A. Ü. Vet. Fak. Derg.
36 (1): 213-225, 1989

MEASUREMENT OF UREA KINETICS WITH A SINGLE INJECTION OF [^{14}C]-UREA USING A TWO-COMPARTMENT MODEL IN SHEEP[1]

Ahmet Öncüer[2]

Koyunlarda Üre kinetiğinin C^{14} işaretli ürenin tek enjeksiyonu ve iki kompartman modeli kullanarak ölçülmesi

Özet: *Bu çalışmada rumen kanülü takılmış 4 dişi koyun kullanıldı. Bütün hayvanlar tamamen mide içi infüzyon ile beslendiler. Üre kaybolma oranı ve üre kütle boyutu, [^{14}C] – ürenin damar içi tek enjeksiyonu ile ve plazma üre spesifik aktivitesinden tahmin edildi. Üre parçalanması ile üre kaybolma oranı arasında yakın ilişki bulundu. Fakat plazma üre veya rumen amonyak konsantrasyonu ile ilişkisi saptanamadı. Çalışmada normal beslenenlerin üre metabolizması arasında fark olmadığı ve mide içi infüzyon metodunun konak hayvanların üre metabolizmasındaki rollerinin araştırılmasında uygun bir yöntem olarak kullanılabileceği sonucuna varıldı.*

Summary: *Four female sheep with a rumen cannula were used in this study. All animals were nurished wholly by intragastric infusion of nutrients. Urea irreversible loss rate (ILR) and urea pool size were estimated from the decline in specific activity of plasma urea after a single intravenous injection of* [^{14}C] *- urea. Urea degradation was significantly related to ILR but was not related to plasma urea or rumen NH_3 concentration. It is concluded that there are no major differences in the process of urea metabolism between normaly fed sheep and those nourished by intragastric infusion. Sheep nourished by infusion would appear to be suitable models for investigating the role of the host-animal in the control of urea recycling.*

Introduction

Urea is not only a simple waste product of nitrogen metabolism but also an important precursor of protein biosynthesis (5). Ammo-

1. This work was carried out in the Rowett Research Institute.

2 DVMS, PhD, Lalahan Nuclear Research Institute of Animal Health.

nia can be absorbed from the digestive system where formed in excessive quantities and enhance formation of urea, or it can be derived from urea of blood plasma when its formation from feed sources is small. In this way ruminants conserve nitrogen when dietary supplies are low by utilising endogenous urea via microbial protein synthesis (7). A major problem in identifying the control mechanisms involved in urea recycling in ruminants is that of separating those factors which derive from the activity of rumen microorganisms and those which are specific attributes of host-animal metabolism. The system of total intragastric nutrition (11) appeared to offer a method whereby urea metabolism could be examined under controlled, Steady-State conditions and without the complications which ensure from the presence of an active rumen microbial population. The tracer method can be used to investigate certain properties of large populations of atoms or molecules (the tracee) by making observation of the behaviour of small numbers of tracer atoms or molecules. In principle and practice both stable and radioactive isotopes can be equally sensitive as tracers. However, to achieve high sensitivity with stable isotopes [^{15}N] very expensive mass spectrometric equipment is required, whereas radioisotopes can be measured with equal sensitivity in relatively cheap scintillation spectrometers and with analytical methods also simpler than ^{15}N. Urea metabolism can be measured in vivo using either a single injection of isotope or continuous infusion.

The objective of this study was to measurement of various parameters of urea kinetics in sheep nourished by intragastric infusion. It was estimated from the decline in specific activity of plasma urea after a single injection of [^{14}C] – urea into jugular vein.

Before considering the method used for Studying the kinetics of urea metabolism it is convenient to describe briefly the various terms used in vivo studies employing isotopic tracers. The fallowing list conform to the recommendation made by Nolan et al. (9).

Tracee: The defined substance whose movement and behaviour in the system is under study.

Tracer: The defined substance, labelled (e.g. by use of isotopes) so that its movement and behaviour in the system can be studied.

Pool size: The total mass of tracee distributed through all compartmens within the biological system.

