The effects of intraruminal infusions of sodium bicarbonate, ammonium chloride and sodium butyrate on urea metabolism in sheep

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1. Three sheep fitted with rumen cannulas were fed hourly a daily ration of 1000 g pelleted-grass cubes, and during four successive 2-week periods were intraruminally infused (0·45 l/d) with solutions containing sodium chloride (0·47 mol/d), sodium bicarbonate (0·47 mol/d), ammonium chloride (0·47 mol/d) and sodium butyrate (0·47 mol/d). Each solution, except that for $NaHCO_3$, was adjusted to pH 7 before infusion, and provided equal sodium intakes for sheep in all periods.

2. In the final week of each infusion period, a balance trial was conducted and on separate days each sheep was continuously infused with [^{14}C]urea and $NaH^{14}CO_3$ intravenously and $NaH^{14}CO_3$ intraruminally. Carbon transfer rates between blood urea, blood bicarbonate and rumen fluid bicarbonate were calculated from the specific radioactivity of urea and bicarbonate samples and isotope infusion rates during each experimental period.

3. There was no significant effect of intraruminal infusions on N balance, and with the exception of sheep infused with NH_4Cl, all sheep utilized apparently digested N with similar efficiency for N retention. Sheep infused with NH_4Cl (6·2 g N/d) excreted the equivalent of 93% of the infused N as urea in urine.

4. Infusion of $NaHCO_3$, NH_4Cl and sodium butyrate significantly ($P < 0·05$) increased the rumen fluid concentrations of bicarbonate, ammonia and butyric acid respectively, and all infusions significantly ($P < 0·05$) increased total volatile fatty acid concentrations. Both $NaHCO_3$ and sodium butyrate significantly ($P < 0·05$) increased the pH of rumen fluid. There was no significant effect of infusion on the proportions of propionic acid or the osmolality of rumen fluid.

5. Intraruminal infusions of NH_4Cl significantly ($P < 0·05$) increased and infusion of sodium butyrate significantly ($P < 0·05$) decreased plasma urea concentrations. Sheep infused with NH_4Cl had higher rates of urea synthesis and urinary urea excretion compared with sheep on the other treatments, and a significantly ($P < 0·05$) lower proportion of urea synthesized by these sheep was degraded in the digestive tract. Sheep infused with sodium butyrate degraded a significantly ($P < 0·05$) greater amount (3·2 g N/d) and proportion (0·24) of total urea synthesis in the rumen than did sheep infused with NaCl. Corresponding values for the control (NaCl) sheep were 1·5 g N/d and 0·13 respectively. There was no significant effect of other infusions on the amount of urea recycled to the rumen or on the distribution of total urea degradation between the rumen and lower digestive tract. Plasma urea clearance to the rumen was significantly ($P < 0·05$) increased during sodium butyrate infusion, and the clearance of urea to the lower digestive tract was significantly ($P < 0·05$) decreased during NH_4Cl infusion.

6. The mechanism by which urea entry into the rumen is regulated by rumen metabolite levels is discussed.

The rate at which urea is recycled to and degraded in the digestive tract of ruminants has been shown to vary with the protein content of the diet (Cocimano & Leng, 1967; Faichney & White, 1977), the presence of readily fermentable carbohydrate in the ration (Cocimano & Leng, 1967; Potthast *et al.* 1977; Engelhardt *et al.* 1978; Kennedy, 1980; Norton *et al.* 1982), the level of digestible organic matter intake (Kennedy & Milligan, 1978; Kennedy, 1980), the age of the animal (Allen & Miller, 1976), environmental temperature (Kennedy & Milligan, 1978) and with time after feeding (Varady *et al.* 1969).

However, less is known about the specific factors regulating urea entry into the digestive tract of feeding ruminants, although the likely site of increased entry is through the rumen wall from blood (Kennedy & Milligan, 1978; Norton *et al.* 1982). Studies with temporarily isolated rumen preparations have demonstrated that various end-products of fermentation

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affect the rate of urea entry into the rumen. Ammonia concentrations in rumen fluid have been inversely correlated with urea recycling to the rumen (Weston & Hogan, 1967; Thornton, 1970; Harrop & Phillipson, 1974; Kennedy, 1980) and bacterial urease activity in the rumen wall has been suggested as a regulatory factor in urea transport from blood to rumen fluid (Houpt, 1970; Cheng & Wallace, 1979). Volatile fatty acids, particularly butyric acid, stimulate urea entry into the rumen (Dobson *et al.* 1971; Hinderer & Engelhardt, 1976), and temporarily isolated bovine rumen preparations have a high permeability to urea when gassed with CO_2 (Thorlacius *et al.* 1971; Hinderer & Engelhardt, 1976). The synthetic gastric hormone, pentagastrin, has also been shown to stimulate urea entry into the rumen (Harrop & Phillipson, 1971). Although both butyric acid and CO_2 stimulate blood flow to the rumen epithelia (Sellers *et al.* 1964; Thorlacius, 1972), increased blood flow rate alone does not increase the rate of urea passage in the rumen (Dobson *et al.* 1971).

