AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 231, No. 5, November 1976. *Printed in U.S.A.*

Absorption and exchange of water across rumen epithelium

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DOBSON, A., A. F. SELLERS, AND V. H. GATEWOOD. *Absorption and exchange of water across rumen epithelium*. Am. J. Physiol. 231(5): 1588–1594. 1976.—The osmotic pressure of solutions in the ventral sac of the rumen of the conscious cow was varied with NaCl or mannitol. The mucosal blood flow measured by HTO clearance was minimal when the lumen contained an isotonic solution and rose threefold when the rumen was hypo- or hypertonic to plasma by 150 mosmol/kg. Thus osmotic gradients across the rumen epithelium stimulated mucosal blood flow. Using osmotic gradients small enough to avoid blood flow stimulation, the net water flow could be enhanced by butyrate, a chemical stimulator of blood flow. Thus water movement was partially limited by blood flow. This implied an appreciable change in osmotic pressure of the capillary blood toward that of the rumen contents. The relative importance of blood flow, membrane permeability, and solute uptake on water transport was assessed. The osmotic pressure in the rumen was stationary when the rumen solution was distinctly hypotonic to plasma. The absorbate in the absence of an osmotic gradient was thus hypertonic. The net uptake of solute increased rapidly when the solution in the lumen was hypertonic to plasma. This gave rise to a more rapid rate of change of osmotic pressure in the rumen under this condition.

tritiated water fluxes; osmotic pressure; osmotic equilibration; mucosal blood flow; hydraulic conductivity; solvent-solute coupling; bovine forestomach

THE RUMEN PROVIDES a readily accessible model situation for the study of absorption from the gut in vivo, uncomplicated by secretion. Since the volume in this organ is only a little less than the volume in the extracellular space of the ruminant (5), any substantial water transfer between these two compartments could be of concern both to normal digestion and to altered regulation in disease. The main components of water interchange are the inflow of saliva and ingested water, outflow and subsequent absorption from the lower gut, and net passage through the absorptive epithelium lining the organ.

There is no consensus concerning a fundamental aspect of water absorption, namely, the relation between net transfer and ruminal osmotic pressure. A linear relationship, unusual for an epithelium, has been observed (7, 18). In contrast, Engelhardt (9, 11) described a more complicated situation in which apparent resistance to osmotic flow was very high for iso- and slightly hypotonic, moderate for hypertonic, and large for hypotonic solutions.

We found that the exchange of HTO was limited by blood flow to the epithelium (8). Since natural membranes (and many artificial ones) generally have a hydraulic conductivity higher than the diffusivity, net water movement could also be expected to be so limited. Nevertheless, when blood flow was stimulated by incorporating butyrate in the rumen solution, no decrease in the resistance to osmotic flow was observed (7).

The present work began by attempting to measure the unidirectional and net movements of water from solutions of differing tonicity in the ventral rumen sac of the conscious cow. Absorption of the volume marker used, [^{51}Cr]EDTA, was found to introduce an artifact into the net water measurements (6). Once this problem was circumvented, a more coherent description of the absorption of water was possible.

METHODS

Observations were made on two nonlactating Jersey cows each provided with a 25-cm fistula into the dorsal sac of the rumen at least a year prior to experimentation. The 21 experiments reported here were made on Gerda. Five confirmatory experiments made on a similar animal, Doris, are not reported. The only differences noted in this smaller cow were the somewhat lower unidirectional and net fluxes of water.

The temporary isolation of the ventral sac of the rumen to permit absorption experiments has been described in detail (7). Briefly, the contents of the ruminoreticulum were removed through the fistula, and the epithelium was rinsed free of debris with warm 150 mM NaCl. A cofferdamlike structure mounted on a small tire inner tube was floated beneath the cranial and caudal rumen pillars. Solutions placed in the ventral sac were thereby effectively isolated within this region. Salivary contamination was continually monitored by marking the saliva with creatinine. It was minimized by aspiration of the saliva from the cranial dorsal rumen sac. The mixing time of the 3-liter solution in the ventral sac was less than 1 min when vigorously stirred with N_2. Water uptake was measured using [^{51}Cr]EDTA as a marker. In eight experiments made before the nature of the absorption of this substance was established, corrections were calculated for marker absorp-

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tion from the regression of its absorption on the mean ruminal osmotic pressure (6). Absorption of [^{51}Cr]EDTA was measured directly in each period of the remaining 13 experiments. When the regression was applied to the later experiments, the two methods agreed within the experimental error. Further details of the procedure will be found in the companion paper to this (6).