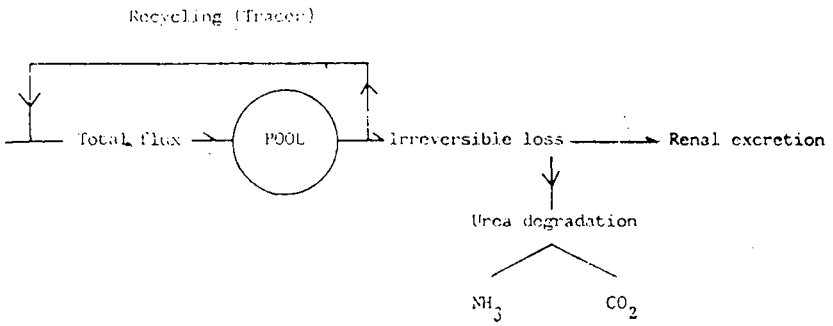
Space: The apparent volume of distribution of the tracee in the pool (i.e. in the biological system).

Total entry rate (Flux-rate): The rate mass/unit time at which all tracee enters and leaves a compartment which is in steady state. This parameter may be divided into:

Irreversible loss rate (ILR): a fractionel flux-rate which leaves the compartment and does not return to it during the experimental period.

Recycling rate: a fractional flux-rate which leaves the compartment and returns to it during the experimental period.

Urea degradation rate: Measured as the difference between ILR and total urea excretion in the urine. This is taken to be the quantity of urea which is hydrolysed to Co_2 + NH_3 within the digestive tract. The above parameters can be described diagramatically.



Material and Methods

Four female lambs were used in this experiment. They were housed indoors in metabolism cretes under conditions of continuous lighting and constant ambient temperature. All sheep were prepared with rumen cannulas and abomasal infusion catheters. After surgery recovery all lambs were nourished wholly by intragastric infusion of volatile fatty acids, buffers, minerals and casein .(11) Feeding provided sufficient energy for maintenance and total nitrogen intake were 6.71 g/d.

The body urea pool was labelled with a single intravenous injection of [^{14}C] – urea to study changes in urea pool size, irreversible loss rate (ILR) and degradation rates.

All animals were weighed and prepared with jugular catheter on the day before the experiment, and a syringe was prepared containing [^{14}C] - urea in 10 ml isotonic sterile saline (0.9 % NaCl) to give a dose of 50 μci on the day of injection the full syringe was weighed and pre-dose blood and urine sample were taken. The [^{14}C] - urea was injected at the same time and approximately at the same speed to all animals. The syringe was then disconnected, the dose "washed in" with 10 ml saline containing 50 iu /ml heparin, and the empty syringe was the re-weighed to calculate the precise dose injected.

A total of 15 blood samples (8 ml) were taken from each animal at frequent intervals over the first 2 hours and then decreasing intervals up to 24 hours. Urine samples were collected over 6, 6 an 12 hour intervals. On the same day rumen samples (40 ml) were taken from each animal at intervals for measurement of pH, osmotic pressure, urease activity and ammonia. Blood samples were centrifuged soon after withdrawal and the plasma stored at 20 °C until analysed for urea concentration and for specific activity (SA) of urea.

The kinetics of urea metabolism in lambs were assumed to conform to a two-compartment model, consisting of a main pool and a side pool (13).

This model, in which the plasma represents the primary pool, is similar to that adopted by Bruckental et al. (1) The observed values for plasma urea SA were first normalized by dividing each by the injected dose of radioactivity and the Maximum Likelihood Programme of Ross (12) was used to establish the parameters of the double exponential curve which best fitted the decline in plasma SA with time.

Urea pool size and ILR from the plasma urea pool were estimated from the parameters of the exponentials using the relationships given by Nolan & Leng (10). It was assumed that the quantity of ^{14}C recycled to urea following urea degradation is negligible and that the ILR of urea-C as estimated with is also an estimate of total flux of urea out of the body urea pool. Urea degradation rate was taken to be the difference between urea ILR and the rate of excretion of urea in urine. Urea space was calculated as urea pool size (mg) ÷ plasma urea concentration (mg / L) and expressed as a percentage of bodyweight (L / kg): it represents the theoretical volume of distribution of the body urea pool on the assumption that urea is an equlibrium throughout the body at the concentration observed in plasma.

