that the membrane adjacent to the ruminal fluid is permeable to acetic acid and its anion, and that a membrane on the blood side of the epithelium is relatively impermeable to the acetate anion (Figure 1c). Stevens (1970) postulated that the acetate ion combined with a H^+ in the cells of the epithelium, rather than in the ruminal fluid as depicted in Figure 1a. The source of the H^+ was dissociation of carbonic acid in the cells, which was supported by the presence of carbonic anhydrase in the isolated bovine ruminal epithelium (Aafjes, 1967). The Stevens model had CO_2 passing from the ruminal fluid and blood into ruminal epithelial cells, where it combined with water to ultimately yield H^+ and HCO_3^-. The H^+ reacted with the acetate anion to form acetic acid, which diffused out of the cells into plasma. The HCO_3^- diffused back into the ruminal fluid.

According to Masson and Phillipson (1951), the rate of appearance of the VFA in venous blood draining the rumen was acetate > propionate > butyrate. Part of the reason for this is because acetate concentration is high and butyrate concentration is low in ruminal fluid. Butyrate also is extensively metabolized by the ruminal epithelial tissue, where it is converted mainly to β-hydroxybutyrate (Stevens and Stettler, 1966). Propionate can be converted to lactate in the ruminal epithelium (Leng et al., 1967). The rate of absorption of propionate into portal blood was recently reported to be .3 of the measured production rate (Seal et al., 1989). This was attributed to

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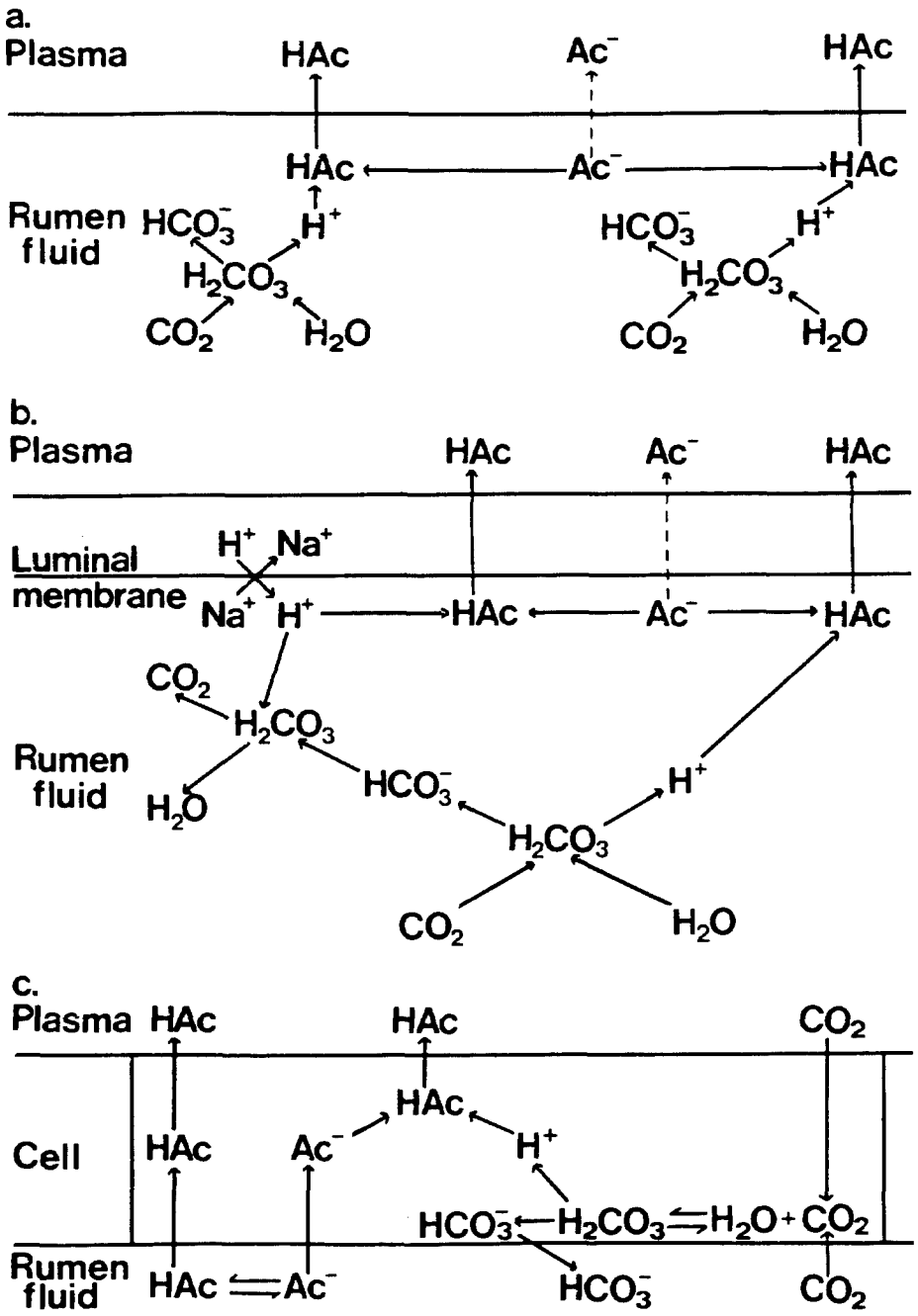

Figure 1. Acetic acid absorption from the rumino-reticulum. (a) Acetic acid absorption in the associated form with some absorption of the acetate anion across the ruminal epithelium (Ash and Dobson, 1963). These authors did not define which membrane in the epithelium was permeable to acetic acid. (b) The model of Ash and Dobson (1963) with modifications proposed by Argenzio (1988) (i.e., a Na^+/H^+ transport system in the luminal membrane supplying H^+ for the conversion of acetate anion to acetic acid and HCO_3^- to H_2CO_3 in ruminal fluid). (c) Acetic acid absorption across the ruminal epithelium as proposed by Stevens (1970). Epithelial tissue is depicted as a single layer of cells. The luminal membrane is proposed to be permeable to both forms of the acid, but the serosal membrane is permeable only to the associated form. The CO_2 from intracellular metabolism or absorbed from the lumen or blood combines with water to produce carbonic acid, which, in turn, serves as a H^+ donor to increase the net movement of acetic acid into blood.

