British Journal of Nutrition (2001), **86**, 691–696
DOI: 10.1079/BJN2001448

Estimation of allantoin flux using continuous infusion of [^{14}C]allantoin: sensitivity to plasma loading with unlabelled allantoin

L. P. Kahn[1]*, D. Ouellet[2] and J. V. Nolan[1]

[1]*School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351, Australia*
[2]*Canada Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Lennoxville, Quebec, Canada*

(Received 8 January 2001 – Revised 18 June 2001 – Accepted 17 July 2001)

Allantoin net flux through the plasma allantoin compartment was determined in sheep given a roughage diet by means of a continuous infusion of [4,5-^{14}C]allantoin for 17 h. Unlabelled allantoin was infused intravenously during the last 7 h of the tracer infusion to increase the allantoin flux by approximately 75 %. When unlabelled allantoin was infused, the specific radioactivity of allantoin in plasma and urine declined exponentially to approach a lower plateau some 2–3 h later. The estimate of net flux during the infusion of unlabelled allantoin, estimated from blood plasma and urine, was on average 79 and 90 % of expected values. Expected values of allantoin net flux during infusion of unlabelled allantoin were calculated as the sum of allantoin net flux pre-loading plus the known rate of infusion of the allantoin load. It is probable that endogenous allantoin synthesis was decreased by the infusion of allantoin: allosteric inhibition of uricase appears a plausible explanation for this observation, and for lower estimates of net flux. Appearance of labelled allantoin-C in ruminal or blood bicarbonate was negligible. Our results indicate that net flux of allantoin through blood plasma is a good predictor of the entry rate of allantoin into the primary compartment and should be a better predictor of rumen microbial outflow than urinary allantoin excretion. However, measurements of allantoin-specific radioactivity, during continuous infusion, should be taken after a period of 24 h, at which time the true plateau specific radioactivity value of allantoin in plasma would be attained.

Allantoin: Purine: Microbial nitrogen: Sheep

The nucleic acids present in digesta leaving the rumen are predominately microbial in origin (Smith & McAllan, 1970; McAllan & Smith, 1971). Thus, if the nucleic acid-N:total N ratio, or, more particularly, RNA-N:microbial-N of mixed rumen bacteria is known, the flow of microbial N from the rumen may be determined from the flow of nucleic acids in rumen digesta outflow and their subsequent intestinal absorption. The latter flow can be predicted from excretion of purine derivatives (PD) in urine, as Chen *et al.* (1990*a*) and Balcells *et al.* (1991) have shown that purine absorption from the gut and urinary excretion of PD (predominantly allantoin) are closely related. Consequently, urinary excretion of PD such as allantoin can be used as a means of estimating the outflow of microbial N from the rumen without the need for surgical intervention in the animal, as is required by other methods. However, one potential problem affecting confidence in the prediction of purine flow from the rumen is that the recovery of PD in urine in response to infusions of nucleic acids into the gut (Chen *et al.*1990*a*; Balcells *et al.* 1991; Chen *et al.* 1997) and allantoin (Chen *et al.* 1991; Surra *et al.* 1997) and [^{15}N]uric acid (Chen *et al.* 1998) into the blood has been variable and consistently less than unity.

Transfer of allantoin into the gut via saliva could be one route of non-renal loss of PD (Chen *et al.* 1990*b*), but Surra *et al.* (1993, 1997) and Kahn & Nolan (2000) concluded that the fraction of the total loss of PD by this route was negligible. Kahn & Nolan (2000) also found only negligible amounts of radioactivity in saliva samples of sheep following the intra-jugular injection of labelled allantoin and calculated that less than 1 % of the radioactivity injected was transferred to the rumen via saliva. Further, the transfer of allantoin-C to rumen or blood bicarbonate amounted to only about 1 and 4 % plasma allantoin net flux respectively. Thus, we concluded that very little allantoin is secreted into the gut.

Abbreviations: PD, purine derivative; SR, specific radioactivity.

