UREA METABOLISM IN SHEEP

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Summary

The rate at which urea enters the body pool of urea in sheep was investigated using isotope dilution techniques. Both single injection and constant infusion of carbon-14 urea were used. Urea excretion rates were calculated by measuring the urea in urine collected from a catheter in the bladder. Urea entry and excretion rates were measured in sheep fed rations containing 3.5, 9, 17 and 27% crude protein. The entry and excretion rates on each diet in mg/min were 2.5 ± 0.23 and 0.2 ± 0.09 (3 experiments), 8.5 ± 0.77 and 4.6 ± 1.13 (8), 26.4 ± 1.3 1 and 17.7 ± 0.52 (8), 39.4 ± 2.45 and 28.3 ± 2.37 (8), respectively, showing that of the urea entering the body pool of urea in sheep 8, 56, 67 and 70% respectively was excreted on each ration. The entry rate and the excretion rate of urea both appeared to be linearly correlated with the concentration of urea in the blood of sheep.

I. INTRODUCTION

The ability of ruminants to exist on diets of low protein content (i.e. 3-4% crude protein [C.P.]) is generally attributed to reutilization of urea nitrogen by the ruminal microorganisms (Phillipson 1964). Ammonia absorbed from the alimentary tract and ammonia produced in the tissues by deamination of amino acids is converted to urea in the liver and passed to the systemic circulation, either to be excreted in the urine or to pass into the rumen, either in the saliva (McDonald 1948) or across the rumen wall (Houpt 1959). The amount of urea conserved by ruminants is still uncertain, particularly under conditions of low protein nutrition. The investigations now described were designed to determine in sheep the quantities of urea entering the body pool and, by estimating excretion rates in the urine, to determine the amounts of urea nitrogen degraded in the gastro-intestinal tract.

II. MATERIALS AND METHODS

(a) Experimental animals

Groups of four Merino ewes were individually fed on one of the following rations:

Ration 1: 450 g chopped wheaten straw + 50 g chopped lucerne hay. C.P. 3.5%.
Ration 2. 800 g chopped wheaten hay + 200 g chopped lucerne hay. C.P. 9%.
Ration 3. 800 g chopped lucerne hay. C.P. 17%.
Ration 4. 700 g chopped lucerne hay + 100 g casein. C.P. 27%.

All animals had been on the ration for at least 12 weeks prior to the infusion experiments. Animals were given the ration in 12 equal amounts at hourly intervals (from 8 a.m. to 7 p.m.), a feeding regime that was found to produce a constant level of urea in blood between 12 noon and 10 p.m.

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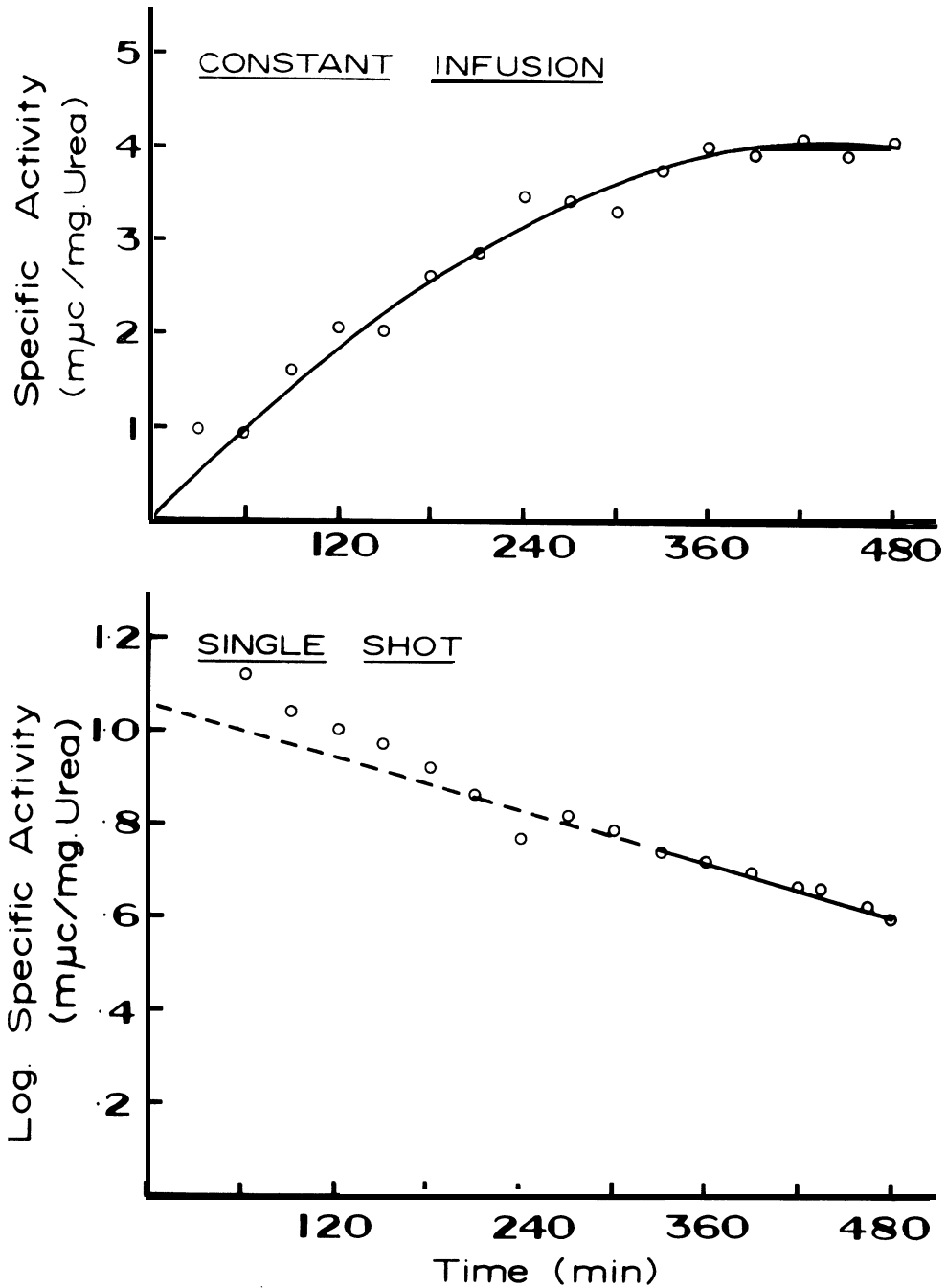