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metabolism of propionate in the rumen and ruminal epithelium. Whereas Elliot (1980) considered the conversion of propionate to lactate in the rumen epithelium to be quantitatively insignificant, Stevens (1970) calculated that metabolism in isolated sheets of ruminal epithelium could account for 65% of propionic acid absorbed from the luminal side. The propionate and butyrate not metabolized in the ruminal epithelium are removed largely by the liver (Cook and Miller, 1965). Acetate is utilized mainly by extra-hepatic tissues (Annison et al., 1957). After a large meal the capacities of the ruminal wall and liver to metabolize butyrate and propionate are exceeded; these VFA, along with acetate, then appear in the systemic circulation (Theurer and Wanderley, 1987) and contribute to the post-prandial increase in plasma osmotic pressure. There also is a post-prandial increase in ruminal fluid osmotic pressure, which may reduce the absorption rate of the VFA (Oshio and Tahata, 1984) but not affect the relative proportions of VFA remaining in the rumen (MacLeod and Ørskov, 1984).

Sodium Absorption

The ruminal epithelium contains the Na^+-K^+ ATP-ase enzyme (Schnorr et al., 1969, cited by Warner and Stacy, 1972), and a system explaining the active absorption of Na^+ based on the cellular organization of the ruminal epithelium was proposed by Steven and Marshall (1970). Figure 2 shows a diagrammatic cross-section of the ruminal wall. The theoretical scheme for Na^+ absorption relies on Na^+ being pumped by the Na^+-K^+ ATP-ase enzyme from the cells of the stratum basale into the large intercellular spaces, across the basement membrane and into blood. This would create a concentration gradient facilitating the diffusion of Na^+ from the lumen through the cells and tight junctions of the strata corneum, granulosum and spinosum.

Sodium absorption from the rumen is thought to be the major mechanism giving rise to a hypotonic ruminal fluid in fasted sheep (Warner and Stacy, 1972). Chloride ion also would be transported out of the rumen, but the electroneutral and coupled transport of these two ions may not be the only system operating (Martens and Blume, 1987). Potter et al. (1972) recorded increases in ruminal Cl^- concentration that were much greater than the combined increases in Na^+ and K^+ concentrations when a NaCl solution (1.3%) was consumed with a chaffed or pelleted diet. They proposed that Na^+ and Cl^- were being removed from the rumen differentially. In a recent review, Martens and Gabel (1988) proposed a double exchange system involving Na^+/H^+ and Cl^-/HCO_3^- and concluded that mucosal Na^+/H^+ exchange was of major importance in the mechanism for Na^+ transport across the ruminal epithelium in sheep.

Sodium is the principal inorganic constituent of mixed saliva in sheep, with concentrations ranging from 161 to 201 meq/liter (McDougall, 1948). Although the concentration of Na^+ in the ruminal fluid of cows fed a variety of diets was lower than that of saliva (Bailey, 1961a), Na^+ was the principal cation of ruminal fluid from cattle fed various diets with values from 74.5 to 157.8 meq/liter (Ward et al., 1976). Stacy and Warner (1966) calculated that increasing the tonicity of ruminal fluid in sheep led to an increase in the absorption rate of Na^+, although the increase was small when tonicity was raised with non-ionic sources (mannitol plus urea or glycerol) compared with potassium as KCl or K^+ salts of the VFA (Warner and Stacy, 1972). Stimulation of Na^+ absorption by raising the K^+ concentration in the rumen has been demonstrated in other studies (Stacy and Warner, 1966; Scott, 1967; Warner and Stacy, 1972). However, osmotic pressure per se still was thought to be a significant factor contributing to Na^+ absorption from the rumen (Warner and Stacy, 1972). More recently, Gaebel et al. (1987) concluded that raising the tonicity of artificial ruminal fluid in sheep to 422 mOsmol/liter with mannitol did not affect the net absorption rate of Na^+, Cl^- or Mg^{++}. This lack of response may be explained by the use of a non-electrolyte osmotic source and by the basal concentration of Na^+ in the artificial ruminal fluid being markedly less than that in the study of Stacy and Warner (1966) and very much lower than that normally present in ruminal fluid. Apart from osmotic effects, the Na^+ absorption rate is increased by increasing its concentration in ruminal fluid (Warner and Stacy, 1972; Martens and Blume, 1987). This increased absorption occurred provided that water entry into the rumen was minimal. However, when there was substantial movement of water into the rumen (e.g., 70 to 290

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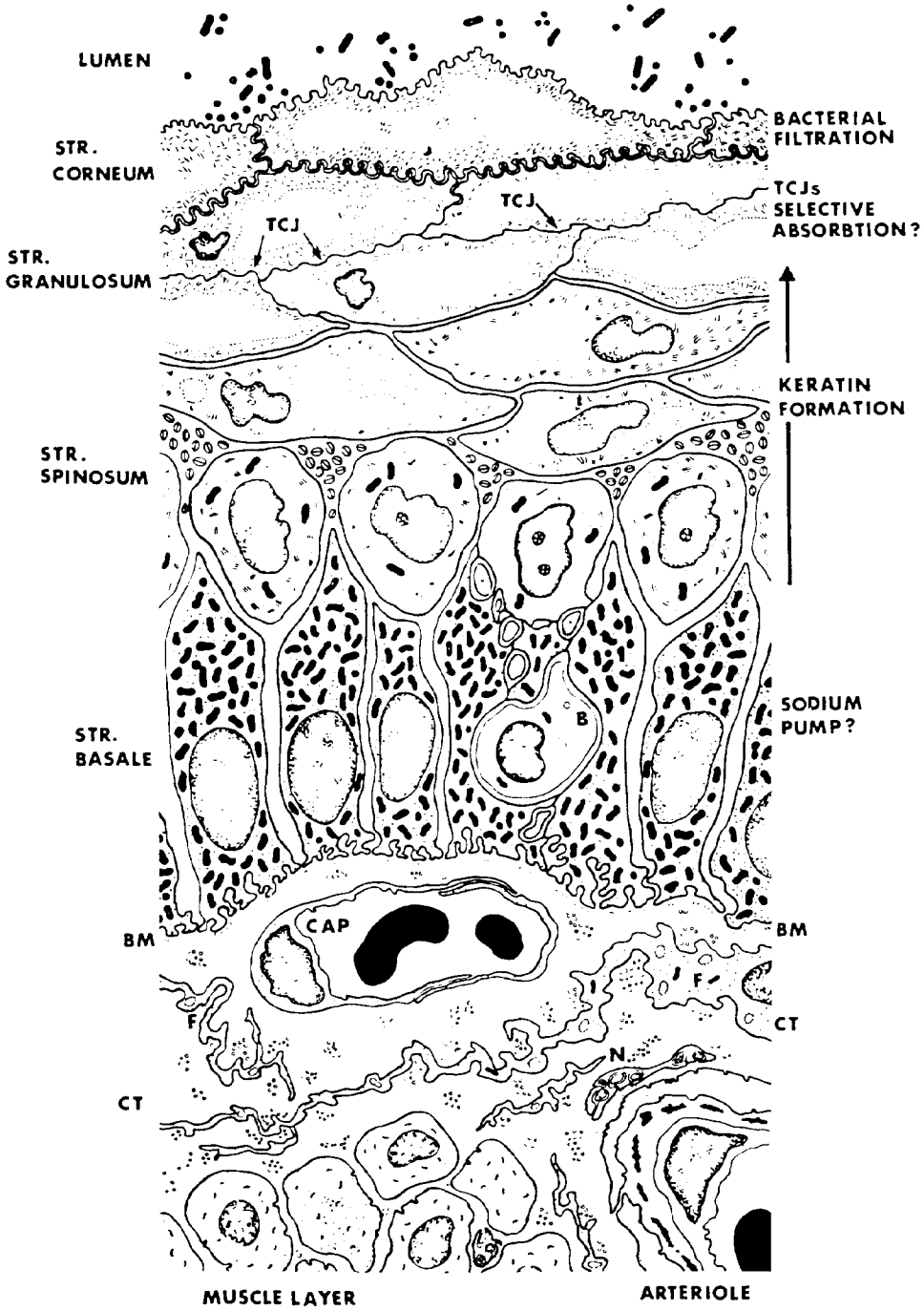