* **Corresponding author:** Dr Lewis P. Kahn, fax +61 2 67733275, email lkahn2@metz.une.edu.au

In the study reported here, the kinetics of allantoin metabolism in body fluids and the quantitative importance of non-renal loss of allantoin in sheep were further investigated. A continuous intra-jugular infusion of ^{14}C-allantoin was established in two sheep and the flux of metabolically derived allantoin was supplemented by an intra-jugular infusion of unlabelled allantoin. The difference between the estimates of net flux of allantoin in plasma made before and after an intravenous infusion of unlabelled allantoin provided a measure of the recovery of unlabelled allantoin. Estimates made using samples derived from plasma were compared with those derived from urine.

Materials and methods

Animals and conditions

Two rumen-cannulated Merino × Border Leicester ewes, 1 year old, weighing 30·4 and 31·2 kg live weight, were used. The animals were fed hourly from an overhead automatic feeder that delivered approximately equal portions of a daily ration of 510 g oaten chaff (*Avena sativa*; 893 g DM and 12 g N/kg) mixed with 190 g lucerne chaff (*Medicago sativa*; 872 g DM and 28 g N/kg) on an as-fed basis. Total N intake was 11·4 g/d. Animals were maintained on this feeding schedule for 14 d prior to the start of the experiment. Throughout the experiment, the animals were kept in metabolism crates with continuous lighting and unrestricted access to water. Indwelling jugular catheters and Foley bladder catheters (5 ml; Paediatric Bard Limited, Crawley, West Sussex, UK) were placed in each sheep 24 and 18 h before continuous infusions of [4,5-^{14}C]allantoin commenced.

The study was approved by the Animal Ethics Committee of the University of New England.

Experimental procedures

An intra-jugular infusion of isotopically labelled allantoin was maintained for 17 h. Details of the infusion are as follows:

1. A solution containing [4,5-^{14}C]allantoin (Amersham International plc; 1·4 MBq/mg) and 12·5 mg allantoin carrier in 180 ml medical-grade physiological saline (9 g NaCl/l) was infused (approximately 1·2 kBq/min; 0·101 ml/min) for 17 h into one jugular vein of each sheep.
2. Medical grade physiological saline was also infused (approximately 0·190 ml/min) into the other jugular catheter of each sheep from 0–10 h.
3. The saline was replaced by a solution of unlabelled allantoin (21·2 mM), dissolved in medical grade physiological saline, that was infused (approximately 0·190 ml/min; 0·196 mg C/min) into the jugular catheter of each sheep from 10–17 h after the start of the tracer infusion.

Sample collection and storage

Samples of urine, blood and rumen fluid were taken immediately before the tracer infusion period and used later to determine the background levels of radioactivity when counting samples to determine the specific radioactivity (SR) of allantoin or rumen or blood bicarbonate.

Blood and urine were sampled two hourly from 0–6 h, then hourly from 6–8 h and then half-hourly from 8–10 h. Blood and urine were sampled hourly from 10–17 h of the tracer infusion during which the load of unlabelled allantoin was also infused. Urine excretion that occurred between sampling times was quantified. Rumen contents were sampled at the same time as blood and urine during the period from 8·5–17 h.

Blood samples were withdrawn from the jugular catheter and placed immediately into crushed ice. Blood samples obtained in the period from 8·5–17 h were evenly divided into two subsamples. One fraction was used for the determination of the SR of blood bicarbonate by the method of Leng & Leonard (1965). The remaining sample was placed into a heparinized tube and the plasma was then separated by centrifugation. Rumen contents were withdrawn through nylon gauze and a portion was used to determine the SR of rumen bicarbonate (Leng & Leonard, 1965).

Urine continued to be collected, hourly, from the bladder catheters for a further 11 h (i.e. 17–28 h) after the end of the tracer infusion. Bladder catheters were removed at 28 h and urine was then collected for the next 18 h (to give one composite sample) and then daily for the following 2 d to give two more composite samples. Urine was preserved with thymol (final concentration of thymol in urine about 2·2 mM) and subsampled (20 ml) at the end of each collection period. Faeces were collected daily and subsampled (10 %). Plasma, urine and faecal samples were frozen immediately after collection or separation, pending later analysis.

Tracer purity

The radiochemical purity of the [4,5-^{14}C]allantoin was determined, by Amersham International plc, to be 96 % by TLC and 96·3 % by HPLC. The purity of the radio-label was checked upon delivery by the method described by Kahn & Nolan (2000). The purity was found to be 96·1 %, which was in close agreement with the product specification.