Blood samples were obtained from each animal via jugular vein catheter. On the day of [^{14}C]-urea injection, blood samples (8 ml) were withdrawn into tubes containing concentrated heparin solution. Tubes centrifuged for 10 min at 2000 g and 3 ml plasma removed then Stored at —20°C until they were used. Collected urine volume was weighed, sampled and Stored at —20°C until analysed. Rumen Samples (10 ml) were taken routinely twice a day and on the day of injection, 6 samples (40) ml) were taken over 24 hours.

For urea in urine and plasma samples were determined by the automated methods of Marsh et al. (8) Rumen ammonia was analysed by the method described by Fawcett — Scott (4). Rumen fluid urease activity was measured by production of NH_3 from urea at 37°C (3). Radioactivity in plasma was measured by liquid scintillation counting (Tri-Carb 460 C, Packart. Instrument Company Ltd): 1 ml sample was mixed with 10 ml of commercial Scintillation cocktail (NE 265, Nuclear Enterprises Ltd.,) and the count rate corrected for quenching by use of automatic external stardard. Radioisotope was obtained from Amersham International plc, and diluted with non-radioactive urea in sterile isotonic saline to give a solution for injection which contained 5 μci / ml and 8 μci / mg urea.

The various indices of urea metabolism measured in this experiment were examined by regression analysis.

Results and Discussion

Nitrogen, Energy intakes, rumen ammonia concentration and urease activity are given Table 1. The parameters of urea metabolism (Urea irreversible loss rate, urea pool size, urinary urea excretion and plasma urea and urea degradation) measured by isotope dilution using a single injection of [^{14}C]—urea are given in Table 2 and typical example of the decline in specific activity (SA) of plasma urea with time, from which the various parameters of urea metabolism were derived, is shown in Fig. 1.

Urea ILR showed a mean overall value of 16.5 g / d (SE ± 0.97). Urea pool size was on 2.47 g urea equivalent to a theoretical mean urea "space" amounting to 57.8 %.

The various indices of urea metabolism measured in this experiment were examined by regression analysis for evidence interrelations-

Table 1. Nitrogen and Energy intake, rumen ammonia concentration and rumen urease activity of sheep (mean value of 4 Sheep)

Mean Liveweight (kg)	Mean* Liveweight (kj / $kg^{0.75}$)	Energy intrake (kj / $kg^{0.75}$)	Nitrogen intake (mg / $kg^{0.75}$)	Rumen PH	Rumen ostomic Pressure (mosmol / kg)	Rumen ammonia concentration (mg / 100 ml)	Rumen activity (mmol / ml / min)
36.83	14.95	444.0	439	6.54	227	10.16	0.22

*Metabolic bodyweight.

Table 2. Various parameters of urea kinetics of sheep. (mean value of 4 Sheep)

Mean Liveweight (kg)	Mean* Liveweight ($kg^{0.75}$)	Plasma Urea Concentration (mg / 100 ml)	ILR (g / d)	Urine Urea excretion (g / d)	Urea degradation (g / d)	Urea pool size (g)	Urea spare (% liveweight)
36.83	14.94	11.77	16.5	9.27	7.26	2.47	57.8

* Metabolic bodyweight

hips. Those variables showing significant regression relationship are shown in Fig. 2.

A highly significant relationship was detected between urea pool size (mg / kg $^{0.75}$) and plasma urea concentration (mg / 100 ml) ($P < 0.001$). Some of those variables showed significant relationships (Fig 2) with plasma urea concentration and serve to demonstrate the central role of plasma urea concentration as an index of urea metabolism. The question arises as to the usefulness of measuring urea concentra-