The experimental solution contained 20 mM KCl, 2.5 mM Na_2HPO_4, 17.5 mM NaH_2PO_4, pH 6.0, 33 μCi/liter HTO, and 20–200 μM ammonium [^{51}Cr]EDTA, 20 μCi/liter. The osmotic pressure was varied by adding 0–230 mM NaCl or 20 mM NaCl + 0–340 mM mannitol. In some experiments, 20 mM butyrate were substituted for an equivalent amount of chloride. During each experiment, three to five experimental solutions with differing osmotic pressures were introduced into the ventral sac for 35–45 min. The ventral sac was washed twice with 3 liters 150 mM NaCl after each experimental period. Fifty-milliliter samples of ruminal solution were removed at 10-min intervals except in the final five experiments, when the intervals were increased to 20 min. Blood samples were removed before the first experimental solution was introduced and about 5 min after each solution was removed from the rumen.

For HTO scintillation counting (Beckman), 1 ml rumen fluid or plasma ultrafiltrate was added to 10 ml scintillation fluid (Aquasol, New England Nuclear Corp.) and counted to an error of <1%. Quench estimations were made by external standard technique, but were generally too uniform to warrant applying a correction. Interference from the Compton scatter of ^{51}Cr was reduced by allowing it to decay for five or six half-lives, and then correcting for the residual Cr activity. This was generally below 10% of the tritium count. Other chemical methods used are described elsewhere (6).

The unidirectional water fluxes across the epithelium of the ventral sac were calculated using the *expressions 8* and *9* of Berger (2). The flow of water from the blood into the rumen is given by

$$\beta = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A0} - \theta_{B0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}}\right]$$

where A is the volume of the solution in the ventral sac, B is the distribution volume of HTO in the rest of the cow at *time t*, and subscript 0 refers to time *0*. θ is the specific activity. The corresponding flow from rumen to blood is given by

$$\alpha = \frac{A - A_0}{t}\left[\frac{\ln\dfrac{(\theta_A - \theta_B)}{(\theta_{A0} - \theta_{B0})} + \ln\dfrac{B}{B_0}}{\ln\dfrac{B}{B_0} - \ln\dfrac{A}{A_0}} - 1\right]$$

In the control experiments (Fig. 1) in which net water movement was not measured and was assumed to be zero, Berger's *expression 12* was used for calculating the exchange. This is given by

$$\beta = \alpha = \frac{-\ln\dfrac{\theta_A - \theta_B}{\theta_{A0} - \theta_{B0}}}{\left[\dfrac{1}{A_0} + \dfrac{1}{B_0}\right]t}$$

The plasma concentration of HTO at the time of taking rumen samples was estimated from the concentration of samples removed between solutions by an interpolation based on the assumption that the mean rise in plasma HTO concentration was proportional to the amount of HTO absorbed from the rumen solution. The flux from blood to rumen was given a positive, and that from rumen to blood, a negative sign. No correction for isotope effects was made. Since variations between experiments on the same animal were generally low (e.g., 7), all the data collected were combined prior to analysis.

RESULTS

Exchange of HTO

Isotonic NaCl. In order to establish how long the exchange of HTO remained low and stable, the time course of the exchange was observed with isotonic solutions in the rumen (Fig. 1). In the two experiments the exchange was low and steady for 120–130 min, then gradually rose. In other experiments, in which the osmotic gradient varied, there were generally low exchanges with isotonic solutions over this time interval. Certain exceptions are noted hereafter. Observations requiring the exchange to be low were confined to this time interval.

Osmotic gradients. NaCl. When the osmotic gradient across the rumen epithelium was varied by altering the concentration of NaCl, the exchange of HTO was minimal when the osmotic pressure was close to that of the plasma. Any marked departure from isotonicity greatly increased both unidirectional fluxes (Fig. 2).