The experiments with urea recycling in feeding animals may be generally criticized for a lack of information on the effects of diet on fermentation patterns in the rumen associated with increased urea recycling rate, and the relevance of studies with temporarily isolated rumen preparations to the feeding animal are questionable. In a previous study, increased urea recycling to the rumen was associated with increased concentrations of butyric acid in rumen fluid and with high rates of CO_2 production from fermentation, but it was not possible from these experiments to relate specific metabolite changes with altered rates of urea recycling (Norton *et al.* 1982).

The following experiment was therefore designed to study the effects of intraruminal infusions of sodium butyrate, sodium bicarbonate and ammonium chloride on urea metabolism in sheep offered a pelleted-grass diet. Infusion rates were selected to produce a change in metabolite concentration in rumen liquor within the normal physiological range, and urea recycling to the rumen and lower digestive tract was determined with an isotope dilution technique.

MATERIALS AND METHODS

Animals, diet and management

Three Scottish Blackface wethers (8 months old), fitted with rumen cannulas, were introduced to a diet of pelleted-grass cubes over a 3-week period and maintained on this diet for the duration of the experiment. The mean live weight of the sheep was 41·2 kg (range 39·4–44·3), and all gained weight (80 g/d) during the experiment. All sheep were held in individual metabolism cages in a temperature-controlled room and were offered 1000 g grass cubes (as fed) daily from an hourly feeding machine. The average composition of the pelleted-grass diet was: 893 g dry matter (DM)/kg air-dry food, 25·7 g nitrogen and 916 g organic matter (OM)/kg DM. The DM, OM and N content of the food varied slightly between the different infusion periods, and actual values for N and OM intakes may be calculated from the results given in Table 1 (see p. 268). Drinking water was available at all times, and with the exception of one sheep in the first experimental period, all food offered was consumed.

Experimental procedure

The experimental period of 8 weeks was divided into four 2-week periods, and during each period, all sheep were intraruminally infused (0·45 l/d) successively with one of the following solutions in the order shown: sodium chloride (1·04 mol/l), sodium bicarbonate (1·04 mol/l), ammonium chloride (1·04 mol NH_4Cl and 1·04 mol NaCl/l) and sodium butyrate (1·04 mol butyric acid and 1·04 mol sodium (as NaOH and NaCl)/l). All solutions,

except $NaHCO_3$ (pH 8·2), were adjusted to pH 7 for infusion. These infusion solutions provided a constant Na intake (10·8 g/d) to all sheep during each infusion period.

In each 2-week period, the first week was allowed for adaptation to infusion treatment and balance trials and isotope infusion experiments were conducted in the second week of this period. Urine was collected continuously by aspiration from a rubber belly tank into a bottle containing glacial acetic acid as preservative, and after subsampling, urine was stored frozen until analysed. Faeces were collected daily and also stored frozen. On the tenth day of each infusion period, each sheep was fitted with a jugular vein catheter and was continuously infused intravenously with a sterile saline solution (9 g NaCl/l) containing [^{14}C]urea (0·12 μCi/ml; 0·8 μmol/ml; 0·3 ml/min) for 24 h. An intravenous infusion of a sterile saline solution containing $NaH^{14}CO_3$ (0·13 μCi/ml; 1·2 μmol/ml; 0·3 ml/min) immediately followed the [^{14}C]urea infusion and was also maintained for 24 h. At the end of the infusion, the jugular catheter was removed and an intraruminal infusion of $NaH^{14}CO_3$ in water (0·13 μCi/ml; 1·2 μmol/min) was maintained for a further 24 h. In the final 8 h of this infusion, the jugular catheter was removed and an intraruminal infusion of $NaH^{14}CO_3$ rumen fluid sampled from an indwelling ruminal probe. After removal of an aliquot for bicarbonate determination, heparinized blood was centrifuged at 1500***g*** for 20 min, and plasma stored at −20°. Rumen fluid was immediately subsampled for bicarbonate determination (0·5 ml), ammonia (10 ml diluted with 10 ml 0·2 M-hydrochloric acid), volatile fatty acids (VFA) (4 ml added to 1 ml metaphosphoric acid) and pH and osmolality were immediately determined on the remaining sample.