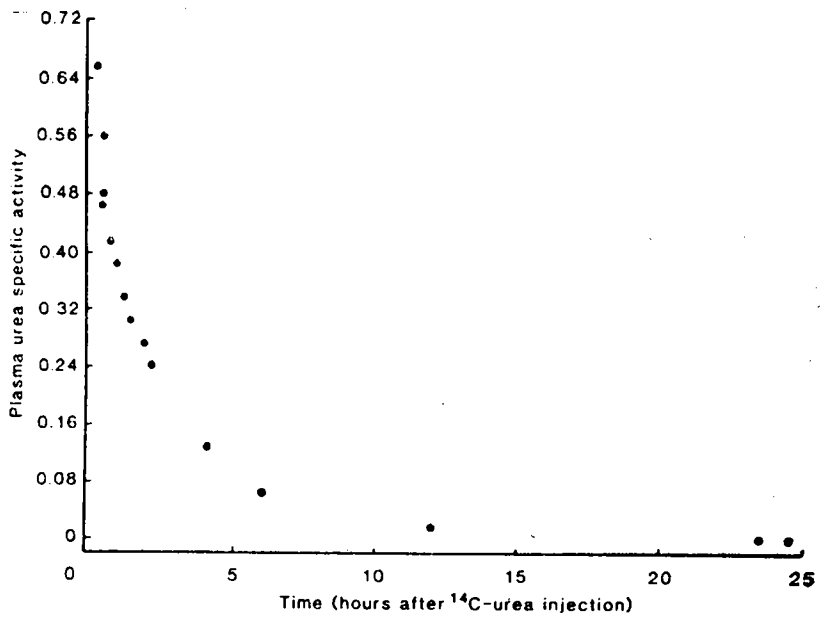


Fig. 1. Typical computer fitted curve of the decline in specificity of plasma urea with time after ^{14}C-urea injection. Specific activity is expressed as μci × 10^1 / mg urea divided by the total dose injected (normally 50 μci). The fitted double-exponential equation in this example was:

