

## Transfer of urea from the blood to the rumen of sheep

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1. The rate of transfer of plasma urea-nitrogen to rumen ammonia was measured by infusion of  $^{15}\text{NH}_4\text{Cl}$  and  $^{15}\text{N}$ urea into sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*) pellets. Urea was infused into the rumen or abomasum of two sheep given brome grass in order to increase the concentration of rumen ammonia.

2. From 6.2 to 9.8 g/d of plasma urea-N were transferred to the rumen of sheep given brome grass pellets and a measurement of 1.3 g nitrogen/d was obtained for a sheep given lucerne pellets. When urea was infused into the rumen of sheep given brome grass pellets the transfer was only 2.8–3.7 g N/d.

3. There was a significant negative correlation between the rate of transfer of plasma urea-N to the rumen and the concentration of rumen ammonia.

Although evidence has been reported (Cocimano & Leng, 1967) that urea is transferred from blood to sites of microbial degradation in the digestive tract of sheep, there is controversy about the extent of transfer to the rumen and the means by which transfer to the rumen may occur (Allen & Miller, 1976). Weston & Hogan (1967) suggested a maximal transfer of blood urea to the rumen of sheep of approximately 5 g N/d, while Nolan & Leng (1972) concluded that only 1.2 g N/d was transferred in sheep given lucerne (*Medicago sativa*) hay. Secretion of urea in saliva contributes to the transfer of blood urea to the rumen (Somers, 1961), but Houpt (1959) estimated that transport of urea across the rumen epithelium could account for up to 0.95 of the total transfer. Nolan & Leng (1972) suggested, however, that in their study virtually all the transfer occurred by way of saliva.

In the present experiments the transfer of urea from the blood to the rumen was studied in sheep given two diets, using  $^{15}\text{N}$  tracer dilution techniques. After it had been found that there were large differences between diets in the transfer of urea-N from the blood to the rumen ammonia pool, urea was infused into the rumen and abomasum of two sheep during two periods in order to establish the effect of increased concentrations of rumen ammonia on urea transfer.

### EXPERIMENTAL

#### *Animals and feeding regimen*

Five adult Suffolk wethers (aged 1.5–2.5 years), weighing 50–65 kg were used. They were fitted with rumen and abomasal cannulas and individually housed in metabolism cages at 18–25° with continuous illumination. Food was given at hourly intervals by means of an interval feeder for at least 3 weeks before measurements commenced. The diets used were brome grass (*Bromus inermis*) pellets and lucerne (*Medicago sativa*) pellets which contained (g/kg dry matter (DM)) 20 N, 900 organic matter (OM) and 26 N and 870 OM, respectively.

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*Experimental procedures*

*Expt 1.* Concurrent with a study of the transformations of sulphur (S) in the rumen of two sheep (A and B) given brome grass pellets at the rate of 33.0 and 66.0 g DM/h (Kennedy & Milligan, 1978a), estimates of N transformations were also obtained during each of two periods in which the animals received an intraruminal infusion of 0 or 4 g S as sodium sulphate/d. The two periods were separated by an interval of 38 d. [ $^{15}\text{N}$ ]Urea (100 mg/d, 0.96 atoms  $^{15}\text{N}$ /atom N, 1.82 mg/ml saline (9 g sodium chloride/l)) was infused intravenously for 48 h. Samples (nine) of blood (10 ml) and rumen fluid (20 ml) were taken at 1 h intervals and urine was collected from 40 to 48 h after the start of the continuous infusion of tracer. After termination of the intravenous infusion, an intraruminal infusion of  $^{15}\text{NH}_4\text{Cl}$  (205 mg/d, 0.99 atoms  $^{15}\text{N}$ /atom N, 0.394 mg/ml water) was made for 6.5 d. During the final 3 d of intraruminal infusion of tracer, nine samples of rumen fluid (20 ml) and three blood samples (10 ml) and urine samples were taken for  $^{15}\text{N}$  analysis.

*Expt 2.* One sheep (C) was given lucerne pellets at the rate of 53 g DM/h. The experimental procedure of the first experiment was followed to accomplish the tracer study.

