Excretion of purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut

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The saliva of sheep was shown to contain significant concentrations of uric acid (16 (SD 4·5) μmol/l) and allantoin (120 (SD 16·4) μmol/l), sufficient to recycle purine derivatives equivalent to about 0·10 of the normal urinary excretion. When allantoin was incubated in vitro in rumen fluid, it was degraded at a rate sufficient to ensure complete destruction of recycled allantoin. In a series of experiments in which allantoin was infused into the rumen of sheep fed normally, or into the rumen or abomasum of sheep and the rumen of cattle completely nourished by intragastric infusion of volatile fatty acids and casein, no additional allantoin was recovered in the urine. These losses were probably due to the degradation of allantoin by micro-organisms associated with the digestive tract. It is concluded that all allantoin and uric acid recycled to the rumen via saliva will be similarly degraded. Therefore, the use of urinary excretion of purine derivatives as an estimator of the rumen microbial biomass available to ruminants will need to be corrected for such losses.

The urinary excretion of purine derivatives by ruminants has been proposed as an estimator of the rumen microbial protein supplied to the host animal (e.g. Topps & Elliott, 1965). This is because the nucleic acids flowing to the small intestines are essentially of rumen microbial origin (McAllan & Smith, 1973). Absorbed purines are degraded to hypoxanthine, xanthine, uric acid and allantoin. These are excreted in urine, and should relate quantitatively to the amount of microbial purines, and hence microbial protein, absorbed.

The quantitative relationship between purine supply and derivative excretion has been examined previously following an abomasal infusion of a nucleic acid concentrate in sheep. Recovery as derivatives of absorbed nucleic acid purines was 0·84 (mmol/mmol) after correction for possible utilization by the animal (Chen *et al.* 1990*a*), with 0·16 of the purines unaccounted. A similar observation was made in steers (recovery of 0·85) (Verbic *et al.* 1990). Since there are no reports of a mammalian allantoinase (*EC* 3.5.2.5), the most likely route of loss would appear to involve microbial degradation within the digestive tract and this hypothesis was tested in the current study.

Three aspects were examined: secretion of purine derivatives into the rumen via saliva; the rate of degradation of allantoin in rumen digesta by rumen micro-organisms; and whether allantoin escaping degradation within the rumen would then be excreted unchanged. A provisional report of recycling of purine derivatives via the saliva has already been made (Chen *et al.* 1989).

MATERIALS AND METHODS

Animals, management and treatments

Expt 1. Salivary purine derivatives. Twenty mature Blackface × Suffolk sheep (50–70 kg live weight (W)) were used. They were given 1200 g/d of a diet containing 0·50 rolled barley and 0·50 grass cubes. The feed was offered in two equal meals at 08·00 and 15·30 hours. Jugular

blood and saliva samples were taken once daily for 4 d between 09·00 and 16·00 hours. Sampling of blood and saliva from each animal was made within 15 min; each animal was sampled in the morning for 2 d and in the afternoon for the other 2 d. Blood was sampled into heparinized tubes, centrifuged, and stored as plasma. Saliva samples were obtained by letting the animals chew on a sponge. The saliva was squeezed out and immediately frozen. All samples were stored at −20° until analysis for uric acid and allantoin. For allantoin assay, both plasma and saliva samples were treated with an equal volume of trichloroacetic acid (TCA; 100 g/l) and centrifuged at 2200 g for 20 min.

Expt 2. in vitro incubation of allantoin. The Rusitec (rumen simulation technique) apparatus (Czerkawski & Breckenridge, 1977) was used. About 1·5 litres rumen fluid were collected from each of three sheep receiving a diet (GP diet) containing 0·50 hay, 0·30 rolled barley, 0·10 molasses, 0·09 fishmeal and 0·01 minerals and vitamins. The rumen fluid was bulked and transported to the laboratory in a Thermos-flask.