Analytical methods

Urea in blood and urine was determined by a modification of the Boehringer test (Fawcett & Scott, 1960). Plasma was protein-precipitated before urea determination (Cocimano & Leng, 1967). The concentration and specific radioactivity (*SR*) of bicarbonate in blood and rumen liquor were determined by methods described by Norton *et al.* (1982). Ammonia in rumen fluid and total N in food, faeces and urine were determined by the Kjeldahl method, and the concentrations and proportions of VFA in rumen fluid were determined on a Pye 204 gas–liquid chromatograph (Cottyn & Boucque, 1968). Osmolality of rumen fluid samples was measured in an Advanced Laboratory Osmometer (model 36: Advanced Instruments Inc., Massachussets) and pH determined on a digital pH meter.

Calculations and statistical methods

The irreversible loss of bicarbonate-C from blood and rumen fluid, and urea-C from blood, the relative contributions of primary (infused) pool to secondary pools and the transfer rates of C between blood urea, rumen fluid bicarbonate and blood bicarbonate were calculated by methods previously described (Norton *et al.* 1982). Analysis of variance was used to determine the significance of differences between sheep and between treatments, and least significant difference (LSD) calculated to distinguish treatment differences. In the first infusion period, one sheep refused food and a missing value was calculated (Steele & Torrie, 1960). The subsequent analysis of variance was corrected for bias caused by the use of a missing plot value in the statistical analysis.

RESULTS

The effect of intraruminal infusions on diet utilization

Small differences in feed N and DM content between treatment periods resulted in small differences in N and OM intakes for sheep in the different infusion periods. Table 1 shows mean values, with their standard errors, for the effects of the different infusions on the

Table 1. *Mean values for the digestibility and utilization of dietary organic matter and nitrogen, and for water intake and urine excretion of sheep intraruminally infused (0·47 mol/d) with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate	SE of mean
Organic matter					
Apparent digestibility	0·709	0·687	0·706	0·718	0·008
Apparently digested intake (g/d)	576_{ab}†	560_a	589_{bc}	612_c	7*
Nitrogen					
Apparent digestibility	$0·678_a$	$0·655_a$	$0·717_b$	$0·647_{ac}$	0·007*
Faecal excretion (g N/d)	7·71	7·89	8·33	7·73	0·16
Urinary excretion (g N/d)	$12·44_a$	$11·36_a$	$18·14_b$	$10·84_a$	0·74*
N balance (g N/d)	3·80	3·61	2·99	3·32	0·84
Apparently digested N retained	0·240	0·238	0·142	0·234	0·051
Water intake (l/d)	2·97	3·03	3·31	2·90	0·12
Urine volume (l/d)	$1·75_a$	$1·78_a$	$1·90_a$	$1·47_b$	0·05*

* Significant difference between treatment means ($P < 0·05$).
† Values within a line with different subscripts differ significantly ($P < 0·05$).

apparent digestibility of OM and N, for N excretion in urine and faeces and for water intake and urine volume. Each treatment supplied the same daily infusion of sodium (10·8 g/d), and treatment effects are described as differences from the NaCl treatment. Sodium butyrate infusion significantly ($P < 0·05$) increased apparently digested OM intake (ADOM) by 36 g/d without affecting either N or OM digestibility, but significantly ($P < 0·05$) decreased daily urine volume.

NH_4Cl infusion increased total N intake by 6·2 g/d, and the significant ($P < 0·05$) increase in urinary N excretion by these sheep accounted for 93% of the N infused. The additional N load was excreted in urine by an increased daily volume of urine (9%) and an increased concentration of N in urine (36%). There was no significant effect of infusion treatment on N balance, and with the exception of sheep infused with NH_4Cl, sheep utilized apparently digested N with a similar efficiency for N retention in tissues.

The effect of intraruminal infusion on rumen fluid constituents

Table 2 shows mean values, with their standard errors, for the concentrations and proportions of VFA in rumen fluid, for bicarbonate and ammonia concentrations and for the pH and osmolality of rumen fluid from sheep infused with the different solutions. $NaHCO_3$ significantly ($P < 0·05$) increased bicarbonate concentrations and the pH of ruminal liquor, but had no significant effect on the concentrations and proportions of VFA, ammonia concentration or the osmolality of rumen fluid. NH_4Cl infusion significantly ($P < 0·05$) increased rumen ammonia concentration, but neither pH nor osmolality were significantly affected by this infusion. Sodium butyrate infusion significantly ($P < 0·05$) increased the concentration and proportion of butyric acid and decreased the proportion of acetic acid in rumen fluid, and increased pH. There was no significant effect of sodium butyrate infusion on either ammonia concentrations or osmolality of rumen fluid.