*Expt 3.* Two sheep (D and E) received brome grass pellets at the rate of 59 g DM/h. During the first of two periods of 12 d, urea (20 g/d, 520 ml/d) was infused into the abomasum. Water (5200 ml/d) infused into the rumen replaced drinking-water. In the second period, urea (20 g/d) was dissolved in the water infusate administered into the rumen. During days 8 and 9 of each period, each sheep received an intravenous infusion of [ $^{15}\text{N}$ ]urea (280 mg urea/d, 4.2 mg/ml, 0.96 atoms  $^{15}\text{N}$ /atom N) in physiological saline for 33 h. During days 11 and 12, an intraruminal infusion of  $^{15}\text{NH}_4\text{Cl}$  (420 mg/d, 0.082 mg/ml, 0.99 atoms  $^{15}\text{N}$ /atom N) was made for 33 h. Samples (nine) of blood (10 ml) and rumen fluid (20 ml) were taken hourly for the final 9 h of infusion during both periods.

All samples of rumen fluid were obtained by suction through a plastic tube covered by fine nylon gauze, immediately treated with two drops of concentrated sulphuric acid, and stored at  $-15^\circ$  before analysis. Plasma was separated by centrifugation from heparinized blood and stored at  $-15^\circ$ . Urine was collected into sulphuric acid to maintain the pH below 2.

*Analytical methods*

Ammonia in rumen fluid was collected into boric acid by steam distillation over magnesium oxide and titrated with 0.01 M-sulphuric acid. Deproteinized plasma samples and urine were treated similarly after addition of urease (EC 3.5.1.5) (Nolan & Leng, 1972).

$^{15}\text{N}$  was measured, using a mass spectrometer (model CEC 21-614; Dupont Instruments Co., Newtown, Conn., USA), in  $\text{N}_2$  prepared from ammonium sulphate by the method of Francis, Mulligan & Workmall (1959). Background abundance of  $^{15}\text{N}$  in samples from each sheep was estimated before each infusion of  $^{15}\text{N}$ . Since there was a minimum (Expt 3) of 2.6 d between estimation of plateau enrichment of plasma urea during [ $^{15}\text{N}$ ]urea infusion and estimation during intraruminal  $^{15}\text{NH}_4\text{Cl}$  infusion, carryover of enrichment from the former to the latter period of measurement would have been less than 1% (Nolan *et al.* 1976) of the level achieved with [ $^{15}\text{N}$ ]urea and would, therefore, have resulted in negligible error.

The concentration of urea in plasma was determined using the urease method of Fawcett & Scott (1960).

*Calculations*

The rates of irreversible loss of plasma urea and rumen ammonia were estimated at plateau enrichment using standard procedures (Nolan & Leng, 1972); plateau enrichments of plasma and urinary urea did not differ. The rate of total derivation of rumen ammonia-N

Table 1. Nitrogen intake, concentrations of plasma urea and rumen ammonia, and dynamic aspects of ammonia and urea metabolism of sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*) pellets

Experiment no. ...	Brome grass								Lucerne
	1				3				2
	A	A*	B	B*	D†	E†	D‡	E‡	C
Sheep									
Dietary N intake (g/d)	15.5	16.3	30.9	32.6	29.6	29.4	29.6	29.4	32.6
Urea infusion (g N/d)	0	0	0	0	9.4	9.4	9.4	9.2	0
Concentration of plasma urea (mg N/l)	143	133	147	152	242	254	216	260	220
Concentration of rumen ammonia (mg N/l)	99	98	98	95	127	90	200	173	150
Irreversible loss of plasma urea (g N/d)	10.7	12.0	17.3	19.6	31.5	29.1	29.6	33.2	24.6
Irreversible loss of rumen ammonia (g N/d)	9.5	9.7	8.1	11.9	22.0	20.6	30.3	31.3	20.7
Proportion of plasma urea-N derived from rumen ammonia	0.49	0.51	0.26	0.30	0.40	0.28	0.49	0.59	0.62
Proportion of ammonia-N derived from plasma urea	0.51	0.49	0.73	0.66	0.25	0.29	0.09	0.11	0.06
Total amount of ammonia-N derived from plasma urea (g/d)	6.4	6.4	7.2	9.8	6.2	6.5	2.8	3.7	1.3

\* Intraruminal infusion of 4.0 g S as sodium sulphate/d.

† Urea infused into abomasum.