Analytical procedures for specific radioactivity of allantoin

Allantoin concentration and SR in urine and plasma were determined as described by Kahn & Nolan (2000).

Calculation of allantoin kinetics

The data were analysed for two periods. The first period was taken from the start of the [^{14}C]allantoin infusion to the commencement of the infusion of unlabelled allantoin (i.e. 0–10 h). The second period was during the infusion of unlabelled allantoin (i.e. 10–17 h). The SR of allantoin in urine was deemed to occur at the midpoint of the collection period and the specific radioactivity *v.* time curves were fitted on this basis.

The SR *v.* time data were fitted by an iterative procedure

based on the Gauss-Newton algorithm that minimized the residual sum of squares for unweighted SR values (GraFit, Data Analysis and Graphics program, version 3, 1992 (RJ Leatherbarrow); Erithacus Software Ltd, Horley, Surrey, UK). The data were unweighted and a measure of the residual variance and a plot of the residuals *v.* time of sampling were used as the criteria to determine which of the models provided the 'best' fit. This procedure gave estimates of plateau SR (B; MBq/g C) and rate constants (k; /min). The net flux of allantoin (g C/min) was calculated by dividing the infusion rate of [^{14}C]allantoin (MBq/min) by the plateau SR (Shipley & Clark, 1972).

Results

There were no feed refusals for either sheep at the end of the period of tracer infusion, that is 17 h after the start of tracer infusion. Sheep 1 accumulated refusals from 11–12 h of this period, but then resumed eating and had ingested all feed offered by the time 14 h of infusion had elapsed, and there were no refusals during the remaining part of the experiment.

Recovery of [^{14}C]allantoin in urine (corrected for dose purity) collected during the 4 d after the commencement of the infusion of [^{14}C]allantoin accounted for 102 % (sheep 1) and 92 % (sheep 2) of that infused. After the start of the [^{14}C]allantoin infusion (period 1), even though the tracer almost certainly mixed in two or more kinetically distinct compartments (Kahn & Nolan, 2000), the SR of allantoin in plasma and urine increased to approach an apparent plateau in a manner that was well described by a first-order exponential equation of the form:

$$SR_t = B(1 - e^{-kt}),$$

where SR_t is the SR of allantoin at time *t*, and B (MBq/g C) and k (/min) are the plateau SR and rate constants respectively. The parameter estimates (B and k) for each sheep (Table 1) generated SR *v.* time curves that were a good and unbiased fit to the data (Figs 1 and 2).

When unlabelled allantoin was infused (period 2), the SR of allantoin in plasma and urine declined exponentially to approach a lower plateau some 2–3 h later. The decline in SR was best described by a single exponential plus offset (B) equation:

$$SR_t = A_0e^{-kt} + B,$$

where A_0 (MBq/g C) represented the hypothetical intercept value at 10 h and k and B were the coefficients described earlier. The parameter estimates for each sheep (Table 1) generated curves that provided a good and unbiased fit to the data (Figs 1 and 2).

The concentration of allantoin in plasma remained fairly constant throughout period 1. Intra-jugular infusion of unlabelled allantoin (0·196 mg C/min) increased the plasma concentration of allantoin in period 2 by 60 % or 2 mg C/l (Table 2). The variability of plasma allantoin concentration, as assessed from the CV from sequential measurements, during periods 1 and 2 ranged from 1·2–7·4 %. Following termination of the loading infusion, allantoin concentration in plasma declined to a level below that during the period 0–10 h. Mean plasma concentration of allantoin in sheep 1 and 2 from samples collected from 23·7–28·2 h (i.e. 6·7–11·2 h after termination of the loading infusion) was 2·7 mg C/l which was on average 17·1 % less than during the period 0–10 h (Fig. 3).