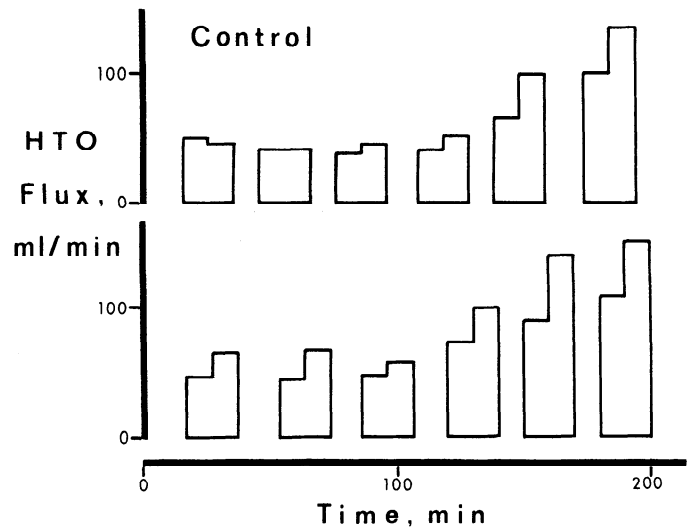

FIG. 1. Trend of HTO exchange across epithelium of ventral sac in absence of known chemical stimulation to perfusion. Two 10-min periods of observation were made with 6 identical solutions made isotonic to plasma with NaCl on 2 separate days. Note rise in flux after 120 min.

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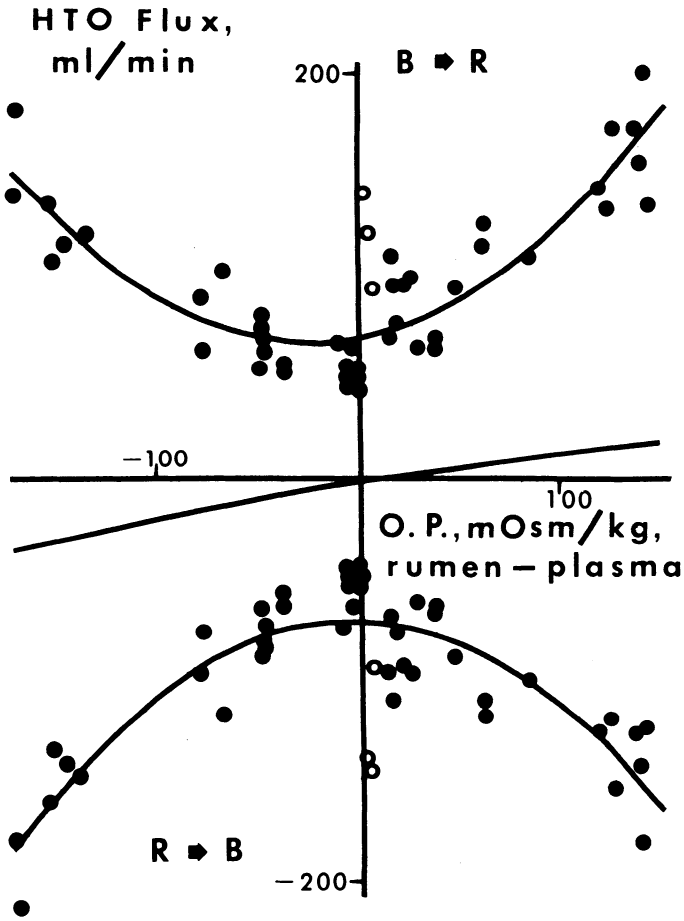

FIG. 2. Relation between HTO clearance and mean osmotic pressure, rumen-plasma. Tonicity was varied with NaCl. Equations of fitted lines are given in Table 1. Middle line is net water flux (cf. Fig. 3). B→R, blood-to-rumen flux; R→B, rumen-to-blood flux. Open circles are points omitted from statistical analysis (cf. text).

When the osmotic difference between plasma and rumen contents was about 150 mosmol/kg, the unidirectional fluxes were about 3 times their value at isotonicity.

When these results were summarized by fitting polynomials of successively higher powers, a significant improvement in fit was observed between first and second orders, with no appreciable improvement thereafter. The coefficients of the quadratics of best fit (Table 1) were calculated with three points close to isotonicity omitted (Fig. 2). These were the only observations which followed immediately an extremely hyper- or hypotonic solution, and two of the three had raised HTO exchange rates. There was, therefore, a suggestion that hysteresis of the HTO exchange could occur. A delayed recovery from enhanced blood flow after butyrate had previously been noted (unpublished observations). Nevertheless, the basic relation between enhanced exchange and increase in osmotic gradient was sufficiently clear.

The flux of HTO across the epithelium has been previously shown to be determined largely by blood flow to the epithelium (8). We assume that the clearance of HTO was a measure of the mucosal blood flow. The visible reddening of the mucosa with departures from isotonicity proved to be accompanied in each case by increased HTO clearance, thereby confirming that epithelial perfusion was changing.

Osmotic gradients. MANNITOL. HTO fluxes when the osmotic pressure in the rumen was altered with mannitol were similar to those observed with NaCl (Table 1). We conclude, therefore, that the changes in HTO fluxes were due primarily to the osmotic effect.