Figure 2. A diagrammatic representation of the cell layers constituting the ruminal wall. B, branching cell; BM, basement membrane; CAP, capillary; CT, connective tissue; F, fibroblast; N, nerve trunk; TCJ, tight cell junction (after D. H. Steven and A. B. Marshall, 1970).

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ml/h, at ruminal osmolalities greater than 400 mOsmol/kg), Na^+ absorption appeared to decrease (Warner and Stacy, 1972). The unchanged Na^+ absorption observed in the study by Gaebel et al. (1987) was associated with a net water flow into the rumen of 65 to 98 ml/h. It has been proposed that moderate hypertonicity of ruminal fluid causes a morphological change in the epithelium leading to an increased entry rate of Na^+ from the rumen into the epithelium (Gemmell and Stacy, 1973). They postulated that Na^+ was being pumped out of the cells in the stratum basale at a greater rate than usual, perhaps because of stimulation of the Na^+-K^+ ATP-ase enzyme system. Very high tonicities in the ruminal fluid, associated with the movement of water into the rumen, create a different situation that can be explained in terms of changes in the electrical potential across the epithelium. An electrical potential difference (PD) exists across the ruminal epithelium, with the luminal side being negative (approximately 30 mV) relative to the serosal side. This PD is created primarily by the Na^+/K^+ pump, which actively moves Na^+ out of the rumen into blood (Keynes and Harrison, 1970). There was no change in the PD across the ruminal epithelium in the studies in which the tonicity of artificial ruminal fluid was raised to 422 mOsmol/liter (Gaebel et al., 1987). When KCl was added to the rumen to elevate the tonicity to 367 mOsmol/kg, the PD increased, but when further KCl was added to raise tonicity to 526 mOsmol/kg, the PD dropped to a level below that for the resting tonicity (Stacy and Warner, 1972). The decrease in PD at very high tonicities, which was replicated with glycerol, was interpreted as being due to an increase in passive diffusion of water and Na^+ from the blood into the rumen. Water moved from blood into the rumen when the tonicity of ruminal fluid exceeded 335 mOsmol/kg (Warner and Stacy, 1972). Transitory swelling of basal cells was observed after short exposure to this elevated ruminal tonicity (Gemmell and Stacy, 1973). A diffusion barrier is thought to exist in the outermost keratinized layer of the stratum granulosum adjoining the stratum corneum on the luminal side of the epithelium (Henrickson and Stacy, 1971; Gemmell and Stacy, 1973). Although zonulae occludentes were postulated as representing an intercellular barrier to permeability in the goat ruminal epithelium (Schnorr and Vollmerhaus, 1967; Schnorr and Wille, 1972, cited by Gemmell and Stacy, 1973) these "tight seals" remained fused after exposure to hypertonic fluid in the studies of Gemmell and Stacy (1973). Tonicities of ruminal fluid that result in larger water movements into the rumen lead to the disruption of the outermost layer of the stratum granulosum, permitting the "leakage" of Na^+ from blood into the rumen and so reversing the PD (Gemmell and Stacy, 1973). Rumenitis is a characteristic finding in ruminal acidosis. The high ruminal acidity and, in particular, the hypertonic ruminal contents occurring with acidosis, were held responsible for the ultrastructural changes in the ruminal epithelium and epithelial desquamation associated with rumenitis (Ahrens, 1965; Engelhardt, 1966, cited by Dirksen, 1970).

Trans-Ruminal Water Flux

The ruminal epithelium is capable of absorbing large volumes of water. In two sheep, when 12 liters/d was infused into the rumen, the net absorption across the rumen wall was 10.8 and 10.1 liters (Harrison et al., 1975). When camels, donkeys and goats drank water after they were dehydrated to 70% of their original BW, plasma tonicity dropped by 31 to 50 mOsmol/kg (Shkolnik et al., 1980). Such a response would not occur with a slow infusion of water, as in the study by Harrison et al. (1975). When dehydrated goats consumed normal saline, the increase in plasma volume was markedly greater than when tap water was consumed (Choshniak and Shkolnik, 1977). The change in plasma tonicity was similar for both fluids, but 5 h after the consumption of tap water, 81% remained in the rumen. Over the same time period, the tonicity of ruminal fluid increased from 90.6 mOsmol/kg immediately following drinking to 131.4 mOsmol/kg. It appears, therefore, that the mechanisms governing the movement of ions and water across the ruminal epithelium operate to minimize insults to the osmotic balance between plasma, interstitial fluid and cellular fluid in the animal.

The control mechanisms for water absorption from the rumen have not been fully identified, but the main force accounting for water movement through the epithelium is the gradient of osmolality between fluid in the lumen and blood perfusing the epithelium (Dobson, 1984). However, there is evidence

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that water can move from the rumen into blood against a small osmotic gradient (Warner and Stacy, 1972; Dobson et al., 1976; Faichney and Boston, 1985). Although this movement of water probably is linked to the active transport of Na^+, Engelhardt (1969, as cited by Warner and Stacy, 1972) suggested that there was a mechanism for water absorption independent of sodium.