Fig. 1.—Specific activity of plasma urea after a single injection [100μc] or continuous infusion [0.08 μc/min] of [^{14}C] urea. Each sheep was given a total of 800 g lucerne in twelve equal hourly feeds.

(b) Estimation of urea entry rates

Animals were prepared with jugular vein catheters the day before an entry rate measurement was made. Bladder catheters were inserted on the day of the experiments.

Entry rates were measured in each group of sheep using two techniques. Preliminary experiments had shown that six hours were required for equilibration of injected [^{14}C] urea with the body pool of urea. Sheep were injected with 10 μmole, 100 μc [^{14}C] urea at 8 a.m. and blood samples were taken at 30 min. intervals from 2 p.m. to 5 p.m. Figure 1 shows that a graph of the log specific activity of plasma urea against time was a straight line after about 300 min., indicating that this was the minimum time for mixing of injected urea with the body pool of urea. From this graph the following values can be calculated.

Extrapolation to zero time gives an estimate of the dilution of activity at the time of injection [i.e. specific activity ($Sp.Act._0$) of plasma urea at zero time] assuming instantaneous mixing. Urea pool size (PO) can be calculated as follows:

$$PO = \frac{\text{Injected dose } (\mu c)}{Sp.Act._0\ (\mu c/g \text{ urea})}$$

The half time ($t\frac{1}{2}$) is the time for half the radioactivity to be lost from the urea pool and can be calculated directly from the graph in Figure 1. The entry rate (E) can be calculated from the formula:

$$E = \frac{PO}{t\frac{1}{2} \times 1.44}$$

Constant infusion experiments were also made and the result of an infusion of [^{14}C] urea are also shown in Figure 1. Comparison of the plateau specific activity ($m\mu c/mg$ urea) with the infusion rate of radioactive urea ($m\mu c/min$) gives values for entry rates (mg/min).

(c) Estimation of excretion rates of urea

Excretion of urea was estimated at the same time as an entry rate by collecting the urine at intervals of 30 min from a catheter placed in the bladder and measuring the urea present.

(d) Isolation and estimation of plasma urea

Plasma (5 ml) plus 50 mg of carrier urea was deproteinised by successive additions of 10 ml H_2O, 5 ml 0.5 N $Ba(OH)_2$, and 5 ml 7% (w/v) $ZnSO_4$; the protein-free filtrate was concentrated on a rotary vacuum evaporator and was finally dried over *concentrated* sulphuric acid under reduced pressure. The dried material was dissolved in 0.5 ml 95% (v/v) ethanol and the urea was crystallised by adding dioxan. The urea was recrystallised three times from ethanol using dioxan and was finally dissolved in water and 0.5 ml of this was counted in 10 ml of a scintillation mixture (Bray 1960) in a Nuclear Chicago Scintillation System 725. Urea was estimated using the urease method of Conway (1950).