Three separate incubations were carried out. In the first run, whole rumen fluid was used. A portion of 750 ml was transferred into each of four reaction vessels. To three vessels allantoin was added (64 mg/50 ml water) and to the fourth 50 ml water (control). The contents of each vessel were mixed and sampled immediately. The GP diet (18 g), contained in a nylon-gauze bag, was put into the perforated 'cage' inside each reaction vessel as substrate for the micro-organisms. The incubation temperature was 39°. Samples of fluid were taken at 1, 2, 3, 4, 5, 6, 7, 9·5, 11·5 and 24 h. Microscopic examination of the rumen fluid at 8 h showed an active microbial population; the pH of the fluid declined, however, from an initial 5·6–5·2 at 24 h.

No buffer had been added to the first incubation. In the subsequent two incubations, 200 ml Akkada Buffer (Abou Akkada & Howard, 1960) were mixed with 600 ml rumen fluid (filtered through two single layers of surgical gauze). Allantoin added was 64 and 32 mg for the second and third runs respectively. The incubation and sampling procedures were as in the first run. The initial pH of the incubated fluid was 6·0, and it was 5·9 at 12 h and 5·6 at 30 h of incubation. An active microflora was still present by 30 h. The dissolved oxygen content (ion-sensitive electrode, Model ISE/97-7089; Russell pH Ltd, Scotland) in the rumen fluid was less than 0·10 μg/l, confirming that the incubation was anaerobic.

All samples were immediately treated with an equal volume of TCA (100 g/l), cooled to 0° and then centrifuged at 2200 g for 20 min. The supernatant fractions were stored at −20° until analysis for allantoin.

Expt 3. Infusion of allantoin into the rumen of normally fed sheep. Three female Blackface × Suffolk lambs (39–42 kg W) and one Finn × Dorset wether (52 kg), each fitted with a rumen cannula, were given 1000 g/d of either grass nut cubes (female lambs) or GP (wether) as two equal meals at 08.00 and 16.00 hours. Consecutive 5 d periods of rumen infusion were as follows: (1) 3·5 litres water/d, (2) 8 mmol allantoin/d in 3·5 litres water, (3) 3·5 litres water/d. Daily urine was collected into about 200 ml 1 M-sulphuric acid and subsampled. All urine samples were stored at 4° before analysis for purine derivatives.

Expt 4. Infusion of allantoin into the rumen or abomasum of sheep nourished by intragastric infusions. Three castrated Finn × Dorset lambs (52–57 kg W) were maintained by daily infusion into the rumen of volatile fatty acids (VFA) (450 kJ metabolizable energy (ME)/kg $W^{0·75}$), and into the abomasum of casein (430 mg casein-nitrogen/kg $W^{0·75}$). Both inputs of energy and protein were of about the maintenance levels for the animals. The details of intragastric infusions were as described by Ørskov *et al.* (1979) and Hovell *et al.* (1987). Five treatments were imposed: continuous infusion of allantoin at 4 or 8 mmol/d either into the rumen (mixed in with the VFA), or into the abomasum (mixed with the casein), and a control, when no allantoin was infused. The five treatments were randomly

allocated to the three animals over five consecutive 4 d periods. Urine collection, sampling and preparation for analysis were as with Expt 3.

Expt 5. Infusion of allantoin into the rumen of steers nourished by intragastric infusions. Two Friesian steers (268–395 kg W) were nourished by daily rumen infusion of VFA (675 kJ ME/kg $W^{0.75}$; equivalent to 1·5 maintenance energy requirement) and abomasal infusion of casein (430 mg casein-N/kg $W^{0.75}$; maintenance N requirement). The details of intragastric infusions were as described by MacLeod *et al.* (1982). Three treatments were imposed: a continuous infusion of allantoin at 31·6 (A) or 63·3 (B) mmol/d into the rumen (mixed with the VFA), and a control (C), when no allantoin was infused. Treatments C, A and B were allocated to the two animals over three consecutive 5 d periods. The methods of urine collection, sampling and preparation for analysis were as for Expt 3.