The osmotically induced influx of water across the ruminal epithelium was reviewed and considered small, being about 200 ml/h with a ruminal fluid tonicity of 370 mOsmol/liter (Engelhardt, 1970). When the tonicity of ruminal fluid was 422 mOsmol/liter, the net water influx to the rumen was determined by Gaebel et al. (1987) to be 62 ml/h in sheep maintained on an all-hay diet but 98 ml/h for a diet made up of 10% hay and 90% concentrate. This increase in fluid influx was small considering the osmotic pressure gradient and the surface area of ruminal papillae, which was increased by almost 400% by the high-concentrate diet. A sigmoidal curve described the relationship of net water movement and ruminal osmotic pressure in the data of Engelhardt (1970); Warner and Stacy (1972) used a straight line and Dobson et al. (1976) use a quadratic curve. In summarizing the published data on the relationship between water absorption and the tonicity of ruminal fluid, Warner and Stacy (1972) showed that the average ruminal fluid osmolality generating a zero net flux was 326 mOsmol/kg, but it ranged from 295 to 360 mOsmol/kg in the studies cited. There is a range over which the net water movement is insignificant, and Engelhardt (1970) suggested this to be from 260 to 340 mOsmol/kg. Tritiated water flux was measured across an isolated portion of the ventral sac of a cow (Dobson et al., 1976) and a net flux of approximately 20 ml/min from blood to rumen was recorded when the osmolality of fluid bathing the epithelium was 150 mOsmol/kg greater than that of plasma. These workers considered that the factors involved in net water movement across the ruminal wall were blood perfusion of the epithelium, the hydraulic conductivity of the epithelium and solute transport. Provided that the contents of the rumen were well stirred, the limiting factor for tritiated water diffusion was epithelial blood flow (Dobson et al., 1971). According to Dobson et al. (1976), if blood perfusion was high, the hydraulic conductivity of the epithelium and its unstirred layers were the limiting factors to diffusion. The conductivity to water movement was restricted by the diffusion barrier in the outer layer of the stratum granulosum.

Artificial saliva and artificial saliva plus 4% and 8% polyethylene glycol (PEG, with a molecular weight of 1,000) infused intraruminally at 4 liters/d increased the dilution rate of ruminal fluid in sheep from a control value of .056 %/h when water was infused to .109, .117 and .14 %/h, respectively (Harrison et al., 1975; data confirmed by personal communication with D. E. Beever). Increasing the osmolality of the infusates by additions of PEG resulted in an increase in the osmolality of ruminal fluid from 312 mOsmol/kg for control to 338 mOsmol/kg for artificial saliva only and to 347 mOsmol/kg and 360 mOsmol/kg for the inclusion of 4% and 8% PEG, respectively. The reason for the increased dilution rate with the addition of PEG to the artificial saliva may be an increase in net water influx to the rumen as the tonicity of ruminal fluid was increased. In another study (Ulyatt et al., 1984), a 50% increase in the daily intake of chopped alfalfa hay increased the 24 h flow of water from the rumen to the duodenum. After considering salivary flow and accounting for increased water consumption and increased reticulo-ruminal water pool size, the increased water flow to the duodenum was attributed to an increase in net flux of water into the rumen across the epithelium. Although the rate of net influx of water into the rumen associated with feeding may not be great (Engelhardt, 1970), it may be biologically meaningful in terms of daily flows to the duodenum. Such a net influx of water, which becomes reflected in an increase in dilution rate, can increase the efficiency of microbial protein synthesis in the rumen and also increase the flow of protein and starch to the duodenum (Harrison et al., 1975). The provision of 1.3% NaCl in drinking water also was associated with an increase in ruminal dilution rate (Potter et al., 1972), as well as with a decrease in the saturated fatty acid content of body fat (Walker et al., 1971).

Some Effects of Hypertonicity on the Ruminant and Its Microbes

Saliva Production

Total saliva production was reported to decrease as the tonicity of both ruminal fluid and jugular plasma were increased by osmotic loads of various solutes into the rumen

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(Warner and Stacy, 1977). However, using anesthetized sheep, Carter et al. (1985) showed that the gastrointestinal tract proximal to the jejunum did not sense osmotic pressure to inhibit parotid secretion. Decreases in the flow rate of both parotid and total saliva have been reported following i.v. infusions of hypertonic solutions (Warner and Stacy, 1977). The inhibition of parotid secretion was found to be greatest when hypertonic infusions were made into the portal vein compared with the caudal vena cava or jugular vein (Carr and Titchen, 1978). This implies that the liver or portal system is the site that senses hypertonicity, but Carter et al. (1985) reported that the head, and probably the brain, was a more important site mediating the inhibitory effect of hypertonicity in blood on parotid flow. The relationship between osmolality of body fluids and salivation is discussed in greater detail below.

TABLE 1. EFFECT OF INCREASING THE OSMOTIC LOAD IN THE RUMEN OF SHEEP ON THE NUMBER OF RUMINAL CONTRACTIONS PER 15 MINUTES[a]

Osmolality of intraruminal infusate[b]	Avg no. of ruminal contractions per 15 min[c]
Water (control)	14
200 mOsmol/kg	15
400 mOsmol/kg	15
800 mOsmol/kg	16
1,600 mOsmol/kg	14

[a]From Phillip et al. (1981).

[b]NaCl was added to either a fresh corn extract or a corn silage extract to achieve these tonicities; ruminal fluid tonicities were increased up to a maximum of 550 mOsmol/kg for the highest osmotic load.

[c]There was no interaction between osmotic load and type of extract; therefore data are pooled values (± .8) for the two extracts.

Feed Intake

Ternouth and Beattie (1971) reported a linear reduction in intake of alfalfa hay after hypertonic solutions of NaCl, KCl and the Na salts of acetic, propionic and butyric acids were added to the rumen immediately prior to feeding. Phillip et al. (1981) also observed a linear decrease in feed intake associated with an increase in the osmolality of ruminal fluid as a result of ruminal infusions of fresh and ensiled whole plant maize extracts and NaCl solutions. Carter and Grovum (1988) identified the rumino-reticular wall as the site mediating the inhibitory effect of hypertonicity on feed intake. The relationship between ruminal osmolality and feed intake is discussed in greater detail in the last section of this review.

Motility of the Rumino-Reticulum

Increasing the tonicity of ruminal fluid to 550 mOsmol/kg did not affect the frequency of ruminal contractions (Phillip et al., 1981; Table 1). Subsequently, infusions of NaCl were made into the reticulum of sheep commencing 4 min prior to feeding and continuing for 10 min into the meal (Carter, 1988). There was no significant effect on either the frequency or amplitude of biphasic reticular contractions in spite of the fact that tonicity of reticular digesta reached 774 ± 287 mOsmol/kg after 10 min of eating and that of the ventral sac reached 445 ± 34 mOsmol/kg after 20 min of eating. However, increasing the tonicity of ruminal fluid to .378 Osmol and .422 Osmol with $KHCO_3$ and PEG, respectively, increased the time to first rumination from 40 min for the control treatment (water added to the rumen resulting in ruminal fluid tonicities of .269 to .291 Osmol) to 706 min and 414 min for the hypertonic treatments, respectively (Welch, 1979). Time spent ruminating by sheep was inhibited to the same extent after ruminal loading with NaCl or PEG-200 compared with controls (J. Wever, personal communication). The effect of hypertonicity on rumination merits further research, in light of the recent finding that the stimulation of α_2-adrenergic receptors, probably located in the rumino-reticulum, evoked rumination (Leek and Stafford, 1987; Stafford and Leek, 1988).