Butyrate solutions. HTO exchange was high at all osmotic gradients when 20 mM butyrate, pH 6.0, was incorporated into the ruminal solutions. Combining observations in two experiments over a range of ±160 mosmol/kg, no dependence of the flux of HTO from the rumen to the blood on the osmotic pressure difference could be detected. The HTO flux from blood to rumen, however, gave a significant regression on the osmotic pressure difference (Table 1). The slope was comparable to that of net water movement.

Additional observations on HTO clearance with low osmotic gradients are given in Table 2. In all experiments the second half of each butyrate period consistently showed a fall in HTO exchange compared to the first half. This was attributed to the fall in butyric acid concentration, due both to absorption and to the rise in ruminal pH (1).

Net Water Movement

Osmotic gradient. NACL. The relation between net water movement across the rumen epithelium and the ruminal osmotic pressure could not be adequately represented by a straight line (Fig. 3), since the second coefficient of a fitted quadratic differed appreciably from zero ($P < 0.001$; col 2, Table 1). When the hypo- and hypertonic sections were separately fitted with straight lines, the slope of the hypotonic section was 0.208 ± 0.011 (SE), whereas the slope of the hypertonic section was 0.134 ± 0.014, a reduction of 36%. Although

TABLE 1. *Coefficients of fitted quadratic and linear equations*

Condition*	Direction	Obs	Coeff $x^2 \cdot 10^4$		Coeff $x \cdot 10^2$		y Intercept		SD
		1	2	3	4	5	6	7	8
NaCl	BTR	44	38.9	±3.2	18.9	±3.4	67	±3.7	19
	RTB	44	−40.2		−2.0		−70		19
Mannitol	BTR	18	44.6	±5.7	19.8	±5.2	55	±7.5	18
	RTB	18	−46.5		−3.0		−57		18
Butyrate	BTR	12			15.4	±6.1	215	±6.9	24
	RTB	12			−0.9	±5.4	−220	±6.1	21
Net water movement, y; mean ruminal osmotic pressure, x									
NaCl	BTR	53	−2.5	±0.5	16.3	±0.5	−2.0	±0.6	3.5
Δ Osmotic pressure, y; mean ruminal osmotic pressure, x									
NaCl		68	−8.5	±0.6	33.3	±0.7	−6.2	±0.7	19
Mannitol		26	−8.5	±0.9	31.6	±0.8	−2.5	±1.6	18

y, HTO flux; x, mean ruminal osmotic pressure. *Osmotic pressure varied with NaCl except for "mannitol." Basal solution without butyrate except "butyrate." Units: net H_2O and HTO flux, ml/min; osmotic pressure, mosmol/kg; ΔOP, ruminal osmotic pressure change in 20 min.

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TABLE 2. *Ruminal osmotic pressure and HTO exchange during a comparison of high (butyrate) and low (chloride) mucosal perfusion rates with low osmotic gradients*

	Treatment			
	Chloride		Butyrate	
	Period 1	*Period 2*	*Period 1*	*Period 2*
Hypotonic soln, ΔOP,* mosmol/kg	−50.0	−44.8	−50.4	−45.0
Error	±2.5			
Hypertonic soln, ΔOP,* mosmol/kg	23.8	12.0	24.6	4.8
Error	±3.2			
HTO clearance, into rumen, ml/min				
Hypotonic soln	68	67	226	185
Hypertonic soln	89	89	257	212
Error	±15		±43	
HTO clearance, out of rumen, ml/min				
Hypotonic soln	79	80	244	202
Hypertonic soln	87	93	258	217
Error	±16		±44	

Two 20-min periods were observed for each solution. OP, osmotic pressure. *Rumen-plasma.

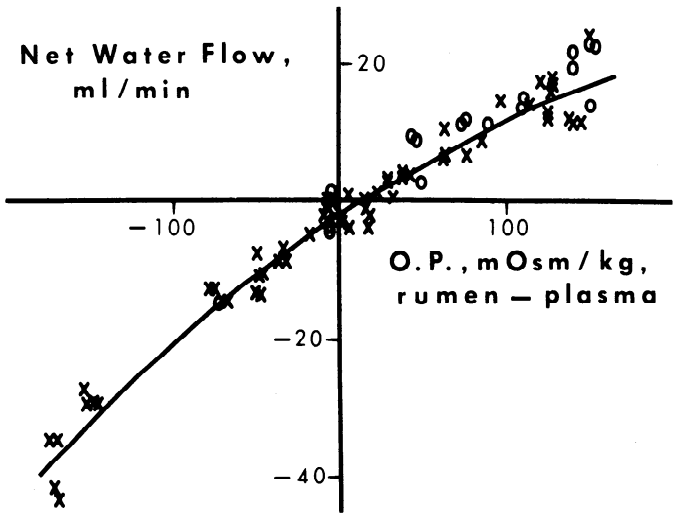

FIG. 3. Relation between net water flow and mean osmotic pressure, rumen-plasma. Tonicity varied with NaCl, crosses; tonicity varied with mannitol, open circles. Quadratic line (Table 1) is fitted to NaCl points.