‡ Urea infused into rumen.

from plasma urea was calculated using the methods presented by Nolan, Norton & Leng (1976) and Kennedy & Milligan (1978a), as follows:

total rumen ammonia-N derived from plasma urea (g N/d) =

$$\frac{a \times \text{irreversible loss of rumen ammonia-N (g N/d)}}{1 - (a \times b)},$$

where *a* and *b* designate the proportion of rumen ammonia-N derived from plasma urea and the proportion of plasma urea-N derived from rumen ammonia, respectively.

## RESULTS

Values for the concentrations of plasma urea and of rumen ammonia, for the rates of irreversible loss of N through both pools and for transfer of plasma urea-N to rumen ammonia for sheep in Expts 1 and 2 are given in Table 1. The coefficients of variation, within trials, for plateau enrichment of rumen ammonia ranged between 16 and 25% and for plasma urea between 9 and 15%.

Clearly, sulphate infusion did not influence movements of N in sheep A and B (Table 1). The rates of irreversible loss of rumen ammonia and of transfer of plasma urea-N to rumen ammonia, and the concentration of rumen ammonia were similar in sheep A and B given brome grass, despite differing N intakes. However, the rate of irreversible loss and concentration of plasma urea appeared greater in the sheep given the larger intake of brome grass. The transfer of plasma urea-N to rumen ammonia was 4.9- to 7.5-fold greater in sheep (A and B) given brome grass than in the sheep (C) given lucerne (Table 1).

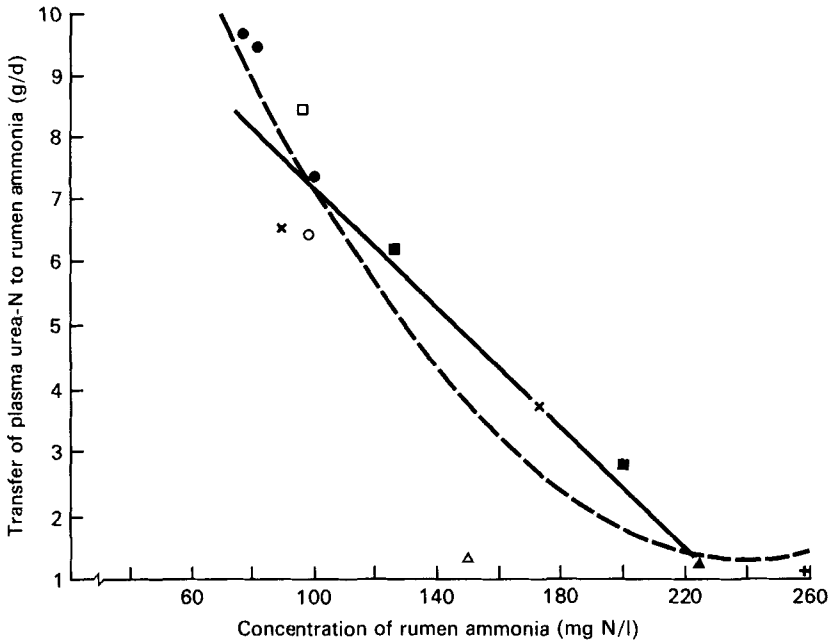


Fig. 1. Relationship between the rate of transfer of plasma urea-nitrogen to rumen ammonia ( $Y$ , g N/d) and the concentration of rumen ammonia ( $X$ , mg N/l) in sheep given brome grass (*Bromus inermis*) pellets: present experiment ( $\circ$ , average for sheep A;  $\square$ , average for sheep B;  $\blacksquare$ , sheep D;  $\times$ , sheep E) or Kennedy & Milligan, (1978*b*) ( $\bullet$ , average of one group of three sheep at each of the three combinations of ambient temperature and level of feeding including 2–5° and 59 g dry matter/h, 2–5° and 98 g dry matter/h, or 22–25° and 59 g dry matter/h) and lucerne (*Medicago sativa*) in the form of pellets ( $\triangle$ , present experiment, sheep C) or hay ( $\blacktriangle$ , Nolan & Leng, 1972, calculated using combined results from four sheep;  $+$ , Nolan, Norton & Leng, 1976, calculated using combined results from two sheep). (—),  $Y = -0.047X + 11.9$ ; (---),  $Y = -0.141X + 0.000291X^2 + 18.4$ .