Bicarbonate metabolism in ruminal liquor and blood

Table 3 shows mean values, with their standard errors, for the concentrations and irreversible loss of bicarbonate-C from blood and rumen fluid of sheep infused with the different solution. Values for the percentage contribution of primary (infused) pool to secondary pools during each isotope infusion are also presented.

Table 2. *Mean values for the concentrations of volatile fatty acids (total and individual), bicarbonate and ammonia in rumen fluid, and for pH and osmolality of rumen fluid from sheep given a pelleted-grass diet and intraruminally infused (0·47 mol/d) with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate	SE of mean
Total VFA (mmol/l)	91	108	111	117	8
Individual VFA (mmol/mol total VFA)					
Acetic acid	618_a†	600_a	625_a	545_b	14*
Propionic acid	239	244	246	252	14
Butyric acid	113_a	112_a	95_a	170_b	6**
Higher acids	30	44	34	33	7
Ammonia (mg N/l)	71_a	65_a	171_b	53_a	12**
pH	$6·22_a$	$6·63_b$	$6·17_a$	$6·58_b$	0·09*
Osmolality (mosmol/kg)	307	308	307	319	11

* $P < 0·05$, ** $P < 0·01$.
† Values within a line with different subscripts differ significantly ($P < 0·05$).

Table 3. *Mean values for the concentrations and irreversible losses of bicarbonate from rumen fluid and blood, and for the relative contributions of bicarbonate and urea-C to the different pools of sheep given a pelleted-grass diet and intraruminally infused (0·47 mol/d) separately with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate	SE of mean
Rumen fluid bicarbonate					
Concentration (g C/l)	$0·386_a$†	$0·543_b$	$0·326_a$	$0·447_{ab}$	0·032*
Irreversible loss (g C/d)	132·0	118·7	115·9	113·3	7·8
Percentage of rumen bicarbonate from					
Blood bicarbonate	33·6	33·9	37·7	47·3	4·8
Blood urea	$1·3_a$	$1·1_a$	$1·3_a$	$2·5_b$	0·2*
Blood bicarbonate					
Concentration (g C/l)	$0·268_{ac}$	$0·315_b$	$0·241_a$	$0·286_c$	0·007**
Irreversible loss (g C/d)	261·8	274·8	247·1	270·1	19·2
Percentage of blood bicarbonate from					
Rumen bicarbonate	49·3	34·3	37·8	35·1	6·0
Blood urea	1·0	0·8	1·1	2·2	0·4

* $P < 0·05$, ** $P < 0·01$.
† Values within a line with different subscripts differ significantly ($P < 0·05$).

Although there was a significant ($P < 0·05$) effect of treatment on the concentrations of bicarbonate-C in rumen fluid, there was no significant effect of infusion treatment on the irreversible loss of bicarbonate-C from rumen fluid. There was a highly significant correlation (r 0·90, $P < 0·01$) between bicarbonate concentrations in rumen fluid and pH. The infusion of $NaHCO_3$ significantly ($P < 0·05$) increased bicarbonate concentrations in blood. The irreversible loss of bicarbonate-C from blood was significantly correlated (r 0·83) with blood bicarbonate concentrations. A significantly ($P < 0·05$) greater proportion of rumen bicarbonate-C was derived from blood urea-C when sodium butyrate was infused, and for this infusion there was a trend for a greater proportion of rumen bicarbonate to be derived from blood bicarbonate than that found for the other infusion treatments.

Table 4. *Mean values for plasma urea concentration, irreversible loss of urea from blood, urinary urea excretion and degradation of urea in the rumen and lower digestive tract of sheep given a pelleted-grass diet and intraruminally infused (0·47 mol/d) separately with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate	SE of mean
Blood urea					
Concentration (mg N/l)	111_a†	108_a	162_b	99_a	6**
Irreversible loss (g N/d)	$19·98_a$	$20·83_a$	$24·92_b$	$20·25_a$	0·62**
Urinary urea (g N/d)	$8·98_a$	$7·75_a$	$15·91_b$	$7·31_a$	0·59**
Total urea degradation‡					
g N/d	11·25	13·31	9·33	13·44	1·38
As a percentage of urea synthesized	$55·6_a$	$63·2_a$	$37·0_b$	$64·8_a$	3·2*
Degradation of urea in the rumen					
g N/d	$1·51_a$	$1·39_a$	$1·79_a$	$3·21_b$	0·36*
Clearance (l/d)§	$13·6_a$	$12·9_a$	$11·0_a$	$32·4_b$	3·4*
As a percentage of total urea degradation	13·4	10·4	19·2	23·9	3·9
Degradation of urea in lower digestive tract					
g N/d	9·74	11·92	7·54	10·23	1·48
Clearance (l/d)	$87·7_{ab}$	$110·4_a$	$46·5_b$	$103·3_a$	12·1*
As a percentage of total urea degradation	86·6	89·6	80·8	76·1	3·9

* $P < 0·05$, ** $P < 0·01$.