Expt 6. Outflow of allantoin from the rumen. Two female Blackface × Suffolk lambs (about 30 kg W) nourished by intragastric infusions as in Expt 4 were used. A single dose of 1·58 mmol allantoin and 10 g polyethylene glycol (molecular weight 4000; PEG), dissolved in 100 ml water, was injected into the rumen of the animals via a rumen cannula. The rumen contents, which were completely liquid, were mixed immediately (by 'pumping' for 2 min with a 60 ml syringe) and sampled. Subsequent samples were taken at intervals over a 24 h period. All rumen samples were frozen immediately and stored at $-20°$ until analysis for PEG and allantoin. The whole procedure was repeated the next day but with a larger dose of allantoin (1·90 mmol) and PEG (15 g).

Chemical analysis

Methods for analysis of allantoin and uric acid were as described in Chen *et al.* (1990 *b*). PEG was determined by the method of Malawer & Powell (1967).

Statistical analysis

Statistical analysis was by means of the package 'GENSTAT 4' (Lawes Agricultural Trust, 1984). In Expt 1, the least-squares analysis of variance was used to examine the effects of animal, day and time of day on the plasma and salivary allantoin and uric acid, and whether there was any relationship between saliva and plasma concentrations. In Expt 2, linear regression analysis was used to examine the loss of allantoin with time during in vitro incubation. In Expts 3 and 4, analysis of variance was performed to compare allantoin excretion between different infusion treatments. Expt 4 was analysed as randomized block (animal as block and infusion as treatment). Values of Expt 5 were not subjected to statistical analysis due to the limited number of animals used and, therefore, the variance of individual means was presented. The maximum likelihood programme (MLP) (Ross, 1987) was employed in Expt 6 for fitting the data into a mono-exponential curve using the least-squares method.

RESULTS

Expt 1. Salivary purine derivatives. The concentrations of allantoin and uric acid averaged, respectively, 52 (SD 5·7) and 6 (SD 1·1) μmol/l in plasma, and 120 (SD 16·4) and 16 (SD 4·5) μmol/l in saliva. With the salivary measurements, the between-animal difference contributed about 20% to the total variance, and was significant ($P < 0.01$). Over the 4 d of measurements, there was a significant ($P < 0.01$) decline in salivary concentrations of allantoin and uric acid with the difference between days contributing about 60% of the total variance (Table 1). However, within a day, there was no evidence that time of sampling affected salivary concentrations of either uric acid or allantoin. The daily measurements of the plasma concentrations showed a slight increase over the 4 d (Table 1).

Table 1. *Expt 1. Allantoin and uric acid (μmol/l) in saliva and plasma of normally fed sheep measured on four consecutive days*

(Means for twenty sheep)

Day...	1	2	3	4	SED (df 16)
Plasma					
Allantoin	49·5	48·0	55·1	56·1	2·82
Uric acid	5·17	6·30	6·66	6·97	0·56
Saliva					
Allantoin	145·9	121·5	113·7	97·6	7·20
Uric acid	23·20	14·57	15·88	11·20	2·00