In Expt 3, there were substantial increases in the concentration, and rate of irreversible loss, of rumen ammonia when the site of urea infusion was changed from the abomasum to the rumen (Table 1). The mean increase in the rate of irreversible loss of rumen ammonia-N was equivalent to the quantity of infused urea-N. In sheep (D and E) given urea into the abomasum, the transfer of urea from the plasma pool to the rumen (6.4 g N/d) was nearly twice the mean value of 3.3 g N/d measured for the same sheep when receiving the intraruminal infusion.

Using the results of the present experiments and from Kennedy & Milligan (1978*b*), a relationship was found between the rate of transfer of plasma urea-N to rumen ammonia ( $Y$ , g N/d) and the concentration of rumen ammonia ( $X$ , mg N/l) for sheep given brome grass (Fig. 1) as described by the equation:

$$Y = -0.051X + 12.6 \quad (r \ 0.93, \text{residual SD } 0.84).$$

When values were included for sheep given lucerne pellets (this experiment, sheep C) and lucerne hay (Nolan & Leng, 1972); Nolan *et al.* 1976), the relationship was:

$$Y = -0.047X + 11.9 \quad (r \ 0.90, \text{residual SD } 1.46).$$

The precision of description of the relationship was improved ( $P = 0.06$ ) by inclusion of the quadratic term (Fig. 1):

$$Y = -0.141X + 0.000291X^2 + 18.4 \quad (r \ 0.94, \text{residual SD } 1.25).$$

No further increase in precision was achieved when the rate of transfer of plasma urea-N to rumen ammonia was expressed on the basis of body-weight (g N/d per kg) or metabolic body size (g N/d per kg<sup>0.75</sup>).

#### DISCUSSION

The inverse relationship found in the present experiments between the rate of transfer of plasma urea-N to the rumen ammonia pool and rumen ammonia concentration (Fig. 1) may explain the differences obtained for estimates of urea transfer in Suffolk wethers given brome grass and Merino ewes given lucerne hay (Nolan & Leng, 1972; Nolan *et al.* 1976). It is possible that the results for lucerne diets are described by different relationships than those for brome grass diets, however, this suggestion is dependent on the reliability of one result point for the sheep given lucerne pellets in this study, and further experimental results would clearly be required for substantiation.

In sheep given lucerne hay or pellets, the transfer of approximately 1.3 g N/d is probably maintained by salivary secretion of urea (Nolan & Leng, 1972). In sheep given brome grass pellets, salivary secretion attains 5–7 l/d (Kennedy & Milligan, 1978*b*) which would contribute 0.4–0.6 g urea-N/d, assuming a concentration of plasma urea of 140–150 mg N/l and that salivary urea concentration was 0.6 that of plasma urea (Somers, 1961). Therefore transport of urea across the rumen epithelium may contribute up to 9 g N/d, or approximately 0.9 of the total transfer. This is in agreement with the conclusions of Houpt (1959) about the relative importance of transfer of urea across the rumen epithelium and by salivary secretions.

The rate of transfer of urea into the rumen of sheep was not significantly correlated with concentration of plasma urea in the range 145–250 mg N/l in sheep given brome grass. Similar results were obtained by Weston & Hogan (1967), Vercoe (1969) and Thornton (1970), who found that increases of plasma urea concentration above 160–180 mg N/l in sheep and 90–100 mg N/l in cattle respectively, during intra-abomasal or intravenous infusion of urea did not result in an increase in the net transfer of urea to the rumen. On examination of these reports and of our present results, it is evident that rumen ammonia concentrations of 70–100 mg N/l in sheep and 50–80 mg N/l in cattle are associated with maximal urea transfer. Other authors (Varady, Boda, Havassy, Bajo & Tomas, 1967; Harrop & Phillipson, 1974) have found that the increase in rumen ammonia concentration after intravenous injection of urea was negatively related to the pre-injection ammonia concentration, while Houpt (1970) has proposed from studies with rumen pouches that urea transfer across the epithelium is dependent on the concentration gradient between rumen ammonia and ammonia derived from hydrolysis of endogenous urea in the cornified layer of the epithelium. Using tracer techniques we have provided support for the conclusion that urea transport is dependent on rumen ammonia concentration, but the mechanism by which ammonia inhibits urea transport, which could conceivably entail effects on diffusion, or active transport in the rumen epithelium, changes of blood flow to the epithelium, or factors associated with dietary differences, remains uncertain.

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