The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flux

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(*Received* 11 *May* 1979 – *Accepted* 9 *July* 1979)

1. The rumen urea concentration in gnotobiotic lambs lacking ureolytic bacteria was equal to that of blood.
2. Bacterial urease (*EC* 3.5.1.5) activity in sheep fed by intraruminal and intra-abomasal infusion was inversely related to rumen ammonia concentration.
3. A model is proposed for the facilitation and control of urea flux by wall-bound ureolytic bacteria.

The recycling of endogenous urea to the rumen is an important means of supplying ammonia for microbial protein synthesis in ruminants on low-protein diets (Chalupa, 1972; von Engelhardt *et al.* 1978). Most evidence suggests that its passage across the rumen wall occurs by simple diffusion (Juhász, 1963, 1965; Houpt & Houpt, 1964; von Engelhardt & Nickel, 1965; Várady *et al.* 1969; Allen & Miller, 1976). Von Engelhardt *et al.* (1978) proposed that the rate of diffusion was influenced mainly by the permeability of the rumen wall and that the permeability in turn depended largely on diet. However, the rumen NH_3 concentration has also been shown to influence the rate of recycling of endogenous nitrogen (Várady *et al.* 1969; Harrop & Phillipson, 1974; Kennedy & Milligan, 1978).

This paper describes experiments in which ureolysis and urea and NH_3 levels were measured in gnotobiotic lambs and in sheep fed by intraruminal and intra-abomasal infusion (Ørskov *et al.* 1979). It is proposed that urea passes through the rumen wall by diffusion, that this process is accelerated by its hydrolysis on the inner face of the wall by adherent facultative ureolytic bacteria (Cheng, McCowan *et al.* 1979; Wallace *et al.* 1979) and that, since NH_3 was found to regulate the expression of urease (*EC* 3.5.1.5) activity by these bacteria, the rumen NH_3 concentration thereby regulates the rate of transfer of endogenous urea across the rumen wall.

METHODS

Gnotobiotic lambs. Six gnotobiotic lambs were used in these experiments (Table 1). Lambs C1, D1, E1 and F1 were (Finnish Landrace × Dorset Poll) × Suffolk. Lambs G1 and H1 were (Border Leicester × Cheviot) × Suffolk. The lambs were obtained by hysterectomy and reared in cages inside flexible-film isolators using the equipment and techniques described by Alexander & Lysons (1971) and Alexander *et al.* (1973*a*, *b*). The feeding schedule was as described by Mann & Stewart (1974), except that the final diet, used during the experimental period, was dried grass sterilized by gamma-radiation (Ethicon Ltd, Edinburgh). Animals received the dried grass *ad lib.* Lambs C1, E1, F1, G1 and H1 were inoculated with strains of *Streptococcus bovis*, *Megasphaera elsdenii*, *Veillonella alcalescens*, *Staphylococcus* spp. and *Lactobacillus* spp. at age 15–18 d. Further inoculations of laboratory cultures of *Selenomonas ruminantium*, *Bacteroides ruminicola*, *B. amylophilus*, *Butyrivibrio fibrisolvens*, *Anaerovibrio lipolytica*, *Bacteroides succinogenes*, *Methanobacterium ruminantium*, *Ruminococcus albus* and *R. flavefaciens* were made during days 31–33. Lamb D1 was not inoculated. Samples of rumen fluid were obtained aseptically by stomach tube.

Infusion. Three Suffolk × (Finnish Landrace × Dorset Horn) sheep (B, C, D) aged 6–9

0007-1145/79/3342-0907 $01.00

Table 1. *Urea concentration in the blood and rumen fluid of gnotobiotic lambs*

Lamb	Urea in blood (mmol/l)	Urea in rumen fluid (mmol/l)	Urease (*EC* 3.5.1.5)* in rumen fluid
D1†	4·53	5·03	0·00
C1	5·36	4·88	0·00
E1	5·09	5·33	0·00
F1	6·36	6·46	0·00
G1	5·45	4·75	0·00
H1	5·58	6·00	0·00

* Units of urease are μmol NH_3 released/ml rumen fluid per min at 37°.
† Control, uninoculated animal.

months and one Suffolk × Cheviot sheep (A), aged 9 months, were surgically prepared and fed entirely by infusion of volatile fatty acids (VFA), mineral salts and bicarbonate buffer into the rumen and casein into the absomasum, by the method of Ørskov *et al.* (1979). Casein was infused at a rate of 65 g/d and VFA solution (Ørskov *et al.* 1979), which contained acetic, propionic and butyric acids in molar proportions 65:25:10, was infused at a rate which provided the animal's maintenance requirement. In order to obtain different rumen NH_3 concentrations in the Suffolk × Cheviot sheep (sheep A), ammonium bicarbonate solution was pumped into the rumen at different rates. Samples of rumen fluid were removed via the rumen cannula. The animal had been fed entirely by infusion for 1 month before sampling.