SED, standard error of difference.
* For details of procedures, see p. 197.
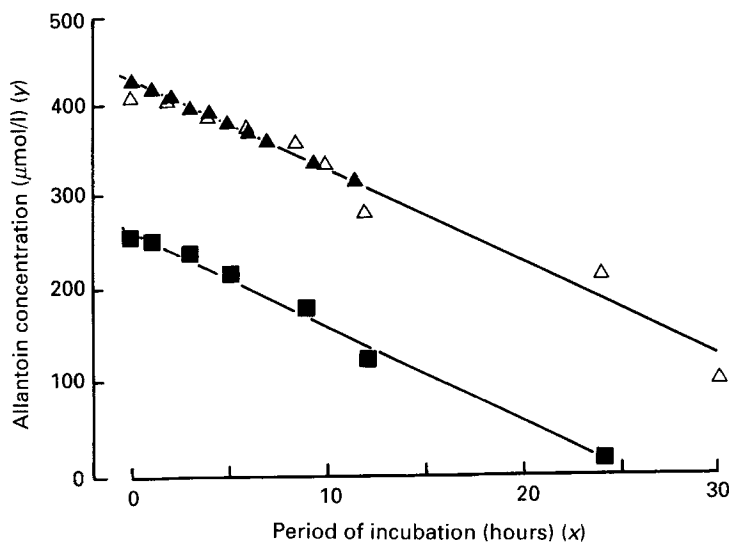
Fig. 1. Expt 2. Disappearance of allantoin during in vitro incubation with rumen fluid from sheep.
(▲), run no. 1, $Y = 438 - 11·1$ (SE 0·41) X (r 0·995);
(△), run no. 2, $Y = 426 - 10·0$ (SE 0·72) X (r 0·983);
(■), run no. 3, $Y = 264 - 10·3$ (SE 0·45) X (r 0·995).
For details, see p. 198.

Time of sampling did not affect plasma uric acid concentration, but did affect plasma allantoin ($P < 0·01$). The plasma allantoin averaged 48·4 and 59·0 μmol/l for samples taken at 09·00–11·00 and 14·00–16·00 hours respectively. The between-animal variance for plasma uric acid was not significant, whereas that for plasma allantoin was ($P < 0·001$), contributing nearly 50 % of the total variance. Salivary concentrations of neither uric acid nor allantoin correlated with plasma concentrations.

Expt 2. in vitro incubation of allantoin. There was a slight decline in allantoin concentration in the control vessels during the first 4 h (by an average of 7 μmol/l) but not thereafter, when the concentration remained at 38 (SD 5) μmol/l. Concentrations in the

Table 2. *Expt 3. Effect of an intraruminal infusion of allantoin on the excretion of purine derivatives (PD) (mmol/d) by normally fed sheep**

(Means for four sheep)

| Period† ... | 1 | 2 | 3 | SED |
Allantoin infused (mmol/d) ...	0	8·0	0	(df 6)
Allantoin	10·2	9·8	8·9	0·78
Other PD‡	3·18	2·98	2·68	0·20

SED, standard error of difference.
* For details of procedures, see p. 198.
† Mean of last 4 d of each period.
‡ Uric acid and xanthine plus hypoxanthine.

Table 3. *Expt 4. Effect of a ruminal or abomasal infusion of allantoin on the urinary excretion of purine derivatives (PD) (mmol/d) by sheep nourished by intragastric infusions of volatile fatty acids and casein**

(Means for three sheep)

| | Rumen infusion | | | Abomasal infusion | | SED |
Allantoin infusion (mmol/d) ...	0	4	8	4	8	(df 8)
Urinary						
Allantoin	1·73	1·69	1·90	2·08	2·24	0·076
Other PD†	1·79	1·63	1·79	1·89	1·77	0·108

SED, standard error of difference.
* For details of procedures, see p. 198.
† Uric acid and xanthine plus hypoxanthine.

allantoin-dosed vessels declined linearly with time. The rate of loss of allantoin during the incubation was very similar in the three runs (Fig. 1), even though the first run was not buffered. The rate of loss was equivalent to 15 μmol/h per 1 rumen fluid.

Expt 3. Infusion of allantoin into the rumen of normally fed sheep. Exogenous administration of allantoin did not cause any changes in urinary excretion of purine derivatives (Table 2).

Expt 4. Infusion of allantoin into the rumen or abomasum of sheep nourished by intragastric infusions. In the lambs nourished by intragastric infusions, urinary allantoin excretion was not significantly affected by the rumen infusion of allantoin (Table 3) even at an infusion level (8 mmol) equivalent to fourfold the basal urinary excretion. The abomasal infusion of allantoin, however, resulted in a 20–29 % increase in allantoin excretion ($P < 0.001$, Table 3). This increase was equivalent to only 6–8 % of that infused. The daily excretions of uric acid, xanthine and hypoxanthine were not affected by either the rumen or abomasal infusion of allantoin.