† Values within a line with different subscripts differ significantly ($P < 0·05$).

‡ Total urea synthesis − urinary urea excretion. Total urea synthesis rate derived from solution to three pool model and was on average 1·0% greater than measured irreversible loss rate.

§ Clearance (l/d) = g urea N transferred from blood to degradation site/plasma urea N concentration (g N/l).

The effect of intraruminal infusions on urea metabolism

Mean values, with their standard errors, for plasma urea concentration, irreversible loss of urea from blood, excretion of urea in urine and for urea degradation in the rumen and lower digestive tract are shown in Table 4. Infusion with NH_4Cl significantly ($P < 0·05$) increased plasma urea concentration and urinary urea excretion. Sodium butyrate infusion significantly ($P < 0·05$) increased both the amount and proportion of total urea synthesis that was degraded in the rumen. The clearance of urea from blood into the rumen was also significantly ($P < 0·05$) increased during sodium butyrate infusion. Urea degradation in the lower digestive tract was not affected significantly by treatment when expressed as either amount degraded (g/d) or as the proportion of total urea degradation occurring in the whole digestive tract although the clearance of urea into the lower digestive tract was decreased when NH_4Cl was infused.

Models of carbon metabolism in sheep during infusion with the different treatments

The values shown in Tables 3 and 4 were used to calculate the rates of C transfer between blood urea, blood bicarbonate and rumen fluid bicarbonate pools for sheep during each infusion treatment. Table 5 gives mean values, with standard errors, for the rates of C movement between the above-mentioned pools and for the rates of production and excretion of C from each pool. As described previously, sodium butyrate infusion significantly ($P < 0·05$) increased urea recycling to the rumen, and NH_4Cl infusion significantly ($P < 0·05$) increased the rate of urea synthesis and urinary urea excretion. However, there were no significant effects of infusion treatments on the quantitive exchange of bicarbonate-C between the ruminal and blood pools, or on the amounts of bicarbonate-C arising from tissue metabolism or rumen fermentation.

Table 5. *Mean values for the transfer rates (g C/d) between blood urea, rumen fluid bicarbonate and blood bicarbonate in sheep given a pelleted-grass diet and intraruminally infused (0·47 mol/d) separately with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate	SE of mean
Blood urea (g C/d)					
From blood bicarbonate	8·67	9·02	10·82	8·90	0·65
To degradation in rumen	$0·65_a$†	$0·60_a$	$0·77_a$	$1·37_b$	0·15*
To post-ruminal tract	4·17	5·10	3·23	4·40	0·64
To urine	$3·85_a$	$3·32_a$	$6·82_b$	$3·13_a$	0·24**
Blood bicarbonate (g C/d)					
From tissue metabolism	207·8	203·2	179·5	210·4	11·2
From rumen bicarbonate	105·1	107·4	109·4	113·8	15·3
To expiration and excretion	228·3	259·0	228·9	257·8	10·5
To rumen bicarbonate	78·8	45·2	51·7	63·4	11·6
Rumen bicarbonate (g C/d)					
From fermentation	79·2	89·4	83·0	71·5	9·7
To eructation	53·6	27·8	26·2	22·4	8·9

* $P < 0·05$, ** $P < 0·01$.

† Values within a line with different subscripts differ significantly ($P < 0·05$).

DISCUSSION

In these experiments, sodium butyrate infusion stimulated urea recycling to the rumen, and contrary to expectation neither ammonia nor bicarbonate concentration in rumen fluid affected the rate of urea recycling. However, there was no significant effect of any infusion treatment on N balance or on efficiency of N utilization. When NH_4Cl was infused, rumen ammonia concentrations increased threefold, and the equivalent of the additional N infused (6·2 g/d) was almost entirely excreted in urine, indicating that rumen ammonia concentrations (50–70 mg N/l) were probably not limiting fermentation rate and microbial growth in the rumen (Satter & Slyter, 1974). The additional N recycled to the rumen during sodium butyrate infusion was also apparently in excess of microbial requirements since tissue N balance remained the same as that in the control infusion period.