the net water uptake in the absence of an osmotic gradient was quite small, 2.0 ml/min (col 6, Table 1), it differed significantly from zero ($0.005 > P > 0.001$).

Osmotic gradient. MANNITOL. A similar relation between net water movement and osmotic gradient was observed when the osmotic pressure was varied with mannitol instead of NaCl (Fig. 3). The regression lines for the two substances could not be distinguished over the hypertonic region, whereas there were insufficient data for comparison over the hypotonic region.

We conclude that net water movements were determined largely by the osmotic gradients and were similar whether NaCl or mannitol was used to vary the osmotic pressure in the rumen.

Blood flow. The change in net water movement with osmotic pressure was compared at high and low rates of epithelial perfusion by comparing treatments with and without 20 mM butyrate. Two osmotic gradients were chosen close to isotonicity in order to avoid osmotic stimulation of blood flow. Since low osmotic gradients gave low net water movements, the time between samples was increased to 20 min, and each solution was kept in the rumen for 45 min. Chloride treatments preceded butyrate treatments in order properly to use the available time. The order of imposing the osmotic pressures was alternated between experiments. The relevant conditions were summarized by analysis of variance of appropriate groups, with each experiment constituting an independent observation (Table 2). The following description is based on these analyses with all differences discussed being at $P < 0.05$ level.

The rumen osmotic pressure moved toward the plasma between the first and second pairs of samples, with both chloride and butyrate treatments. The rate of equilibration of HTO altered between sample pairs only with the butyrate. This fall in rate of equilibration was doubtless related to the fall in concentration of butyric acid noted previously. The osmotic pressure of the slightly hypertonic solutions altered more with the butyrate than with the chloride, probably because the butyrate penetrated more rapidly. With NaCl, HTO clearance from rumen to blood was enhanced for the hypertonic situation compared with the hypotonic situation. The important difference between chloride and butyrate was that seen in HTO exchange, chloride being, on an average, 36% of the butyrate for each direction of HTO movement. This indicated a substantial difference in epithelial perfusion between the two treatments.

The effect of the two treatments on net water flux was tested by calculating the regression line of net water movement on mean osmotic pressure difference for each pair of samples in each experiment. The mean slope of these regression lines of net water movement on osmotic pressure difference, rumen − plasma, was 0.160 ± 0.018 (SE) for chloride and 0.244 ± 0.122 ml·kg/mosmol·min for butyrate. The mean net water movement when the osmotic pressure vanished was −4.0 ± 1.57 (SE) for chloride and −6.3 ± 0.85 ml/min for butyrate. The differences in slope between the treatments were analyzed in a paired-*t* test. The butyrate exceeded the chloride by 0.084 ± 0.024 (SE, 5 pairs of observations, $P \simeq 0.025$). We conclude, therefore, that epithelial perfusion limited the net flow of water across the epithelium at low perfusion rates. The intercepts of the butyrate experiments differed significantly from zero ($P < 0.002$). Both the corresponding chloride intercepts, and the paired differences between the intercepts, butyrate − chloride, just failed to be significantly different from zero ($P = 0.05$).

Osmotic Pressure Change

The change of osmotic pressure in the rumen reflects the movement of both water and osmotically active particles. Since the precision with which it can be

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measured compares favorably with that of individual solutes, it provides a useful insight into solute movements which themselves change the osmotic gradients and may directly affect water movements. The relation between osmotic pressure change and ruminal osmotic pressure (Fig. 4) was complex. The osmotic change was more rapid for hypertonic than the corresponding hypotonic solutions in the rumen: the slope of the regression line through the hypertonic points was 2.6 times that of the line through the hypotonic points. The other striking feature was the fall of osmotic pressure when the osmotic gradient across the rumen was zero. The quadratic line of best fit had a y intercept differing from zero ($P < 0.001$, Table 1).