Expt 5. Infusion of allantoin into the rumen of steers nourished by intragastric infusions. There was no evidence of any increase in the excretion of allantoin in the urine of the two steers when infused with 31·6 or 63·3 mmol allantoin/d (Table 4)

Expt 6. Outflow of allantoin from the rumen. Both allantoin and PEG concentrations (Y)

Table 4. *Expt 5. Effect of a rumen infusion of allantoin on the urinary excretion of purine derivatives (mmol/d) by steers nourished by intragastric infusions of volatile fatty acids and casein*

(Means with their standard errors for five daily measurements)

Period...		1		2		3	
Allantoin infused (mmol/d)...		0		31·6		63·3	
	Steer no.	Mean	SE	Mean	SE	Mean	SE
Urinary (mmol/d)*							
Allantoin	1	26·6	0·46	32·3	1·43	31·4	0·89
	2	33·2	2·75	30·8	1·70	24·2	0·97
	Mean	29·9		31·6		27·5	
Uric acid	1	4·5	0·17	4·5	0·17	5·8	0·51
	2	4·6	0·28	4·3	0·13	3·7	0·08
	Mean	4·6		4·4		4·8	

* For details of procedures, see p. 199.

Table 5. *Expt 6. The rate of disappearance (proportion/h) of allantoin and polyethylene glycol (PEG) pulse-dosed into the rumen of sheep nourished by intragastric infusions**

	Sheep no.	Trial no.†	Allantoin	PEG
	1	1	0·155	0·090
		2	0·162	0·094
	2	1	0·170	0·082
		2	0·162	0·060
	Mean		0·162	0·082
	SE		0·0031	0·0076

* For details of procedures, see p. 199.
† Dose of allantoin was 1·58 and 1·90 mmol, and of PEG 10 and 15 g in trial nos. 1 and 2 respectively.

in the rumen of the lambs declined exponentially with time (t). The data were fitted into a mono-exponential equation:

$$Y = a + b\,e^{-kt}.$$

The 'k' values, which represented the fractional rate at which allantoin or PEG disappeared from the rumen, are shown in Table 5. In all four runs, allantoin disappeared from the rumen (0·162(SD 0·06) /h) much more rapidly than PEG (0·082(SD 0·015) /h).

DISCUSSION

Salivary and plasma purine derivatives

The presence of allantoin and uric acid in sheep saliva demonstrated that purine derivatives in plasma can be recycled via salivary secretion to the rumen. Saliva flow in sheep has been estimated to range from 8 to 16 litres/d (Kay, 1966), and assuming a saliva flow of 10 litres/d for the animals of Expt 1, then 1·4 mmol allantoin plus uric acid /d could have been secreted into the rumen. This would be equivalent to about 0·10 of the urinary excretion by similar animals (10–15 mmol/d; Chen, 1989).

The higher concentrations of both allantoin and uric acid in saliva, compared with those of plasma (Expt 1), are in agreement with the observation of Katz & Sorensen (1968) who noted in humans that salivary uric acid concentration was 0·2–3·6 times the plasma concentration, depending on the rate of salivary flow. If allantoin and uric acid are not actively excreted into saliva, the salivary glands must have the ability to concentrate the salivary fluid by re-absorption of water, which would reduce or eliminate any direct relationship between the plasma and salivary concentrations, and explain the absence of any such relationship in Expt 1.

It is not clear why there was a decline in salivary concentrations over the 4 d. During sampling, the saliva of the last 2 d appeared more dilute than that obtained in the earlier 2 d and it is possible that the action of sampling acted as a conditioning stimulus to the animals to produce a larger volume of saliva, resulting in reduced concentrations of the derivatives.

The significant between-animal variance for both plasma and salivary allantoin may have been partly due to differences in the microbial biomass originating from the rumen and, hence, of purine-derivative production by the host animal. The diurnal variation could be interpreted as the result of a varying flow of microbial biomass throughout the day, due to the peaks of microbial biomass production related to the feeding times of the meal-feeding management system used.