Ruminal Microbes

Cellulose digestion in vitro was not significantly inhibited until the osmolality of the culture medium was raised to 400 mOsmol/kg (Bergen, 1972); beyond this value, differences in the extent of the inhibition were thought to be related to the compounds used. The in vitro digestibility of DM in ruminal fluid was decreased when the tonicity of the medium was increased from 250 to 500 mOsmol/kg by addition of $NaHCO_3$, $KHCO_3$ or PEG to the medium (Okeke, 1978). The reduction in digestibility was greater with PEG than with

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the ionic salts. This difference was thought to be related to the bacteria's requirement for Na^+ and K^+ and, therefore, their ability to utilize and concentrate these cations within the cell with the $NaHCO_3$ and $KHCO_3$ treatments but not with PEG. Perhaps more importantly, PEG could not move through the bacterial cell membrane, as could Na^+ and K^+. The internal osmolality of bacteria is higher than that of the surrounding medium, at least during growth in dilute media, but the rigid cell wall offers protection from rupture (Mackie and Therion, 1984). Increasing the osmolality of an in vitro growth medium with Na^+, K^+ (anions not stated) or non-metabolizable sugars (adonitol, sorbitol, sorbose), showed that, from 200 to 500 mOsmol/kg, the growth rate of *Selenomonas ruminantium* was unaffected (Mackie and Therion, 1984). However, tonicities from 500 to 900 mOsmol/kg appeared to be inhibitory. When 800 to 900 g NaCl was added to the diet fed to cows, or was directly added to the ruminal contents, no change was recorded in the total digestibility of cellulose, protein or energy (Cardon, 1953). Furthermore, provided that drinking water is readily available, sheep can adapt to the consumption of feed containing a high NaCl concentration with unimpaired digestion (Wilson, 1966). It appears, then, that the ruminal microflora can tolerate or are unaffected by short-term increases in osmolality. Gram-negative bacteria, normally present in approximately equal numbers to gram-positive bacteria in the rumen (Alonso, 1979), generally are more sensitive to high ruminal osmolalities than are gram-positive bacteria (Mackie and Therion, 1984). When an animal was fasted for up to 4 d, the osmolality of ruminal contents decreased (Dehority and Males, 1974), and the numbers of gram-negative bacteria increased relative to gram-positive bacteria (Alonso, 1979). However, it is not known whether a cause and effect relationship exists.

Protozoa generally are more sensitive to elevated tonicities than are bacteria (Mackie and Therion, 1984), but this does not mean that protozoa are completely intolerant to increases in osmotic pressure. For example, altering the tonicity of ruminal contents through the manipulation of feed and water intake did not influence either the occurrence or numbers of the holotrich protozoa (Dehority and Males, 1974). Although holotrichs contribute more to the ruminal protozoal mass, the *Isotricha* have been shown to be more numerous and active under the adverse conditions that are present in grain overload when pH would be decreased and osmolality increased (Eadie et al., 1970). Furthermore, high protozoal populations have been observed in cattle fed high-molasses diets when the tonicity of ruminal contents may be raised due to the high osmotic pressure of molasses and to the readily fermentable nature of sucrose (Bird and Leng, 1978).

Ruminal microbiota seem to be resilient to the normal short-term changes in tonicity of ruminal fluid, but more research is needed to understand mechanisms of adaptation (Mackie and Therion, 1984). Presumably, fermentation becomes self-limiting when the contents of the rumen become hypertonic after the consumption of a meal (Bennink et al., 1978). Therefore, tonicity is raised, in part, by an increased fermentation rate until a tonicity is reached that inhibits microbial activity. This allows time for the ruminant to absorb electrolytes and VFA, and for an influx of water into the rumen to occur. These processes will cause the osmotic pressure of the ruminal fluid to decrease. The inhibition of feed intake caused by hypertonicity also would help to restore tonicity values of ruminal fluid.

Effects of Hypertonicity on Salivation

General Role of Saliva

Saliva plays a critical role in the digestive physiology of the ruminant (Kay, 1966) because it is required for mastication and swallowing, provides a fluid environment for ruminal fermentation, aids in buffering the VFA produced by microbial fermentation, provides nutrients for the ruminal microflora (e.g., urea as a N source) and facilitates passage from the rumino-reticulum. The bilateral glands contributing to the total saliva output are the parotid, submandibular, inferior molar, sublingual, buccal and labial. The pharyngeal and palatine glands also contribute secretions (Kay, 1960). Submandibular secretion is associated with eating only and is thought to be involved primarily with lubrication of the mouth, esophagus and swallowed feed (Carr, 1984). Parotid secretion is elevated markedly at the start of a meal; unilateral parotid secretion rate in sheep increased from

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1.6 ml/min prior to eating to a peak of 6.4 ml/min at 3.2 min into meals of alfalfa hay ranging from 69 g to 328 g DM (Carter et al., 1990). Secretion rate then declined rapidly despite the continuation of enthusiastic feed consumption. Saliva for the ruminant and, in particular, parotid secretion is considered to be the fluid supply for the non-secretory ruminoreticulum (Kay, 1966). Sectioning the parotid ducts and parotid nerves abolishes this source of saliva and leads to a reduction in feed intake associated with a decreased rate of passage (Wilson, 1964). Although parotid saliva constitutes 50 to 60% of the total production under normal circumstances, sectioning the parotid ducts and nerves led to an increase in the saliva output from the remaining glands (Wilson, 1964).

Saliva is the major digestive secretion in the ruminant with daily outputs of 6 to 16 liters in sheep (Kay, 1960) and 98 to 190 liters in cattle (Bailey, 1961b). Approximately 70 to 90% of all the fluid entering the rumen of cattle is saliva (Bailey, 1961b). A recent study estimated that 92 to 96% of ruminal fluid in heifers originated from saliva and water flux through the rumen wall (Garza and Owens, 1989). It also was estimated from this study that water in the rumen originating from drinking water was only 7.5% of total ruminal water for a hay diet and 5.3% for a concentrate diet.