When the osmotic pressure was increased with mannitol, a similar shape of relation between osmotic pressure change and mean osmotic pressure was observed (Table 1). The y intercept did not differ significantly from zero. Moreover, the individual observations in the iso- and hypertonic regions appeared to fall slightly higher than the NaCl observations. This was confirmed by testing the coefficients of quadratics with suitable adjustment for points common to both lines (the basal solution). The intercept was significantly higher for mannitol than NaCl ($P < 0.05$), and the first-order coefficient for mannitol only just failed to be significantly above that of NaCl at the same probability level.

DISCUSSION

Three factors could be identified which affected the relation between the net water movement and the osmotic pressure gradient across the epithelium of the rumen of the conscious cow. The perfusion of the epithelium became important when the epithelial blood flow was low because it partially limited the net water movement. The second factor was the hydraulic conductivity of the epithelium and its unstirred layers, which became more important at high perfusion rates. Finally, transport of solute provided a minor effect.

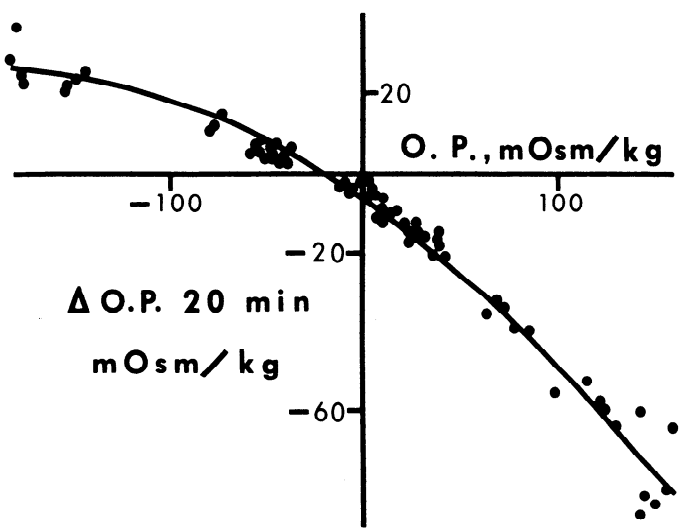

FIG. 4. Relation between fall in ruminal osmotic pressure and mean osmotic pressure, rumen-plasma. Tonicity varied with NaCl. Equation of fitted line is given in Table 1.

Many workers (e.g., 12) have interpreted the fluxes of the isotopes of water in terms of the structure of the epithelial membranes of the gut, tacitly assuming that the epithelium represented the major barrier to the flux in vivo. When the rumen contents were well stirred, the major constraint upon HTO diffusion appeared to be the epithelial blood flow (8). For the purpose of this discussion, we shall tentatively assume that HTO diffusion is limited entirely by the blood flow and ignore the contribution of the membrane. Whereas this approach risks underestimating the mucosal perfusion by an undefined amount, the arguments employed should readily be modified when a quantitative relationship between mucosal flow and HTO clearance becomes available.

Previous efforts to detect an effect of epithelial perfusion on net water movement (7) were confounded by our failure to recognize that large osmotic gradients stimulate blood flow. The large increase in exchange of HTO across the rumen epithelium with gradients of osmotic pressure in either direction (Fig. 2) provides evidence of this increase in epithelial perfusion. Although working with small osmotic gradients placed severe constraints on the experimental design, the net water movement was consistently lower when the exchange of HTO was low (Table 1). Blood flow limited net water movement, therefore, within the range of flow encountered in the quietly standing animal, once chemical stimulation was minimal. This state would correspond to the normal animal when fasting.

Once the dependence of net water flow on blood flow was established, the question of the relative importance of blood flow and the hydraulic conductance of the epithelium in limiting this flow arose. If HTO equilibration and net water movement were equally dependent on blood flow, a change in blood flow would give the same relative change in clearance of both HTO and net water. This is clearly not the case, since the blood flow decrease from butyrate to chloride depressed the flow of water for a unit change of osmotic gradient by 34%, whereas simultaneously the clearance of HTO dropped by 64% (Table 2). An additional barrier, presumably the epithelium and its adherent unstirred layers, prevents the equilibration of water under its osmotic gradient compared with HTO diffusion.

By considering how closely the blood draining the rumen epithelium equilibrates osmotically with the rumen contents, the situation may be further analyzed. If Q_a and Q_v are the water flow in the arteriolar and venular blood supply to and from the epithelium and O_a and O_v are their respective osmotic pressures and solute addition by absorption is ignored, then $Q_a \times O_a = Q_v \times O_v$. The net water entering the rumen, $W = Q_a - Q_v$. Therefore, $W = Q_a(1 - O_a/O_v)$. For complete osmotic equilibration of the venular blood with rumen contents, the rumen osmotic pressure $O_r = O_v$. Thus, W can be predicted for this case if we use the clearance of HTO as a measure of Q_a.