The mechanisms regulating urea entry into the rumen have been recently reviewed (Engelhardt *et al.* 1978; Kennedy & Milligan, 1980), and there is general agreement that the nature of rumen fluid metabolites significantly affects the extent to which blood urea is recycled to the rumen. However, the specific site of action of these metabolites and dietary conditions leading to increased urea recycling are still not clear. Thorlacius *et al.* (1971) demonstrated that gaseous CO_2 increased urea entry through the wall of temporarily isolated rumen of cattle. High rates of urea entry were localized to the area of CO_2 application and increased as both plasma urea concentration and the partial pressure of CO_2 increased. Both acetamide and thiourea entry into the rumen were also stimulated by gaseous CO_2 indicating that urease [*EC* 3.5.1.5] activity was not involved in the stimulation of transport rate. In the absence of gaseous CO_2, urea entry into the rumen was independent of plasma urea concentration, and the previously mentioned observations suggest that gaseous CO_2 affects the permeability of either the capillary wall or the rumen mucosa to urea. Sellers *et al.* (1964) observed that both CO_2 and VFA stimulated blood flow to the rumen arteries, but in later studies by the same group, urea entry into the rumen was found not to alter during a fourfold increase in blood flow rate (Dobson *et al.* 1971).

Rumen gas varies from 10 to 80% CO_2, depending on the feeding regimen and time after

Table 6. *The effect of pH on the carbonic acid and bicarbonate concentrations (mmol/l) in rumen fluid of sheep intraruminally infused (0·44 mol/d) with sodium chloride, sodium bicarbonate, ammonium chloride and sodium butyrate*

Infusion treatment	NaCl	$NaHCO_3$	NH_4Cl	Sodium butyrate
$[H_2CO_3+HCO_3^-]$	32·2	45·3	27·2	37·3
pH	6·22	6·63	6·17	6·58
Proportion as H_2CO_3	0·63	0·40	0·66	0·43
$[HCO_3^-]$*	11·8	27·1	9·3	21·3
$[H_2CO_3]$*	20·4	18·2	17·9	16·0

* Calculated from the Henderson–Hasselbach equation: $pK_a = pH + \log_{10}([H_2CO_3]/[HCO_3^-])$, where $K_a = 3{\cdot}5 \times 10^7$.

feeding (Washburn & Brody, 1937; Barry *et al.* 1977). In our experiments, all sheep were fed hourly which would result in constant and probably low partial pressures of CO_2 in rumen gas, and these conditions would not be conducive to CO_2-stimulated urea recycling to the rumen. Furthermore, as shown in Table 6, although $NaHCO_3$ infusion had a significant effect on bicarbonate ion concentrations in rumen fluid, there was little effect of any treatment on carbonic acid concentrations in rumen fluid. If carbonic acid was in equilibrium with gaseous CO_2, then the infusion of $NaHCO_3$ was not effective in increasing the partial pressure of CO_2 in rumen gas, and it is perhaps not surprising that urea recycling was not increased during this infusion period. Neither blood flow nor urea entry into the rumen are responsive to bicarbonate ion concentrations as shown in the studies of Dobson *et al.* (1971) where phosphate buffers were substituted with bicarbonate buffer with little effect on the above measurements. Under practical feeding conditions, CO_2-induced urea recycling would occur shortly after feeding and continue until low CO_2 tensions in rumen gas prevailed. Diets containing readily fermentable carbohydrate and fed once daily would provide the greatest potential for increased urea recycling. Varady *et al.* (1969) observed a change in the rate of urea recycling to the rumen of sheep with time after feeding, and low rates of recycling are usually found in starved ruminants (Engelhardt *et al.* 1978). Both studies support the hypothesis that the permeability of the rumen wall to urea is either directly or indirectly controlled by the partial pressure of CO_2 in rumen gas which is in turn related to the diet and physiological state of the animal.

Many workers have reported a correlation between blood urea concentrations, rumen ammonia concentrations and urea entry into the rumen (Weston & Hogan, 1967; Vercoe, 1969; Thornton, 1970; McIntyre, 1971; Kennedy & Milligan, 1978; Norton *et al.* 1978; Kennedy, 1980), and these results have been interpreted by some as evidence that rumen ammonia concentrations regulate the rate of urea entry into the rumen (Varady *et al.* 1967; Harrop & Phillipson, 1974; Kennedy & Milligan, 1978; Cheng & Wallace, 1979; Wallace *et al.* 1979; Kennedy, 1980). But low rumen ammonia concentrations are not always associated with high rates of urea recycling to the rumen (Norton *et al.* 1978; Macrae *et al.* 1979; Kennedy, 1980), nor do high rumen ammonia concentrations necessarily inhibit urea recycling (Norton *et al.* 1982; present study).