The Na^+ contained in ruminal fluid in the sheep or goat represents more than five times the Na^+ content of the animal's plasma (Blair-West et al., 1965). The control of salivary secretion in the ruminant therefore is linked closely with fluid and electrolyte homeostasis, which includes the involvement of aldosterone in Na^+-depleted ruminants (Blair-West et al., 1965). Ion transport accounted for 66.5% of the total energy expenditure of the sheep parotid gland in vivo (Summers et al., 1989). A negative relationship exists between osmolality of body fluids and saliva production because of a priority to maintain fluid and electrolyte homeostasis in blood.

Neurophysiology of Salivation

Salivation is under neural control. The superior and inferior centers located bilaterally in the medulla oblongata process afferent information from stimuli originating in the mouth, esophagus and areas of the ruminoreticulum. Higher centers also are involved in salivary output; we know that secretion can be elicited as a conditioned reflex by the sight, smells and sounds associated with feeding (Denton, 1957). Stimuli from the mouth and pharyngeal regions are transmitted to the brain by the glossopharyngeal (Comline and Kay, 1955) and lingual nerves (Kay, 1958). Stretching the terminal esophagus, cardia and reticulo-omasal orifice stimulates parotid secretion (Ash and Kay, 1959). The receptors responsible for this reflex are not the epithelial receptors, but the in-series tension receptors in the musculature of the reflexogenic areas (Grovum and Leek, 1985). The afferent fibers are in the vagus nerve. The parotid and submandibular glands have both parasympathetic and sympathetic innervation. However, the parotid gland exhibits secretion independent of this neural supply. Secretion continued after section of the parotid nerve and after chemical blocking of synaptic transmission in the parasympathetic and sympathetic nervous systems using atropine, hexamethonium bromide, phentolamine (Rogitine) and acetazolamide (Diamox; Kay, 1958). The parasympathetic secretory fibers reach the parotid gland by the buccal branch of the mandibular nerve which, in turn, contributes fibers to the parotid nerve (Getty, 1975). Sympathetic fibers are found in the vascular plexuses and branches of the auriculotemporal nerve. Stimulation of both parasympathetic and sympathetic nerves separately increased the secretion rate from the submandibular gland (Ariyakulkaln and Carr, 1980). Parotid nerve activity stimulates parotid secretion (Kay, 1958). After 4 min of continuous stimulation, the ultrastructure of the parotid gland essentially was unchanged (Yamashiro et al., 1987). However, during stimulation, there was moderate dilation of the canalicular system indicative of increased fluid movement into the acini. Sympathetic stimulation has a more complex effect on the parotid gland. Stimulation of the cervical sympathetic nerve led to expulsion of preformed saliva followed by a cessation of flow (Kay, 1958). This was attributed to contraction of the myoepithelial cells located around the acini in the gland. More recently, Patterson and Titchen (1979) repeated this observation with high-frequency electrical stimulation. However, with lower frequencies of shorter duration, they reported an increase in secretion without mechanical expulsion. There also was an

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increase in protein content of the saliva that was due to stimulation of β-adrenergic receptors. This probably was mucoprotein, because the protein elicited did not contain amylase, lipase or albumin (R. R. Carter and W. L. Grovum, unpublished data). Attempts to relate this increased protein content to the microscopic anatomy of the gland have been made. Thus far, it is clear that sympathetic stimulation increased the number and size of intercellular spaces in the gland (S. Yamashiro, R. R. Carter, T. Bast and W. L. Grovum, unpublished data).

Salt Loading the Rumen and Salivation

The effects of the consumption of saline drinking water and hypertonicity of ruminal contents on salivary secretion has been addressed by research conducted in Australia. Parotid salivary flow in sheep was recorded from 5 to 10 d after the provision of drinking water with increasing NaCl content (Tomas and Potter, 1975). As the salinity of drinking water increased, salivary flow rate decreased. This result is associated with the negative relationship between total salivary flow in conscious sheep and the tonicity of ruminal fluid over the range of 200 to 500 mOsmol/kg using ionic and non-ionic solutes (Warner and Stacy, 1977). However, in this work, samples of ruminal fluid and salivary flow measurements were taken between 1 and 5 h after solute loading, by which time the tonicity of plasma also would have increased. Based on findings in anesthetized sheep, the gut is not involved in mediating the response, but the brain and perhaps the liver senses the rise in tonicity of blood to inhibit parotid secretion (Carter et al., 1985).

Effects of Urea Loading and NH_3 on Salivation

Total salivary flow in cattle was found to be 30% lower at 2 h than at 6 h after feeding a purified diet containing urea (Oltjen et al., 1969). Adding urea to the rumen directly also led to a depression in salivary flow, with the depression being more pronounced when the pH was greater than 7.5. High ruminal pH would result in an increased conversion of NH_4^+ to NH_3, which is readily absorbed from the rumen, thus contributing not only to plasma tonicity but also the NH_3 in the systemic circulation when the capacity for uptake by the liver is exceeded. An inverse relationship was found between parotid secretion and ruminal NH_3 concentrations after intraruminal injections of increasing levels of urea in sheep (Obara and Shimbayashi, 1979). There were, however, higher negative correlation coefficients between parotid flow and jugular blood ammonia concentration than between parotid flow and ruminal ammonia concentration. When ammonium salts were injected into a jugular vein, both parotid flow and motility of the rumino-reticulum were inhibited when the NH_3 concentration in blood reached .28 m*M*. There was no apparent effect on parotid flow when urea was dosed into the rumen and blood NH_3 levels were increased up to this concentration. Therefore, inhibition in salivation following the i.v. injections probably was associated with an inhibition of ruminal motility. This would remove a source of stimulation to parotid flow due to reduced inputs to the salivary centers from tension receptors in the rumino-reticulum. Obara and Shimbayashi (1979) suggested that the effects on motility and parotid salivary flow may be signs of ammonia toxicity. These responses probably are patho-physiological rather than physiological.