III. RESULTS

Urea production and excretion rates

Figures 2 and 3 include the results for both single injection and constant infusion experiments.

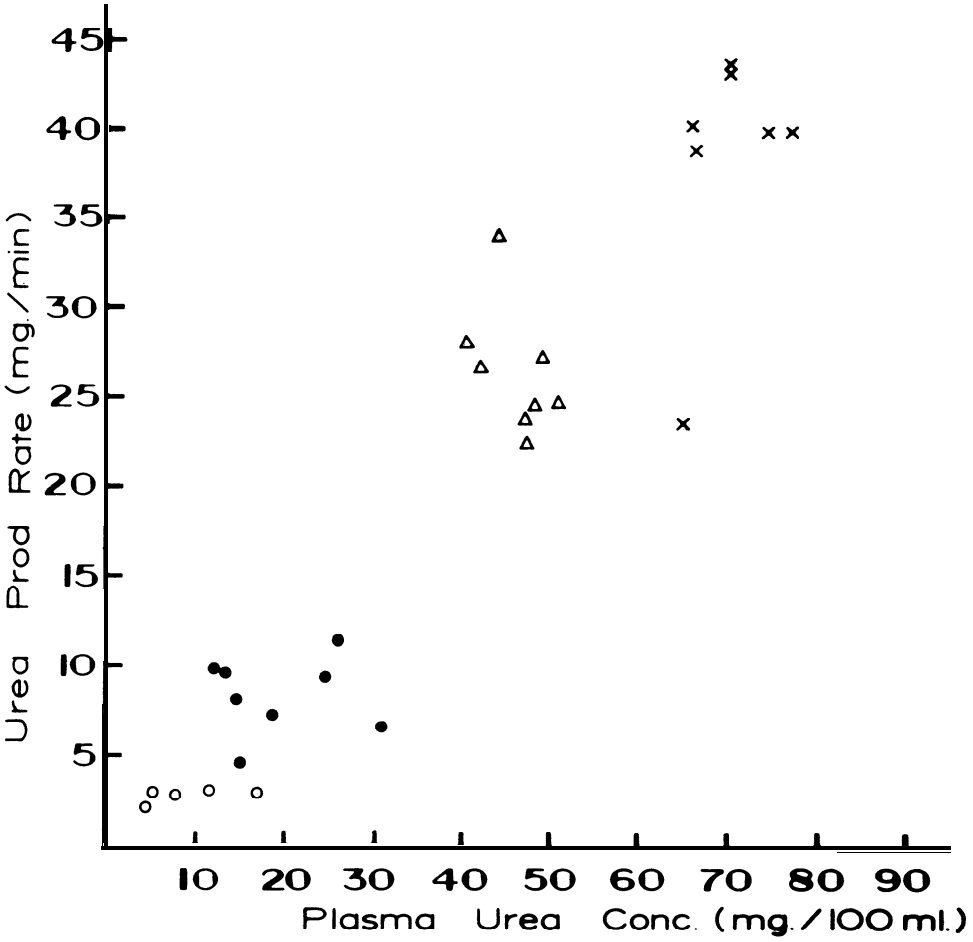

Fig. 2.—Relationship between plasma urea concentration and entry rate of urea in sheep fed various diets. ○, sheep given ration 1; ●, ration 2; △, ration 3; X, ration 4.

There appeared to be good correlations of urea production or excretion rates with plasma concentrations (Figures 2 and 3 respectively). The average urea entry and excretion rates for each ration are shown in Table 1. The extent of degradation of urea in the alimentary tract is estimated from the difference in entry and excretion rates (Table 1). At low protein intakes (i.e. 3.5% C.P. in the diet), only 8% of the urea entering the body pool was lost in the urine, but this percentage was increased at high protein intakes.

IV. DISCUSSION

No differentiation has been made between degradation of urea in the rumen or in the lower intestinal tract, and at present methods are not available for estimating the quantities that enter and are degraded at the different sites.