$$ye = 0.59\text{–}7.38x - 0.41e\text{–}0.34x$$

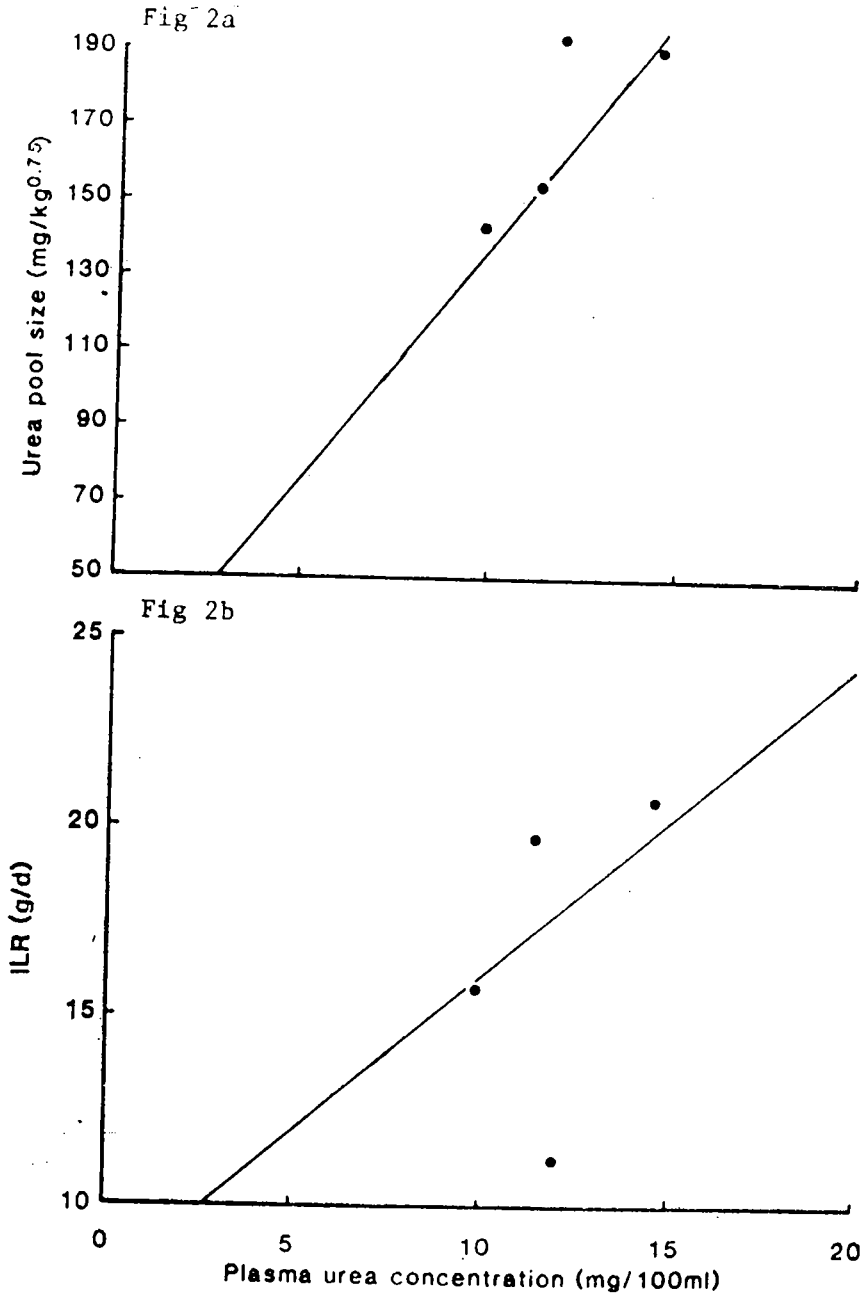


Fig. 2. The regression relationship between the variables, with plasma urea concentration (mg / 100 ml), rumen ammonia (mg / 100 ml), and irreversible loss rate (g / d) being the independent variables (x) were:

Fig. 2a: $y = 12.22 (\pm 1.95)x + 15.32$ r = 0.89, RSD = 15.79 $P < 0.001$

2b: $y = 0.82 (\pm 0.351)x + 7.83$ r = 0.59, RSD = 2.85 $P < 0.05$

Fig c

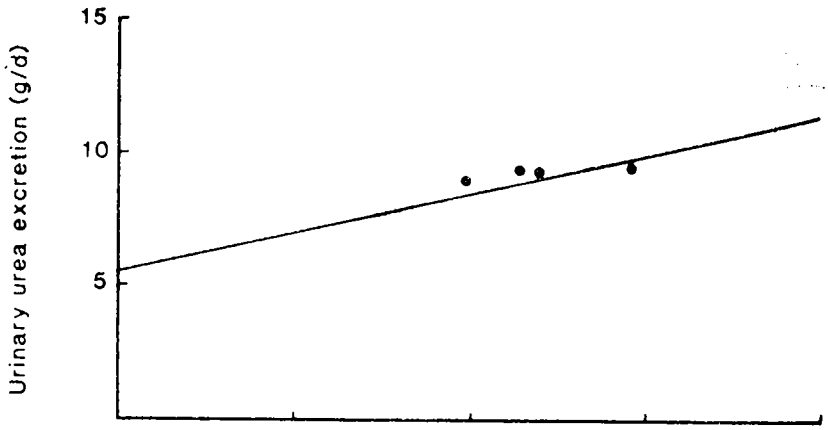
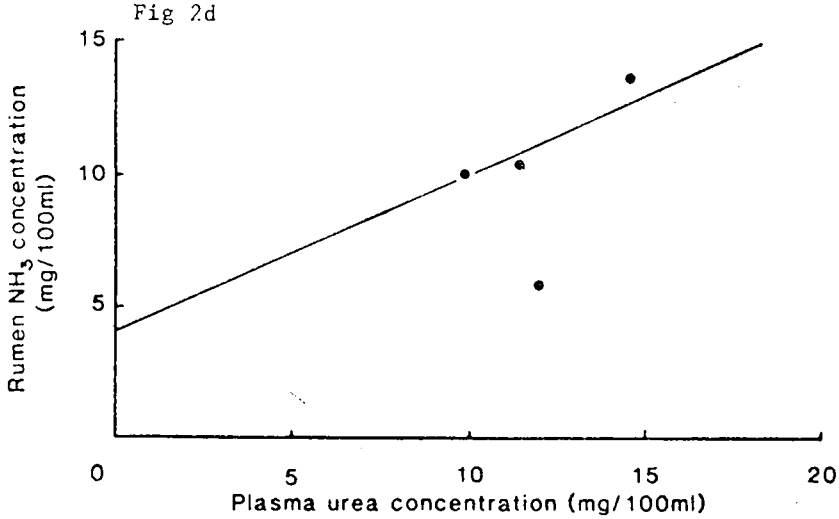