The estimates of net flux of allantoin were similar for both sheep during period 1 but differed depending on whether they were derived from blood or urine (Table 3). Net flux of allantoin increased in period 2 and the estimates made from samples of plasma or urine became similar. The recovery of the infused load of unlabelled allantoin was calculated as the difference between the estimates of allantoin net flux (mg C/min) made in periods 1 and 2 expressed as a fraction of

Table 1. Parameter estimates for plasma and urine allantoin specific radioactivity *v.* time curves from two sheep that received a continuous infusion of [^{14}C]allantoin without (period 1) or with (period 2) an intravenous load of unlabelled allantoin*

(Parameter estimates with their standard errors)

	Sheep 1		Sheep 2	
Parameter	Estimate	SE	Estimate	SE
Period 1				
First order rate model				
Plasma				
B (MBq/g C)	4·14	0·096	5·29	0·096
k (/min)	0·015	0·0027	0·015	0·0020
Urine				
B (MBq/g C)	5·73	0·104	6·22	0·089
k (/min)	0·014	0·0011	0·014	0·0009
Period 2				
Single exponential plus offset model				
Plasma				
B (MBq/g C)	2·95	0·067	3·59	0·070
k (/min)	0·025	0·0082	0·020	0·0058
Urine				
B (MBq/g C)	3·22	0·044	3·29	0·104
k (/min)	0·014	0·0005	0·011	0·0008

* For details of procedures, see p. 692.

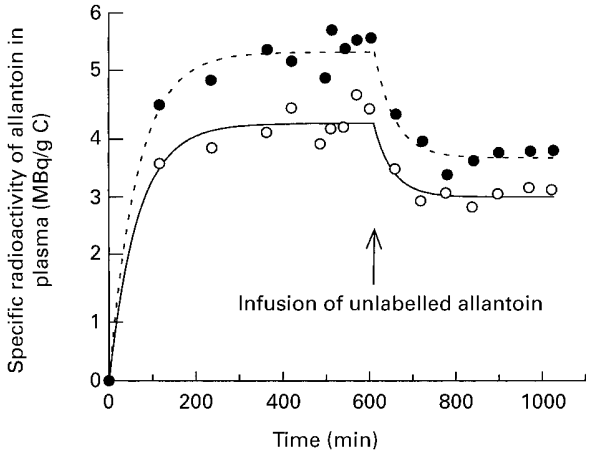

Fig. 1. Specific radioactivity of allantoin in blood plasma over the experimental period. For details of procedures, see p. 692. Data for sheep 1 (0) were fitted by a solid line (—) and for sheep 2 by a dashed line (– –). Infusion rates of [^{14}C]allantoin were 1·1 and 1·3 kBq/min for sheep 1 and 2 respectively.

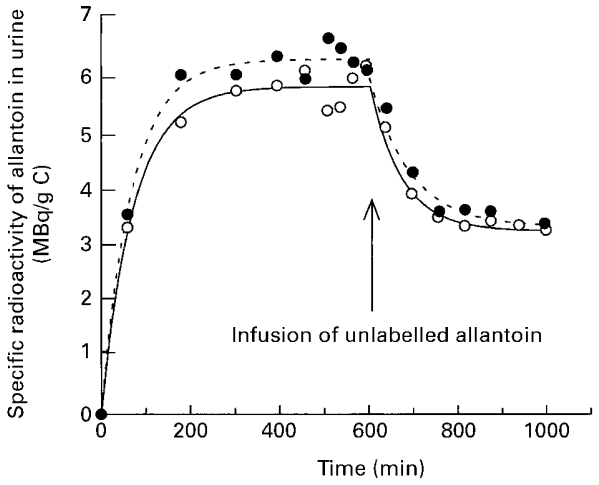

Fig. 2. Specific radioactivity of allantoin in urine over the experimental period. For details of procedures, see p. 692. Data for sheep 1 (0) were fitted by a solid line (—) and for sheep 2 by a dashed line (– –). Infusion rates of [^{14}C]allantoin were 1·1 and 1·3 kBq/min for sheep 1 and 2 respectively.

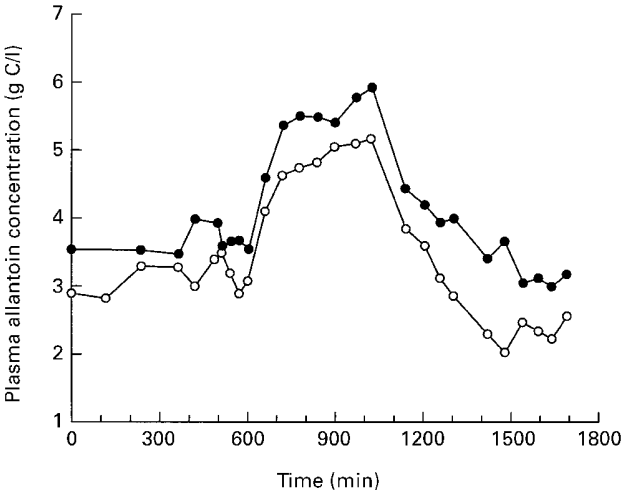

Fig. 3. Plasma concentration (g C/l) of allantoin in sheep 1 (○—○) and sheep 2 (●—●) prior to (0–600 min), during (600–1020 min) and after termination (1020–1700 min) of the infusion of a load of unlabelled allantoin. For details of procedures, see p. 692.