Vascular Loading of Solutes and Salivation

When ionic or non-ionic hypertonic solutions were infused into a jugular vein to raise the plasma tonicity without affecting that of ruminal fluid, there was an inverse relationship between total salivary flow and plasma osmolality over the range 290 to 330 mOsmol/kg (Warner and Stacy, 1977). In anesthetized sheep, inhibition of parotid salivary flow was greater when hypertonic infusions of ionic and non-ionic solutions were made into the portal vein compared with the same infusions into the caudal vena cava or jugular vein (Carr and Titchen, 1978). The authors suggested that the response from the portal system was neurally mediated with afferent fibers in the vagus nerve and also perhaps the splanchnic nerve. However, it was not possible in any of their experiments to abolish the inhibitory effect on parotid flow, even after sectioning the vagal and splanchnic nerves, and the cervical sympathetic trunks. Carter et al. (1985) identified the

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head, and probably the brain, as being important in sensing hypertonicity to inhibit parotid secretion. Moreover, whereas there is evidence for hepatic osmoreceptors in the guinea pig (Niijima, 1969) and the rat (Adachi et al., 1976), they have not been identified in the ruminant. There was a latency in the parotid response of 10 s for single rapid injections and .5 to 1.5 min for infusions of hypertonic saline into the portal vein of anesthetized sheep lasting 2 to 4 min (Carr and Titchen, 1978). Inhibition of ipsilateral parotid secretion after injections of hypertonic saline into the carotid artery of sheep was associated with an almost instantaneous decrease in the efferent activity in the ipsilateral parotid nerve (Carter, 1988). This supports the conclusion that the brain senses hypertonicity to inhibit parotid secretion. Intravenous injections of arginine vasopressin in conscious sheep did not inhibit parotid salivary flow; therefore, its involvement in the response was ruled out (Carr and Titchen, 1978). An inhibition in parotid salivary flow in sheep was reported when a hypertonic KCl solution (.5 *M*) was infused into the carotid artery contralateral to the parotid gland (Beal, 1977). This response could not be duplicated with NaCl. The osmolality of plasma from the ipsilateral carotid artery was elevated only by 1.3 and 1.8 mOsmol/kg with the KCl and NaCl solutions, respectively. Beal (1977) concluded that K^+ was acting at a site located in the head, because i.v. infusions were less effective in inhibiting parotid secretion than were ipsilateral and contralateral carotid infusions (both produced equivalent inhibitions). The possibility exists that K^+ ions from KCl would depolarize neurons in the brain and thereby interfere with the neural impulse transmissions to the gland. The possibility of reduced blood flow to the gland being involved in the response was dismissed when the response was unchanged after sectioning the ipsilateral cervical sympathetic trunk. Decreased blood flow also was found to be unrelated to an inhibition in secretion from the parotid gland when tonicity of the blood flowing through the gland was increased (Carter, 1988). In this instance, the blood was made hypertonic with a close arterial infusion of NaCl into the blood supply of a parotid gland that had been surgically isolated except for one arterial supply and one vein. However, the tonicity rise required to inhibit secretion by this direct effect on the gland (42 to 70 mOsmol/kg) was greater than that required to invoke the central inhibitory mechanism (7 to 12 mOsmol/kg; Carter et al., 1985). This central mechanism may be a contributing factor in the observation that feeding 800 g of alfalfa hay as eight meals of 100 g compared with one meal of 800 g resulted in 34% and 68% increases in total and unilateral parotid saliva production during eating, respectively (Carter et al., 1990b). The rise in osmolality of plasma with the smaller meals would not be as great as with the one large meal and, therefore, the inhibitory effect of hypertonic blood on parotid secretion would be reduced. This argument is supported by the observation that when sheep were offered a set amount of alfalfa silage-based diets as one meal or four separate meals, the rise in systemic plasma osmolality was only 4.5 mOsmol/kg for the small meals compared with 12 mOsmol/kg for the single large meal (A. Ruiz, personal communication).

The hypothalamic receptors regulating thirst were shown to be sensitive to the concentration of Na^+ in the cerebrospinal fluid (Andersson, 1971). This also was the case in the goat, and infusions of hypertonic saline were associated with a reduction in parotid secretion (Olsson, 1976). Parotid secretion is inhibited after hypertonic NaCl injections into either a carotid artery or intracerebroventricularly.

Effects of Hypertonicity on Feed Intake

General Information on the Control of Feed Intake

Reviews on the control of feed intake abound in the literature, and have recently been categorized as being general, orientated to the dairy cow or the grazing animal, related to forages, concerned with short-term and long-term controls, focused on the involvement of the brain, peptides and hormones or fat depots on intake or being of an integrative nature relating feed intake to ruminal function and rate of passage (Grovum, 1988). Grovum differentiated between controls over intake and what may be called influences on intake. Feed intake is controlled by signals that can be detected by the animal, whereas influences operate by indirectly affecting factors that, in turn, have an effect through the controls over

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intake. For example, distension of the reticulum and cranial sac was considered to be a control over the intake of roughages because this can be sensed by tension receptors located in the musculature of these regions (Leek and Harding, 1975; Grovum, 1979). An increased rate of passage of digesta through the ruminoreticulum would, however, be an influence over intake, because it reflects the ratio of throughput to volume of ruminal contents, which, in turn, is determined by the nature of the diet, rate of digestion, motility, etc. Short-term and long-term controls over intake also should be distinguished. Factors sensed by the animal that initiate and terminate discrete meals are defined as short-term controls. These have been studied to a greater extent than long-term controls, which relate more directly to the energy balance of the animal over periods greater than a day and are important in matching intake to various physiological states such as growth to mature BW, pregnancy, lactation and body condition attained in different seasons.

De Jong (1986) stated that for a component of the blood or gut contents to have a regulatory role in meal initiation or termination, it must change in concentration upon the ingestion of feed, and the change in concentration produced experimentally to test its biological significance must be within the physiological range. These requirements have not been satisfied for many metabolites and hormones that have been ascribed roles in the control of feed intake, except for insulin and perhaps glucagon (de Jong, 1986). This is partly because intake is highly variable and requires supraphysiological levels of the test substance to attain statistically significant depressions in intake. A subject for future research is the possibility that a signal for satiety may come from the change in concentration of a metabolite or hormone, rather than its absolute concentration. The rate of increase in glucose concentration in rats has been shown to be more important than the glucose level per se in signalling the release of insulin (Grodsky, 1972).

Tonicity and the Nonruminant

The osmolality of body fluids fluctuates during the feeding cycle and is determined by solute and water fluxes as well as by the ingestion and excretion of water and electrolytes. The homeostatic regulation of tonicity is fundamental to cellular integrity and function. The greatest change in tonicity during a meal occurs in the contents of the gut, as has been described. The elevated osmotic pressure excites intestinal receptors to inhibit feed intake in humans (Hunt, 1980) and in pigs (Houpt et al., 1979, 1983a,b; Gregory et al., 1987). The intestinal mechanism inhibiting intake appears to act by slowing gastric emptying, thus leading to gastric distension and a depression of intake (Gregory et al., 1987). In the preruminant calf, the gastric emptying response was shown to be mediated by an inhibition of antral motility (Sissons et al., 1988). This response prevented additional substrate from entering the small intestine and further contributing to the osmotic stimulus.