Allantoin in the rumen

The results of the in vitro incubation of allantoin in rumen fluid confirmed that allantoin is degraded by the rumen microbes. Belasco (1954) showed both allantoin and uric acid to be used as a source of N by rumen bacteria in vitro. The rate of degradation at about 15 μmol/h per l rumen fluid reported here is sufficient, in theory, to degrade all the allantoin recycled in the saliva (assuming a rumen volume of 5–10 litres, 15 μmol/h per l rumen fluid is equivalent to 1·8–3·6 mmol/d). This was confirmed by Expt 3 when none of the 8 mmol allantoin/d infused into the rumen of normally fed sheep was excreted in the urine.

Surprisingly, with the animals nourished by intragastric infusion and, therefore, without a microbial fermentation in the rumen, it was also found that allantoin infused into the rumen was not recovered in the urine of either sheep (Expt 4) or cattle (Expt 5). Expt 6 gave some information as to where these losses occurred. When allantoin and PEG were dosed into the rumen of sheep also nourished by intragastric infusion, the allantoin disappeared at a rate (k_1) which was double that (k_2) of PEG. The latter represented the rumen-fluid outflow rate. There was evidently a net loss of allantoin from the rumen, other than by outflow. The proportion (Z) of the dosed allantoin that had been lost (cumulatively) as such by time t, can be expressed as

$$Z = \frac{k_1 - k_2}{k_1}(1 - e^{-k_1 t}),$$

which indicates that 0·49 of the dosed allantoin could be lost other than by outflow, at a fractional rate of ($k_1 - k_2$) = 0·08 /h. This loss could be due to either degradation by bacteria in the rumen epithelium, or to absorption from the rumen. The former was the more probable since allantoin infused into the rumen was not recovered in the urine and allantoinase is not present in mammalian tissues. There is a microbial population intimately associated with the rumen epithelium of animals nourished by intragastric infusion (Wallace et al. 1979). Presumably these bacteria live on epithelial debris and, therefore, would be well-adapted to degrade cellular material including nucleic acids and their derivatives. They would act as an effective barrier against the absorption of any purines not degraded by micro-organisms in the digesta of normally fed animals.

Allantoin in the abomasum

Expt 4 showed that even when relatively large amounts of allantoin were infused directly into the abomasum, less than 10 % of that infused was recovered in urine. The failure to recover, in urine, allantoin infused into the abomasum could have been due to the combined effect of a poor absorption of allantoin from the lower digestive tract and an extensive degradation by bacteria associated with the gut wall. Although purine free-bases and nucleosides are readily absorbed from the intestines, some purine metabolites may not be (McAllan, 1980). Berlin & Hawkins (1968) studied the transport of purines in hamster intestine in vitro and showed that xanthine and uric acid were secreted into, but not absorbed from, the intestinal lumen. In human subjects given injections of labelled uric acid into the blood, 23 % of the radioactivity was present in urea in the urine and no uric acid was found in faeces. However, when bacteriostasis was achieved in the gut, a similar proportion of the injected radioactivity was found in uric acid in faeces (Sorensen, 1960). This suggests that uric acid secreted into the gut is not re-absorbed, and under physiological conditions is decomposed by bacteria associated with the gut. Presumably allantoin is similar to uric acid in its fate in the gut of ruminants.

These experiments demonstrated the presence of allantoin and uric acid in the saliva of normally fed sheep in amounts which could result in the recycling of these purine derivatives into the reticulo-rumen in quantitatively significant amounts. Non-significant amounts of this recycled allantoin (and presumably also uric acid), would be re-absorbed for subsequent excretion in the urine and, therefore, in normal physiological conditions would be completely lost. The use of purine-derivative excretion as an estimator of the microbial biomass made available to the ruminant host animal will, therefore, have to be corrected for such losses.

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