the unlabelled allantoin infusion rate. Based on estimates of net flux in plasma and urine, the recovery of unlabelled allantoin averaged 0·79 and 0·90 respectively. Transfer of allantoin-C to either blood or rumen content bicarbonate pools was negligible (Table 3) as reported previously (Kahn & Nolan, 2000).

Discussion

At the time when the intra-jugular ^{14}C-allantoin infusion was terminated (17 h), excretion of [^{14}C]allantoin in urine had accounted for 90 and 78 % [^{14}C]allantoin infused into sheep 1 and 2 respectively. However, excretion of [^{14}C]allantoin in urine continued over the next 66 h, by which time 102 and 92 % of the tracer infused had been recovered. Prolonged excretion of [^{14}C]allantoin in urine was also observed in our previous study (Kahn & Nolan, 2000) in which about 80 % of the intravenously administered [^{14}C]allantoin was excreted after 24 h, increasing to 95 % over the next 4 d.

In sheep, the mass of allantoin in the primary plasma compartment (33–43 mg C) is small in relation to the total flux of allantoin and turns over approximately once per h (Kahn & Nolan, 2000). A rapid passage of allantoin from blood to urine would therefore be expected given the limited capacity for tubular reabsorption of allantoin in sheep (Chen *et al.* 1991). However, the data from this and our previous study indicate that, in sheep, excretion of [^{14}C]allantoin in urine continues for a considerable period following cessation of its intra-jugular administration.

One possible explanation for the prolonged excretion of [^{14}C]allantoin in urine is that allantoin in plasma is able to exchange with other secondary or tertiary compartments in body fluids that sequester tracer for extended periods. Labelled allantoin entering such compartments will take longer to be excreted via the primary compartment, i.e. the site of its administration, and each kinetic compartment will potentially be present as an additional exponential component in the equation describing the SR *v.* time curves. This notion is supported by the studies of Kahn & Nolan (2000) which indicated that the SR *v.* time data for blood allantoin appeared to contain at least two and probably three exponential components.

Plasma concentrations of allantoin averaged 66 and 77 μM (3·1 and 3·7 mg allantoin-C respectively) for the period prior to infusion of unlabelled allantoin. These values

Table 2. The mean concentration of allantoin in blood plasma during period 1 and during the final 2 h of period 2*†

(Mean values, standard deviations and coefficients of variation)

	Sheep 1			Sheep 2		
Parameter	Mean	SD	CV (%)	Mean	SD	CV (%)
Allantoin in plasma (mg C/l)						
Period 1 (0–10 h)	3·1	0·23	7·4	3·7	0·18	5·0
Period 2 (15–17 h)‡	5·1	0·06	1·2	5·7	0·27	4·7

* For details of procedures, see p. 692.
† During period 2, animals received an intravenous load of unlabelled allantoin.
‡ Calculations were made using data from 15–17 h of period 2 when the plasma allantoin concentration was approaching a higher and constant concentration.

Table 3. Kinetic data derived from blood plasma and urine of two sheep following intra-jugular continuous infusion of [^{14}C]allantoin (0–17 h) and unlabelled allantoin (10–17 h)*†

Parameter	Sheep 1	Sheep 2
Plasma		
Net flux (mg C/min)		
0–10 h	0·257	0·237
10–17 h	0·357	0·350
Unlabelled allantoin infusion (mg C/min)	0·195	0·196
Recovery (fraction of unlabelled allantoin infusion rate)	0·79	0·80
Net flux (mg C/kg intake)	528	487
Proportion of C derived from allantoin (transfer quotient $\times 10^5$)		
In blood bicarbonate	4·86	5·73
In rumen bicarbonate	3·17	4·69
Urine		
Net flux (mg C/min)		
0–10 h	0·185	0·201
10–17 h	0·328	0·380
Unlabelled allantoin infusion (mg C/min)	0·195	0·196
Recovery (fraction of unlabelled allantoin infusion rate)	0·86	0·96
Net flux (mg C/kg intake)	381	413