Urea enters rumen fluid from blood by salivary secretions and by passive diffusion through a selectively permeable membrane in the rumen wall. Although urea diffuses through this membrane at a slower rate than water (Dobson *et al.* 1971), in the absence of microbial urease activity, urea concentration in rumen fluid will equilibrate with that in blood (Cheng & Wallace, 1979). Houpt (1970) suggested that the permeability of the

cornified (keratinized) epithelium limited urea diffusion into rumen fluid, and that bacterial urease imbedded in the cornified layer facilitated urea entry by hydrolysis of urea to the more freely diffusable ammonia. However, this theory assumes, firstly that total urease activity is limiting the rate of urea hydrolysis, secondly that liberated ammonia would exist as ammonia and not ammonium ions and would diffuse against a concentration gradient into rumen fluid rather than down the concentration gradient back to blood, and thirdly that whilst microbial urease can penetrate the epithelial layer, the much smaller urea molecule cannot diffuse from this layer.

Cheng & Wallace (1979) found a significant decrease in urease activity in rumen fluid of sheep when ammonia concentrations were increased and proposed that a similar adaptive response of urease activity in the epithelial layer may regulate the amount of urea entering the rumen from blood.

Calculations from the data of Cheng & Wallace (1979) indicate that for sheep with a rumen volume of 5 l, and with the lowest urease activity found (0·49 μmol ammonia released/ml rumen fluid per min at 37°), there was still sufficient activity to hydrolyse 49 g urea-N daily, an amount far in excess of that likely to be transferred across the rumen wall (Hecker & Nolan, 1971). Tillman & Sidhu (1969) have also reported that urease activity in rumen fluid is in excess of the substrate available for hydrolysis. Since Wallace *et al.* (1979) found that urease activity associated with the rumen epithelium was significantly higher than that in rumen fluid, it is difficult to envisage urease activity *per se* under these conditions as a regulator of urea transport across the rumen wall.

Nolan & Stachiw (1979) have reported that the appearance of $^{14}CO_2$ and $^{15}NH_4^+$ in rumen fluid was in the same proportions as ^{14}C- and ^{15}N-labelled urea in blood, which indicates that all urea degraded in passage across the rumen wall enters ruminal fluid and that there is no substantial evidence supporting a preferential ammonia reabsorption into blood after subepithelial degradation of urea.

Houpt & Houpt (1968) have made the interesting observation that disruption of the keratinized epithelial layer with NaOH without obvious damage to the underlying cells resulted in a fifty-fold increase in the rate of urea entry into the rumen. Under normal conditions, the rate of disintegration of this cornified layer will depend on a number of factors such as the rate of epithelial cell proliferation, its rate of keratinization and cell shedding, and on the extent of physical abrasion and on enzymic destruction of this layer by the microbial population inhabiting the wall. If the permeability of the rumen wall to urea is related to the thickness of the keratinized cell layer as the above evidence suggests, then permeability of the wall to urea may be altered by the effects of rumen metabolites on either microbial degradative activity in the rumen wall and/or by a direct effect on epithelial cell metabolism. For example, high butyric acid concentrations may stimulate microbial activity in the keratinized layer, increasing the rate of disruption of this cell layer resulting in higher rates of urea entry into rumen fluid. Alternatively, since butyric acid is extensively metabolized in the rumen epithelium (Weigand *et al.* 1972) the effects of high butyric acid concentrations in rumen liquor on urea recycling may be related to an increased rate of cell division and shedding. Similarly, the effects of CO_2 on urea recycling may be related to microbial activity, since the effect of CO_2 perfusion on urea entry is localized to the area of application, and is not immediate (Thorlacius *et al.* 1971).

The evidence presented previously suggests that the rate of urea entry into rumen fluid is related to plasma urea concentration and the permeability of the rumen wall to urea. Since neither blood flow rate nor urease activity *per se* appear to exercise control over urea entry, future studies of factors regulating urea passage through the rumen wall should concentrate on the effects of rumen metabolites (CO_2, VFA and NH_3) on both microbial and epithelial cell activity as they relate to the thickness or integrity of the keratinized cell layer. Although

the experiments reported in this paper have established that urea recycling through the rumen wall is inducible by dietary manipulation, and correlated with the concentrations of various rumen metabolites, the specific mechanism by which urea entry is increased still remains speculative.