Analytical methods. Urea in blood and in rumen fluid was determined by the automated procedure of Marsh *et al.* (1965). NH_3 was assayed by the phenol-hypochlorite method (Weatherburn, 1967). Urease activity was measured by the production of NH_3 from urea at 37° (Cook, 1976).

Bacteriological methods. The total viable count and the count of facultative bacteria were done as reported previously (Wallace *et al.* 1979).

RESULTS AND DISCUSSION

The inoculation of a number of strains of different species of rumen bacteria failed to produce ureolysis in the rumen fluid of gnotobiotic lambs (Table 1). Post-mortem examination of the rumen epithelium showed that it too did not possess urease activity, and no urease activity developed in the control, uninoculated, lamb. Thus the transfer of urea across the rumen wall in gnotobiotic lambs was unaffected by any hydrolysis on the inner face, and the problems in the interpretation of results associated with hydrolysis (Houpt & Houpt, 1968) were avoided. The concentration of urea in rumen fluid was very similar to the concentration in blood (Table 1), suggesting that the transfer of urea across the rumen wall occurs by simple diffusion.

In sheep fed entirely by infusion, the urease activity of rumen fluid is comparable with that of a normally fed animal, since the facultative bacteria responsible for this activity remain bound to the rumen epithelium even in the absence of food particles and the normal flora of rumen fluid, and occur in rumen fluid as the result of shedding of dead epithelial cells (Wallace *et al.* 1979). The results of Wallace *et al.* (1979) implied that urease activity varied inversely with rumen NH_3 concentration. Similar findings were made in this study in samples taken from different sheep at different times, and it was clear that the total number of facultative bacteria present in rumen fluid had little influence on this relationship (Table 2).

In order to confirm that the effect on urease activity was a direct result of changes in NH_3 concentration, one sheep (sheep A) was infused with a concentrated solution of

Table 2. *Ammonia concentration, urease activity and counts of facultative bacteria in the rumen fluid of sheep fed entirely by intraruminal and intra-abomasal infusion*

Sheep	Ammonia (mmol/l)	Urease* (*EC* 3.5.1.5)	10^{-7} × no. of facultative bacteria/ml
C	3·60	2·57	0·2
C	4·80	2·06	0·3
C	5·21	1·00	1·2
A	7·71	1·03	0·6
B	8·20	0·96	0·3
A	9·42	1·00	0·9
D	11·57	0·54	1·1
B	13·70	0·49	0·7
B	15·42	0·68	0·8

* Units of urease are μmol NH_3 released/ml rumen fluid per min at 37°.

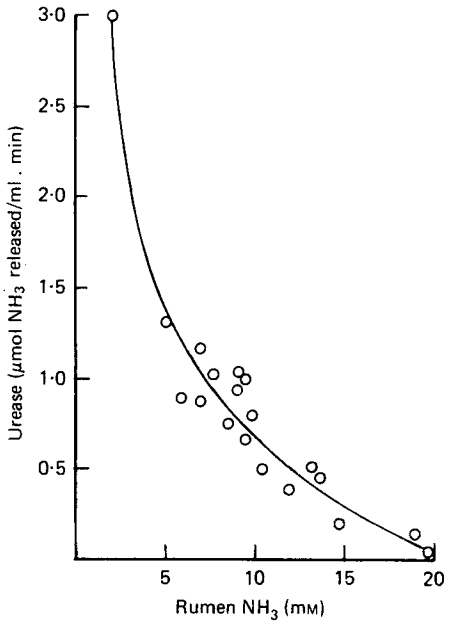

Fig. 1. Influence of rumen ammonia concentration (mM) on urease (*EC* 3.5.1.5) activity (μmol NH_3 released/ml per min) of rumen fluid in a sheep fed by intraruminal and intra-abomasal infusion.