* For details of procedures, see p. 692.
† Allantoin data are corrected for dose purity of 0·961.

are similar to those reported by Chen *et al.* (1990*a*) and Balcells *et al.* (1992) but are lower than those reported by Kahn & Nolan (2000). However, because blood concentrations of allantoin are dependent on both feed intake and protein status of the animal (Chen *et al.* 1992), comparisons between experiments may be expected to show differences.

Plasma concentration of allantoin fell sharply after cessation of the infusion of unlabelled allantoin and remained 17 % below pre-loading infusion levels for at least 10 h. It is possible that such a result was a consequence of allosteric inhibition of uricase initiated during the loading infusion. Allosteric inhibition of uricase would reduce the catabolism of uric acid to allantoin and lower endogenous plasma concentrations of allantoin.

Chen *et al.* (1991) infused unlabelled allantoin into the jugular vein of sheep and compared plasma concentrations of allantoin during and after the infusion. In contrast to our results, those authors did not report any decrease in plasma allantoin concentration after cessation of allantoin loading. There are a number of possible reasons for this, but of most importance was that the post-loading concentrations of allantoin that were reported were a mean of concentrations in samples taken 0, 2, 4, 6, 8, 13 and 24 h after termination of the loading infusion. Our data would suggest that the method by which Chen *et al.* (1991) calculated the post-loading mean concentration of allantoin would have hidden carryover effects similar to those we are reporting. One effect of this carryover would have resulted in elevated allantoin concentration for the samples taken for the 4 h period after cessation of allantoin loading. In addition, the longer intervals between later samples most probably did not allow Chen *et al.* (1991) to detect sub-basal allantoin concentrations as a result of allosteric inhibition of uricase. The net result of these effects would have been to elevate mean plasma allantoin concentration reported by Chen *et al.* (1991). The significance of allosteric inhibition of uricase for the interpretation of results obtained in the present experiment will be discussed later.

Assuming that allantoin metabolism in the sheep was in steady state, the difference between allantoin net flux in periods 1 and 2 should have represented the rate at which the allantoin load was being infused. We tried to overcome potential diurnal variation that might alter allantoin kinetics during period 1 and period 2 by holding the animals under continuous lighting (to eliminate possible photo-period effects) and supplied feed in hourly portions from an automatic feeder (to promote a constant uptake of purines from the gut, and a steady state of purine metabolism by tissues).

Even though the animals were held in steady-state conditions, the estimate of net flux during loading, estimated from blood plasma and urine, was only 79 and 86 % (sheep 1) and 80 and 96 % (sheep 2) of expected values, the expected value in each instance being the sum of the net flux estimate in the pre-loading period plus the known rate of infusion during the loading period. There are a number of reasons why the net flux estimates might have been lower than expected. First, it is possible that after the loading infusion was terminated, continued allosteric inhibition of uricase resulted in reduced rate of conversion of uric acid to allantoin resulting in a 17 % reduction in endogenous allantoin concentration in plasma. (A reduction in allantoin concentration would have had the effect of increasing allantoin plateau SR and reducing estimates of net flux, where net flux (mg C/min) = infusion rate/plateau SR.) It can be calculated that a 17 % reduction in plasma allantoin concentration would reduce net flux by 16 %. Accounting for this potential error would result in estimated values of allantoin net flux through plasma increasing to 92 and 94 % of expected values for sheep 1 and 2 respectively. An

adjustment for the effect of allosteric inhibition on the estimates of net flux from urine would also be warranted but because urinary allantoin concentration is dependent on urine volume and hence may vary independently of allantoin net flux through plasma, an adjustment rate could not be applied with any certainty.