On this basis the conditions in Table 2 would predict a slope of the net water flow for unit osmotic pressure difference of 0.29 and 0.86 ml·kg/mosmol·min for chloride and butyrate, respectively. The observed slopes were 55 and 28% of those expected for complete osmotic

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equilibration at low and high rates of perfusion, respectively. Thus, the epithelium and its stagnant layers limited the equilibration by 45% at low, and 72% at high perfusion rates, thereby indicating that these barriers appreciably restrict net water movement at the lowest blood flows encountered, and became more important at the higher perfusion rates. They would, therefore, become the dominant factor at high osmotic gradients, or with intense rumen fermentations, when the chemical stimulation of blood flow is maximal.

Osmotic gradients across the rumen epithelium can be regarded, together with high concentrations of carbon dioxide or butyric acid (8, 16), as naturally occurring chemical stimulants to epithelial perfusion. The relatively high degree of osmotic equilibration at low rates of perfusion suggests that this might have the useful function of damping down otherwise intolerably large changes in osmotic pressure in the venules draining the epithelium. In the calf, hemoglobinuria is a feature of water intoxication, the excessive intake of water due to poor management practices (3). Perhaps the response in blood flow to osmotic stimulation is insufficiently developed to cope with this situation.

The absorption of solutes, a major function of the rumen, will effect water movement indirectly by altering the osmotic gradient. A direct effect of solute transport on water absorption by coupling of the two fluxes would be manifested by the movement of water in the absence of an osmotic gradient. Under this circumstance, however, the rate of uptake though significant (Table 1) was small enough to be difficult to detect. Indeed, the ruminal osmotic pressure needed to be raised by only 13 mosmol/kg above the plasma to offset this small uptake. It was concluded that direct coupling plays a minor role in water movements across the epithelium when the osmotic gradient is low.

Superimposed on the major changes of HTO flux with ruminal osmotic pressure, a consistent minor pattern was observed under the three experimental circumstances, namely when osmotic pressure was varied with either NaCl in the presence or absence of butyrate, or with mannitol in the absence of butyrate. The flux of HTO into the blood was the same for a hypotonic solution as for a solution hypertonic to the same degree (Fig. 2). Quantitatively this symmetry is reflected by the first-degree coefficients of the fitted quadratics (RTB, col 4, Table 1), none of which differed significantly from zero. In contrast, the first-degree coefficients of the flux into the lumen (BTR, col 4, Table 1) were all significant (various $P < 0.05$) and did not differ from the corresponding coefficient of the net water flux (col 4, Table 1). In other words, the net water flux was subtracted from the HTO flux into the lumen, rather than added to the flux into the blood.

If the exchange of HTO in the absence of net water movement is considered to represent the blood flow (8), it is reasonable to equate the flux of HTO into the lumen with the flow of blood into the capillary bed of the epithelium and the flux of HTO into the blood with the flow of blood from the capillary bed when there is a net movement of water. This interpretation would mean that as water was added to or subtracted from the blood in the capillaries by osmosis, the blood flow from the capillaries into the venules tended to remain constant while the arterial supply was reduced or augmented, respectively. This could be regarded as a manifestation of autoregulation in this capillary bed.

In both sheep and goats (10, 17), the fall in the ruminal osmotic pressure to below that of the plasma has been attributed to solute absorption. The observations on the cow confirm this. The absorbate with zero osmotic gradient is clearly hypotonic (Fig. 4), indicating that osmosis does not produce osmotic equilibrium with the aborbed solute. This could arise specifically from a standing gradient of solute in the interstitial fluid (4) or more generally be related to the inability of the capillary blood to equilibrate osmotically with the rumen contents.

When mannitol replaced most of the NaCl in the rumen solution, the rate of fall in osmotic pressure with isosmotic rumen contents was reduced compared to NaCl. This probably reflected a lower uptake of solute under these circumstances. Presumably the net water flux would be reduced, but the data available were too scattered to show this effect.