ammonium bicarbonate directly into the rumen. Initially, an infusion rate of 3·17 mmol NH_4HCO_3/h increased the NH_3 concentration from 5·1 to 13·7 mM after 5 d. Subsequently, however, the effect of NH_4HCO_3 infusion became progressively less, and it was noted that the rumen fluid became more opaque. As the experiment progressed, it was necessary to increase the concentration of NH_4HCO_3 solution to obtain the same increase in rumen NH_3 concentration. A viable count of bacteria showed no significant change ($1{\cdot}2 \times 10^7$/ml), but the microscopic appearance of the fluid suggested that a precipitate had formed. Urease activity was found to vary inversely with the NH_3 concentration achieved in the rumen (Fig. 1), regardless of whether the precipitate was present. The effect of NH_3 concentration on urease activity was found to be an adaptive response. The addition of 100 ml 1·6

M-NH_4HCO_3 to the rumen caused the NH_3 concentration to rise from 7·9 mM to 35·8 mM after 1 h, but the urease activity (0·58 μmol NH_3 released/ml per min before addition, 0·60 μmol NH_3 released/ml per min after 1 h) was relatively unchanged. After 5 h, by which time the NH_3 concentration had fallen to 13·2 mM, the urease activity had fallen only to 0·52 μmol NH_3 released/ml per min. A 24 h period was required for the urease activity to adapt to a new steady-state NH_3 level.

The bacteria occurring in the rumen fluid of sheep fed by infusion are similar to those which adhere to the rumen wall (Wallace *et al.* 1979). Indeed, many of these bacteria remain attached to the remnants of sloughed epithelial cells (Wallace *et al.* 1979). It can therefore be predicted that the bacterial population attached to the epithelium will respond in the same way to changes in NH_3 concentration, and that the urease activity of the epithelial surface will be inversely related to rumen NH_3 concentration.

These results lead us to propose a model for the transfer of urea from blood across the rumen wall, basically similar to that of Houpt (1970), in which bacterial urease activity associated with the epithelium serves to maintain a localized concentration gradient of urea across the rumen wall and hence increases many-fold the rate of diffusion of urea into the rumen. Our results suggest that this model should be extended to include the control of urea flux by rumen NH_3 concentration. As the expression of urease by the facultative bacteria of the epithelium is regulated by NH_3 concentration, the extent to which the diffusion process is accelerated by bacterial urease will depend on the rumen NH_3 concentration, and so the rate of urea flux into the rumen will be controlled indirectly by NH_3 concentration.

The model may help to explain a number of observations on the recycling of endogenous N in the rumen. For example, von Engelhardt & Hinderer (1976) found that when food was withheld from goats for 48 h, the rate of urea turnover was markedly reduced. Similar findings have been made by Thornton (1970), Várady & Harmeyer (1972), and Harmeyer & Várady (1972). Since starvation of cattle can lead to a reduction in urease activity of more than 97% after 72 h (Cheng, Bailey *et al.* 1979), it would be expected from the model that urea flux to the rumen would therefore be diminished by starvation.

Different diets cause substantial differences in the extent of recycling of urea N to the rumen (Kennedy & Milligan, 1978), and these differences are often associated with the protein content of diets (Várady *et al.* 1969), the rumen NH_3 concentration (Hill *et al.* 1961; Várady, Boda, Havassy *et al.* 1967; Várady, Boda, Tomáš *et al.* 1967) or other related factors (von Engelhardt *et al.* 1978). We suggest that many of these findings may be explained by the effect of diet on the rumen NH_3 concentration and hence on bacterial urease activity and the rate of urea flux across the rumen wall.

The ureolytic flora of the rumen wall is therefore a unique example of a population of symbiotic bacteria whose enzyme activity is involved in the regulation of an essential function of an animal, namely the recycling of endogenous urea N.

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The authors are indebted to Mr G. Cushnie and Mr A. J. Richardson for care of the gnotobiotic lambs, and Mr N. A. McLeod and Mr R. Stirton for care of the sheep fed by infusion. We wish to thank also Drs P. N. Hobson and E. R. Ørskov for their advice and encouragement.

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Printed in Great Britain