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REFERENCES

Allen, S. A. & Miller, E. L. (1976). *Br. J. Nutr.* **36**, 353.
Barry, T. N., Thompson, A. & Armstrong, D. G. (1977). *J. agric. Sci., Camb.* **89**, 183.
Cheng, K. J. & Wallace, R. J. (1979). *Br. J. Nutr.* **42**, 553.
Cocimano, M. R. & Leng, R. A. (1967). *Br. J. Nutr.* **21**, 353.
Cottyn, B. G. & Boucque, C. V. (1968). *J. agric. Fd Chem.* **16**, 105.
Dobson, A. J., Sellers, A. F. & Thorlacius, S. O. (1971). *Am. J. Physiol.* **220**, 1337.
Engelhardt, W. V., Hinderer, S. & Wipper, E. (1978). In *Ruminant Digestion and Feed Evaluation* [D. F. Osbourn, D. G. Beever and D. J. Thomson, editors]. London: Agricultural Research Council.
Faichney, G. J. & White, G. A. (1977). *Aust. J. agric. Res.* **28**, 1069.
Fawcett, J. K. & Scott, J. E. (1960). *J. Clin. Path.* **13**, 156.
Harrop, C. T. F. & Phillipson, A. T. (1971). *Proc. Nutr. Soc.* **30**, 3A.
Harrop, C. T. F. & Phillipson, A. T. (1974). *J. agric. Sci., Camb.* **82**, 339.
Hecker, J. F. & Nolan, J. V. (1971). *Aust. J. biol. Sci.* **24**, 403.
Hinderer, S. & Engelhardt, W. V. (1976). In *Tracer Studies on Non-protein Nitrogen for Ruminants, IIIrd Int. Conf.*, p. 59. Vienna: Atomic Energy Agency.
Houpt, T. R. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
Houpt, T. R. & Houpt, K. A. (1968). *Am. J. Physiol.* **214**, 1296.
Kennedy, P. M. (1980). *Br. J. Nutr.* **43**, 125.
Kennedy, P. M. & Milligan, L. P. (1978). *Br. J. Nutr.* **40**, 149.
Kennedy, P. M. & Milligan, L. P. (1980). *Can. J. Anim. Sci.* **60**, 205.
McIntyre, K. H. C. (1971). *Aust. J. agric. Res.* **22**, 429.
Macrae, J. C., Milne, J. A., Wilson, S. & Spence, A. M. (1979). *Br. J. Nutr.* **42**, 525.
Nolan, J. V. & Stachiw, S. (1979). *Br. J. Nutr.* **42**, 63.
Norton, B. W., Mackintosh, J. B. & Armstrong, D. G. (1982). *Br. J. Nutr.* **48**, 249.
Norton, B. W., Murray, R. M., Entwistle, K. W., Nolan, J. A., Ball, F. M. & Leng, R. A. (1978). *Aust. J. agric. Res.* **29**, 595.
Potthast, V., Prigge, H. & Pfeffer, E. (1977). *Z. Tierphysiol Tierenahr. Futter Mittelkd* **38**, 338.
Satter, L. D. & Slyter, L. L. (1974). *Br. J. Nutr.* **32**, 199.
Sellers, A. F., Stevens, C. E., Dobson, A. J. & McLeod, F. D. (1964). *Am. J. Physiol.* **207**, 371.
Steele, R. G. D. & Torrie, J. H. (1960). *Principles and Procedures of Statistics*. London: McGraw-Hill.
Thorlacius, S. O. (1972). *Am. J. vet. Res.* **33**, 427.
Thorlacius, S. O., Dobson, A. J. & Sellers, A. F. (1971). *Am. J. Physiol.* **220**. 162.
Thornton, R. F. (1970). *Aust. J. agric. Res.* **21**, 337.
Tillman, A. D. & Sidhu, K. A. (1969). *J. Anim. Sci.* **21**, 337.
Varady, J., Boda, K., Havassy, I. & Bajo, M. (1969). *Physiol. Bohemoslov.* **18**, 23.
Varady, J., Boda, K., Havassy, I., Bajo, M. & Tomas, J. (1967). *Physiol. Bohemoslov.* **16**, 571.
Vercoe, J. E. (1969). *Aust. J. agric. Res.* **20**, 191.
Wallace, R. J., Cheng, K. J., Dinsdale, D. & Ørskov, E. R. (1979). *Nature, Lond.* **279**, 424.
Washburn, L. E. & Brody, S. (1937). *Univ. Missouri, Agric. Res. Stat. Bull.* 263.
Weigand, E., Young, J. W. & McGilliard, A. D. (1972). *J. Dairy Sci.* **55**, 589.
Weston, R. H. & Hogan, J. P. (1967). *Aust. J. biol. Sci.* **20**, 967.

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