At low protein intakes, it may be assumed that the majority of the urea re-entering the rumen is utilised by the ruminal organisms for protein synthesis and

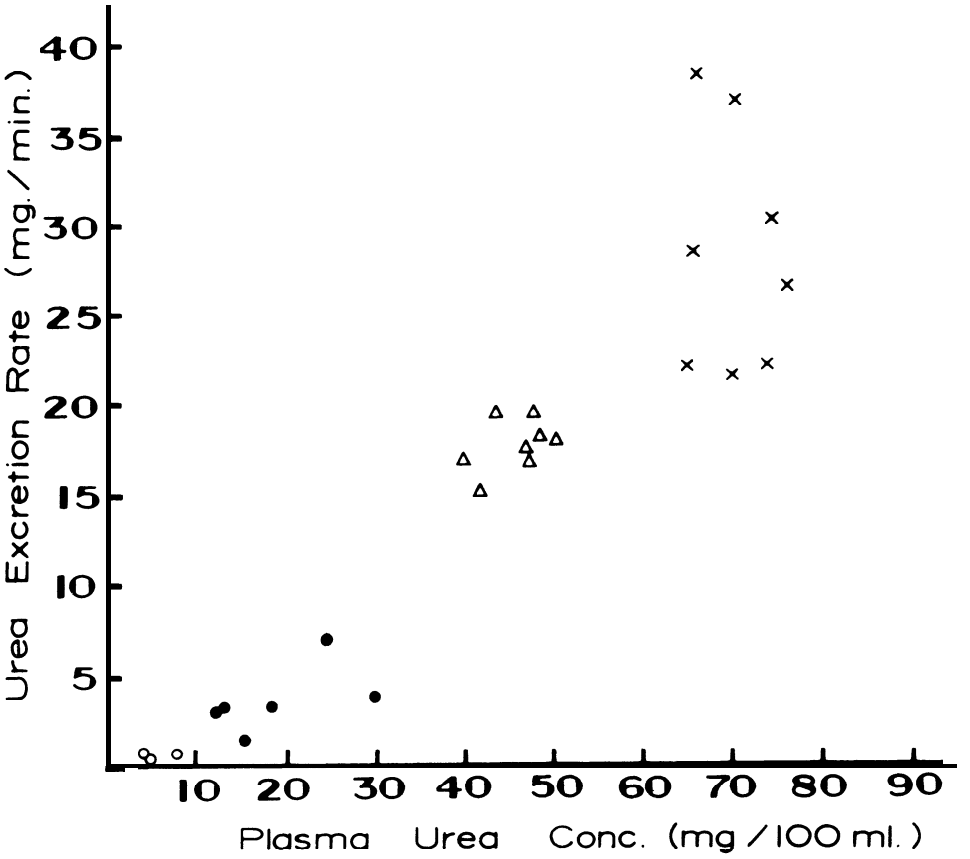

Fig. 3.-Relationship between plasma urea concentration and the excretion rate of urea in sheep. O, sheep given ration 1; ●, ration 2; A, ration 3; X, ration 4.

this protein becomes available to the animal on passage of ingesta to the lower parts of the alimentary tract. These studies clearly show that as the protein content of a ration is reduced there is a decrease in the percentage of the urea entering the body pool that is lost in the urine (Table 1). However, the actual amount of urea returned to the alimentary tract was much higher on the high protein diets (Table 1). It is not known how much of this was utilised for microbial growth.

TABLE 1

Entry ***and excretion rates of urea in sheep fed different rations***

(Results ***expressed as means ± standard errors)***

Ration	No. of Expts.	Plasma Urea Conc. (mg/100 ml)	Urea Entry Rate (mg/min)	Urea Excretion rate (mg/min)	Urea Degradation rate (mg/min)	% of urea entering body pool which is degraded
1	3	5.6 ± 0.67	2.5 ± 0.23	0.22 ± 0.09	2.3 ± 0.32	92.0
2	8	19.4 ± 2.45	8.5 ± 0.77	4.6 ± 1.13	3.9 ± 0.74	44.7
3	8	45.9 ± 1.25	26.4 ± 1.31	17.7 ± 0.52	8.7 ± 1.21	33.0
4	8	70.5 ± 1.53	39.4 ± 2.45	28.3 ± 2.37	11.1 ± 2.74	28.2

On low protein diets, there appears to be a small loss of nitrogen as urea in the urine and there must also be losses in the faeces, since urea equilibrates with all the body water and urea entering the lower intestines may also be utilised by the micro-organisms inhabiting these organs.

At present little information is available as to what proportion of the urea enters the rumen in the saliva and how much passes across the rumen wall. Gärtner, Decker and Hill (1961) have suggested that the contribution of salivary urea to the total amount of urea entering the rumen would be very small. These suggestions, however, were based on the assumption of a saliva flow of 5 litre/day, which is probably an underestimate. The question as to whether plasma urea is actively "secreted" into the rumen is as yet unanswered.

It is concluded that urea recycling is a major process in ruminants, and on low protein roughages (3-4% C.P.) the amount of nitrogen actually entering the rumen may be as much as twice that in the feed.

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