The rate of change of osmotic pressure in the hypertonic region is conspicuously greater than in the hypotonic region (Fig. 4). This can be attributed neither to the differing rate of water movement, nor to the volume changes which in either case would give effects in the opposite direction. To examine further the effects of solute absorption, the uptake of "osmotically active particles" was calculated by treating the osmotic pressure as a concentration and calculating the amount absorbed, using the known rumen volume (Fig. 5). There was an uptake of "osmotically active particles" from all solutions, but this was greatly accentuated from hyper-

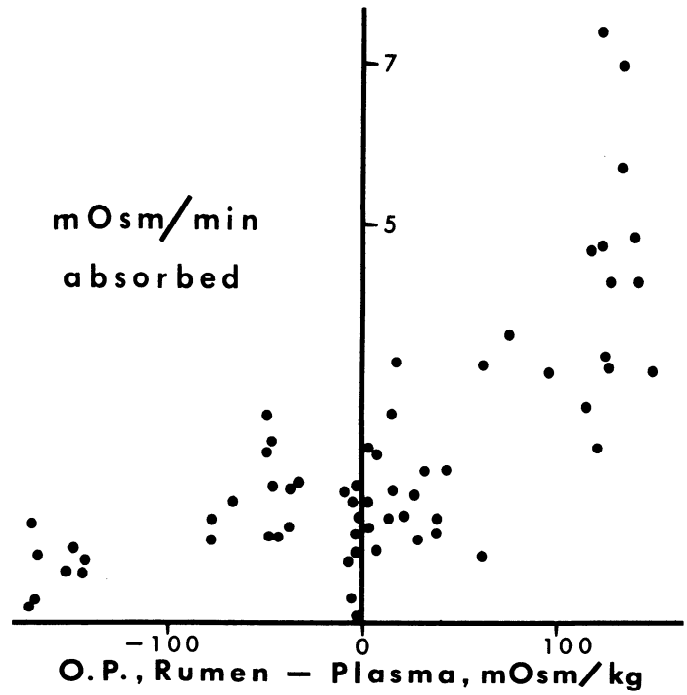

FIG. 5. Relation between absorption of "osmotically active particles" and mean osmotic pressure, rumen-plasma. Tonicity varied with NaCl.

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tonic solutions. The scatter of points increased with tonicity as expected, due to the increase in number of particles with the tonicity at constant volume. A small uptake of osmotically active particles persisted throughout the hypotonic and isotonic regions. When the rumen was hypertonic, there was an appreciably increased uptake, which rose faster than the increase in osmotic concentration in the rumen. It appeared, therefore, that the permeability to major constituents of the ruminal solution has been raised. There is direct evidence (8) that the epithelium becomes much more permeable in the hypertonic range to a minor constituent of the rumen solution, the large anion, Cr-EDTA.

We conclude that the faster osmotic equilibration of hypertonic solutions was due to increased permeability to solute. This would tend to protect the animal from extracellular dehydration into a postfeeding hypertonic rumen, at the expense of transferring an osmotic load to the kidneys. An increase of solute permeability might reduce net water movement into the rumen if the reflexion coefficient were appreciably reduced, but there was no direct evidence that this takes place. These changes in solute permeability take place within a range of hyperosmolality normally encountered during a feeding cycle. They should be distinguished from those changes in permeability produced by high concentrations of KCl within the rumen, which occur at higher ruminal osmotic pressure and result in irreversible epithelial damage (13).

In recent work on the jejunum of the anesthetized rat in which blood flow was recognized as an important factor limiting the flux of HTO, the observations differed in several important respects from those reported here (15, 19). Osmotic gradients which produced a marked increase in both unidirectional fluxes of HTO in the rumen, gave very much smaller changes in the rat jejunum in directions which were consistent with solvent drag aiding or opposing the respective fluxes when the osmotic gradient was reversed. Moreover, no effect of organ blood flow on net water flux could be detected. The basis for these differences is at present not clear.

A reconciliation of the different relations observed between water absorption and ruminal osmotic pressure can now be offered. The previous observations of a linear relationship (7, 18) arose from a failure to appreciate the absorption of the volume marker under hypertonic conditions. The observations on a pouch of rumen epithelium in the goat (9, 11) were similar to Fig. 3, except for inflexions producing a much flatter region where the osmotic gradient was low. If epithelial perfusion were relatively lower in this preparation, water uptake would become much more blood flow dependent. Very little net passage of water to or from the pouch would then occur at tonicities close to the plasma. Blood flow stimulation by osmotic gradient would then cause a disproportionate increase in water flow at larger osmotic gradients.

We thank Dr. D. S. Robson for advice about statistical procedures and Dr. W. J. Arion for providing the facilities to count tritium.

This investigation was supported by National Institutes of Health Grant AM-04679.

Received for publication 4 September 1975.

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