Fig 2d



2c: $y = 0.294\ (\pm 0.123)x + 5.51$ $r = 0.60$, RSD = 1.00 $P<0.05$
2d: $y = 0.595\ (\pm 0.250)x - 4.08$ $r = 0.59$, RSD = 2.09 $P<0.05$

Fig e

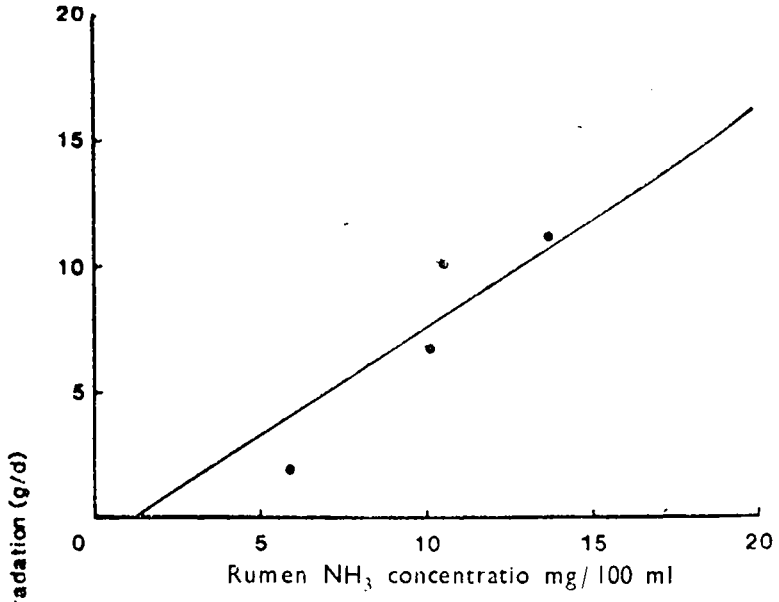
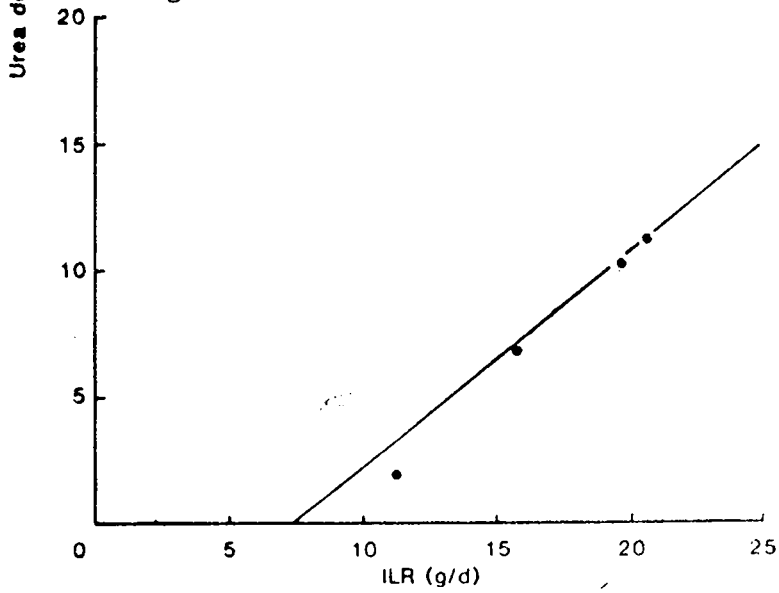


Fig 2f



2e: y = 0.850 (±0.282)x + 0.93 r = 0.69, RSD = 2.31 P<0.05
2f: y = 9.844 (±0.100)x + 6.16 r = 0.94, RSD = 1.12 P<0.001

tion if plasma for the prediction of urea metabolism; Variable findings obtained from various studies of urea metabolism suqqest that these values for predictive purposes may be limited (5).

The regression relationships which were observed in the present experiment were similar to those observed by Whitelaw et al (unpublished) in a comperative study of totally infused and conventionally fed sheep.

Although we did not observe any relationship between plasma urea concentration and degradation rate of urea, these two parameters have previously been shown to be closely related in conventionally fed ruminants.

Regression have been linear between amount of urea degrated in the body and plasma urea concentration elevated by intravenous infusion (6).