Tonicity and the Ruminant

The contents of the rumen become hypertonic during a meal in the functional ruminant. Ternouth (1967) concluded that an increase in osmolality of ruminal fluid helped explain why intakes were lower by ruminants fed ensiled herbage than by ruminants fed the same herbage as hay. This conclusion was strengthened when similar intake reductions were attained when NaCl, KCl and the sodium salts of the VFA were added to the rumen just prior to feeding (Ternouth and Beattie, 1971). This recently has been confirmed by Grovum and Bignell (1988), who reported similar intake reductions of alfalfa pellets after the ruminal loading of hypertonic NaCl, Na-acetate, Na-propionate and PEG (molecular weight 400). These studies indicated that the nature of the osmotically active particles was not important in determining the intake depression. Bergen (1972) also found an intake reduction when either Na-acetate or NaCl was added to the ruminal contents to elevate tonicity above 400 mOsmol/kg. However, he concluded that ruminal fluid tonicity was not important in controlling intake because ruminal fluid tonicities did not reach 400 mOsmol/kg on the diets used in this work and because only tonicities greater than 400 mOsmol/kg inhibited in vitro cellulose digestion. However, Ternouth (1967) found an inverse linear relationship between voluntary feed intake and the tonicity of ruminal fluid over the entire range of 250 to 400 mOsmol. An inverse linear relationship between intake in sheep and ruminal fluid

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tonicity over the range of 200 to 500 mOsmol/kg also was reported after infusions of hypertonic extracts of fresh and ensiled whole corn plant and NaCl solutions into the rumen (Phillip et al., 1981). Tonicity of ruminal fluid increases during a meal; experimentally imposing such increases within the physiological range limits feed intake. Thus, osmolality should be considered as a factor involved in the regulation of meal size. Although Bergen (1972) found that local anesthetic (20 mg carbocaine) added to the rumen just prior to feeding, along with a Na-acetate or NaCl load, blocked the intake depression in the subsequent 2-h feeding period, the site of action of the local anesthetic was unknown because of the long delay between injecting the anesthetic and measuring intake. Lidocaine-HCl (4,000 mg or 8,000 mg) injected into the reticulum of sheep immediately prior to NaCl loading of the rumen did not block the subsequent inhibitory effect of hypertonicity on feed intake (Carter, 1988). In the studies conducted by Ternouth and Beattie (1971) and Bergen (1972), intakes were recorded 1 and 2 h, respectively, after osmotically loading the rumen; in the work of Phillip et al. (1981), ruminal infusions commenced 3 h prior to feeding. The long time delays between ruminal loading and measuring intake in all three of these studies precluded the identification of the organ or site responsible for sensing the increase in tonicity. Sufficient time elapsed for substantial passage and absorption to occur; therefore, tonicity changes could have been registered beyond the rumen. Carter and Grovum (1988) concluded that the wall of the rumino-reticulum was the site mediating the inhibitory effect of hypertonicity on feed intake. The inhibitory effect of salt loading on intake was not mediated by an effect on motility of the rumino-reticulum (Phillip et al., 1981; Carter, 1988).

By monitoring the tonicity of ruminal fluid in animals given ad libitum access to feed, it could be seen whether meals are initiated when tonicity is low, whether meals cease when tonicity is elevated or whether eating rate decreases during meals as the tonicity increases.

Osmoreceptors

In order for osmolality to be considered as a mechanism controlling intake, it must be sensed by the animal. Epithelial receptors in the reticulum, cranial sac, cranial and longitudinal pillars and, occasionally, in other parts of the rumen were found to be sensitive to osmolality in anesthetized sheep (Leek and Harding, 1975). However, exposure to the unphysiological tonicities of 1,700 mOsmol/kg from NaCl were necessary before a convincing proportion of receptors responded. The observation that increases in the tonicity of ruminal fluid within the physiological range were sensed in the wall of the rumino-reticulum to limit feed intake (Carter and Grovum, 1988) indicates that a population of neuronal receptors exist in this region that appears to be different from that studied by Leek and Harding (1975). Leek (1977) also found that mucosal receptors in the abomasum of sheep responded to tonicities of 1,700 mOsmol/kg. In sheep, duodenal mucosal receptors were found to be insensitive to osmolalities over the range 0 to 1,500 mOsmol/kg (Cottrell and Iggo, 1984). However, this does not preclude the possibility that osmoreceptors exist in the small intestine. Hepatic sensing of osmotic pressure has been demonstrated in the guinea pig (Niijima, 1969) and the rat (Haberich, 1968; Adachi et al., 1976). The afferent discharge rate in the hepatic nerve of the rabbit was changed by altering the Na^+ concentration and the oncotic pressure (due to plasma proteins), but not the osmotic pressure of the perfusing medium (Andrews and Orbach, 1974, 1975). The existence of specific ion receptors or osmoreceptors has not been investigated in the ruminant liver.

Practical Relevance

The knowledge that high levels of dietary NaCl can limit feed intake in ruminants has been applied practically to limit the intake of protein supplements at pasture (Weir and Miller, 1952; Riggs et al., 1953). Buffers included in ruminant diets at excessively high levels also inhibited feed intake. This effect overwhelmed their apparently beneficial effects on digestion (Emerick, 1976; Davis and Clark, 1983; Owens et al., 1983). Phillip et al. (1981) concluded that osmolality was a major factor limiting the short-term intake of corn silage. Ruminal fiber degradation of barley straw was increased when it was treated with sodium chlorite, yet voluntary intake was significantly lower than intake of untreated straw (Ford et al., 1987). The addition of

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sodium chlorite may have increased tonicity of ruminal fluid, which could explain the lower intake. Toxic Cl compounds also may be involved. Reduced ruminal volume has recently been suggested as a contributing factor to the low intake of high-salt roughage diets (Zorrilla-Rios et al., 1989). The low intake and frequent consumption of small meals with feeds that are readily fermentable in the rumen, such as molasses (Preston and Leng, 1980) and other energy-dense concentrates, may be due to the high tonicity and acidity generated in ruminal fluid (Kaufmann et al., 1980). Hypertonic ruminal fluid also has been suggested to be a contributing cause of the damage to the ruminal epithelium in cases of ruminal acidosis (Dirksen, 1970).

Increases in salivary flow in sheep and cattle have been produced following consumption of slaframine-contaminated red clover or intramuscular injections of slaframine, a parasympathomimetic secretogogue isolated from *Rhizoctonia leguminicola*; this was associated with an increased ruminal dilution rate (Froetschel et al., 1984, 1987). Pharmacological stimulation of salivation in steers with slaframine has been associated with increases in liquid outflow from the rumen and abomasum, an increase in the efficiency of ruminal bacterial protein production, decreased ruminal digestion coefficients and increased postruminal digestion of DM, ADF and starch (Froetschel et al., 1989). Future studies should assess the potential growth or milk production benefits of pharmacologically stimulating salivary flow in ruminants.

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