A second reason for net flux estimates being lower than expected values is that allantoin SR in plasma is likely to have taken longer than 10 h to reach true plateau values. Modelling of the data of plasma allantoin SR for the period 0–80 h during infusion of tracer suggested that the SR would not have reached plateau during the first 10 h period. This is consistent with earlier observations (Kahn & Nolan, 2000) that allantoin distributes among two or more compartments and hence plateau values in the primary compartment would be dependent on transfer rates between all compartments. The results from the model indicated that plasma SR at 10 h was 9 % lower than that which would have eventually been attained at 25 h. Increasing plateau SR to account for this error reduced estimates of net flux (0–10 h) by 10·5 %. The consequence of a lesser net flux during the pre-loading period was to increase further the estimated values of allantoin net flux through plasma to 98 and 100 % of expected values for sheep 1 and 2 respectively.

The transfer of allantoin-C to bicarbonate-C in blood and rumen contents was negligible and consistent with that reported by Kahn & Nolan (2000). The present study provides further confirmation that degradation of allantoin to bicarbonate in the gut is quantitatively unimportant in sheep given roughage diets.

Our results indicate that net flux of allantoin through blood plasma is a good predictor of the net flux of allantoin through the primary compartment. However, measurements of allantoin concentration and SR, during continuous infusion, should be taken after a period of 24 h at which time the true plateau SR value of allantoin in plasma would be attained. Further, a load of unlabelled allantoin appears to reduce the synthesis of endogenous allantoin in plasma which may be a consequence of allosteric inhibition of uricase.

Acknowledgements

The authors acknowledge the skilled technical assistance of Messrs F. Ball and S. Stachiw. Funding was made available by Australian woolgrowers through the International Wool Secretariat who also provided a scholarship for L.K.

References

Balcells J, Guada JA, Castrillo C & Gasa J (1991) Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum. *Journal of Agricultural Science, Cambridge* **116**, 309–317.

Balcells J, Parker DS & Seal CJ (1992) Purine metabolite concentrations in portal and peripheral blood of steers, sheep and rats. *Comparative Biochemistry and Physiology* **101**B 633–636.

Chen XB, Calder AG, Prasitkusol P, Kyle DJ & Jayasuriya MCN (1998) Determination of ^{15}N isotopic enrichment and concentrations of allantoin and uric acid in urine by gas chromatography/mass spectrometry. *Journal of Mass Spectrometry* **33**, 130–137.

Chen XB, Chen YK, Franklin MF, Ørskov ER & Shand WJ (1992) The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. *Journal of Animal Science* **70**, 1534–1542.

Chen XB, Fujihara T, Nakamura K, Mawuenyegah PO, Franklin MF & Kyle DJ (1997) Response of urinary and plasma purine derivatives to various rates and infusion patterns of purines in sheep nourished by intragastric infusion. *Journal of Agricultural Science* **129**, 343–352.

Chen XB, Hovell FDDeB & Ørskov ER (1990*a*) Excretion of purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut. *British Journal of Nutrition* **63**, 197–205.

Chen XB, Hovell FDDeB, Ørskov ER & Brown DS (1990*b*) Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition* **63**, 131–142.

Chen XB, Kyle DJ, Ørskov ER & Hovell FDDeB (1991) Renal clearance of plasma allantoin in sheep. *Experimental Physiology* **76**, 59–65.

Kahn LP & Nolan JV (2000) Kinetics of allantoin metabolism in sheep. *British Journal of Nutrition* **84**, 629–634.

Leng RA & Leonard GJ (1965) Measurement of the rates of production of acetic, propionic and butyric acids in the rumen of sheep. *British Journal of Nutrition* **19**, 469–484.

McAllan AB & Smith RH (1971) Nucleic acids in ruminant digesta as indices of microbial nitrogen. *Proceedings of the Nutrition Society* **31**, 24A.

Shipley RA & Clark RE (1972) *Tracer Methods for In Vivo Kinetics*. New York, NY: Academic Press.

Smith RH & McAllan AB (1970) Nucleic acid metabolism in the ruminant. *British Journal of Nutrition* **24**, 545–556.

Surra J, Guada JA, Balcells J & Castrillo C (1993) Salivary losses of purine derivatives in sheep. In *British Society of Animal Production Winter Meeting*, pp. 175–176. Penicuik: British Society of Animal Production.

Surra J, Guada JA, Balcells J & Castrillo C (1997) Renal and salivary clearance of purine derivatives in sheep. *Animal Science* **65**, 83–91.