Also, a high energy diet resulted in a decrease of urea concentration in plasma and an increase in urea degradation (5).

In the present experiment of urea degradation and irreversible loss rate of urea highly correlated and it seems likely that irreversible loss rate was a major determinant of the extent of urea degradation ($r=0.94$; $p<0.001$) in animals nourished by infusion.

The implication of these findings is that degradation rate in the animals increases with increasing flow of urea through the body pool and suggests that simple diffusion may account for a large proportion of urea transfer from the blood to the gastrointestinal tract.

A hypothesis that the rate of urea entery into the rumen across the rumen epithelium is decreased as a results of reduced expression of urease activity caused by increasing rumen ammonia concentrations at the epithelial-adherent microbial interface has been proposed by Cheng & Wallace (2). Since urease exist only in the degestive tract, the rate of degradation represents the rate of movement of urea into the areas of bacterial growth, and so is an indicator of the potential for utilisation for urea nitrogen for bacterial synthesis. In the present experiment extremely variable urease and absence of relationship between urea degradation rate and urease activity could not support the theories of urease activity in the mechanism of urea transfer to the gastrointestinal tract.

There is, however, the anomaly of significant relationship being observed between the urea degradation rate and rumen ammonia concentration in the present experiment. This might indicate that rumen ammonia concentration influences degradation by a mechanism which does not involve urease activity. It might also be explained if degradation rather than rumen ammonia concentration was the independent variable, such that increasing concentrations of ammonia simply reflect increasing amounts of urea reaching the rumen by diffusion, as suggested above.

Acknowledgement

I am very grateful to professor James, Director of the Rowett Research Institute, for providing the facilities for this work. My thank to the IAEA for the financial support and the Turkish Atomic Energy Authority for giving me the opportunity to complete this work.

References

1. **Bruckental, I., Oldham J.D. and Sutton J.D.** (1980): *Glucose and urea kinetics in cows in early lactation.* Br. J. Nutr. 44, 33—45.

2. **Cheng, K.J. and Wallace, R.J.** (1979): *The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flux.* Br. J. Nutr. 42, 553—557.

3. **Cook, R.R.** (1976): *Urease activity in the rumen of the sheep and the isolation of ureolytic activity.* J. Gen. Microbiol. 92. 32—48.

4. **Fawcett, J.K. and Scott, J.E.** (1960): *A rapid and precise method for the determination of urea.* J. Clin. Path. 13, 156—159.

5. **Hermeyer, J. and Martens, H.** (1980): *Aspects of urea metabolism in the ruminants with reference to the goat.* J. Dairy Sci. 63, 1707—1728.

6. **Houpt, T.R. and Houpt, K.A.** (1968): *Transfer of urea nitrogen across the rumen wall.* Am. J. Physiol. 214, 1296.

7. **Kennedy, P.M. and Milligan, L.P.** (1980): *The degradation and utilization of endogenous urea in the gastrointestinal tract in ruminants.* Can. J. Anim. Sci., 60, 205—221.

8. **Marsh, W.H., Fingerhut, B. and Miller H,.** (1965): *Determination of urea in urine and serum.* Clin. Chem. 11, 624—627.

9. **Nolan, J.V., Norton, W.B. and Leng, R.A.** (1972): *Dynamic aspects of nitrogen metabolism in sheep.* In: Tracer studies on Non-protein nitrogen for Ruminants, pp. 13—24. IAEE. Vienna.

10. **Nolan, J.V. and Leng, R.A.** (1974): *Symposium on tracer techniques in nutrition isotope techniques for studying the dynamics of nitrogen metabolism in ruminants.* Proc. Nutr. Soc. 33, 1—8.

11. **Orskov, E.R., Grubb, D.A., Wenham, G. and Corrigall, W.** (1979): *The sustenance of growing and fattening ruminants by intragastric infusion of volatile fatty acids and protein.* Br. J. Nutr. 41, 553 558.

12. **Ross, G.J.S.** (1980): *Maximum likelihood Programme.* Version 3.07. Lawes Agricultural trust, Rothamsted Experimental Station, England.

13. **Shipley, R.A. and Clark, R.E.** (1972): *Tracer Methods for in Vivo Kinetics.* Academic Press, New York.