Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep

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The endogenous urinary excretion of the purine derivatives allantoin, uric acid and xanthine plus hypoxanthine were measured in twenty-nine lambs, ten cattle (six steers, one cow and three preruminant calves) and four pigs. The sheep and mature cattle were nourished by intragastric infusion and the calves were given a milk-substitute. The pigs were fed on a purine-free diet. The excretion of total purine derivatives was substantially greater by the cattle, being 514 (SE 20·6) μmol/kg live weight $(W)^{0.75}$ per d compared with 168 (SE 5·0) μmol/kg $W^{0.75}$ per d by the sheep and 166 (SE 2·6) μmol/kg $W^{0.75}$ per d by the pigs. Plasma from normally fed sheep, cows and pigs was incubated with either xanthine or uric acid. Sheep and pig plasma had no xanthine oxidase (*EC* 1.2.3.2) activity whereas plasma from cattle did. Uricase (*EC* 1.7.3.3) was not present in plasma of cattle and pigs and appeared to be present in trace amounts only in sheep plasma. It is suggested that the species differences in endogenous purine derivative excretion were probably due to the different profiles of xanthine oxidase activity in tissues and particularly in the blood. This is because a high xanthine oxidase activity would reduce the chance to recycle purines, by increasing the probability of degradation to compounds which could not be salvaged.

Purine derivatives: Uricase: Xanthine oxidase: Cattle: Sheep

Many studies have suggested that the urinary excretion of purine derivatives (including allantoin, uric acid, xanthine and hypoxanthine) by ruminants could be used as an index of microbial biomass and hence protein supply to the animal (Topps & Elliott, 1965; Rys *et al.* 1975). This is based on the fact that absorbed microbial purines are extensively degraded and excreted as their degradation products. However, some of the urinary purine derivatives originate directly from the animal's tissues, and this endogenous contribution to the total excretion in urine has to be quantified.

The measurement of the endogenous purine derivative excretion of ruminants under normal physiological conditions has been hindered by the technical difficulty of eliminating the contribution of the rumen micro-organisms. Walker & Faichney (1964), Rys *et al.* (1975), Antoniewicz & Pisulewski (1982) and Laurent & Vignon (1983) used animals on prolonged starvation to measure the endogenous excretion. However, it is possible that the metabolic activities of the animals, or the rate of degradation of tissue nucleic acids and, thus, the excretion of purine end-products, could be altered by nutritional restriction. This problem can be resolved by the use of the intragastric infusion technique (Ørskov *et al.* 1979), in which the animals are totally nourished by volatile fatty acids (VFA) and casein infused into the rumen and abomasum respectively. The microbial fermentation in the rumen is eliminated, and a normal nutritional status of the animal can be maintained. Sibanda *et al.* (1982), Giesecke *et al.* (1984) and Fujihara *et al.* (1987*b*) used this technique to measure the endogenous excretion by sheep and steers, but with only a limited number of animals.

In the present study, the endogenous excretions by sheep and cattle were measured in a greater number of animals. For further comparison between species, the measurement was also made on pigs. In consideration of the possible effect of xanthine oxidase (*EC* 1.2.3.2)

in the blood on the magnitude of the endogenous excretion, plasma activities of xanthine oxidase in these three species were measured. A provisional report of some of the findings on the endogenous purine derivative excretion by lambs has been made (Fujihara *et al.* 1987*a*).

MATERIALS AND METHODS
Animals and management

Urinary excretion. With the exception of the calves and pigs, all the animals used for the measurement of urinary purine derivatives were nourished by intragastric infusion as described by Ørskov *et al.* (1979) and Hovell *et al.* (1987). The animals maintained by this method were fitted with a permanent rumen cannula and abomasal catheter. Energy was supplied as VFA continuously infused into the rumen, and protein as casein infused into the abomasum.

Sheep. Observations were made in twenty-nine sheep (Blackface × Suffolk and Finn × Dorset, live weight (W) 28–69 kg). Fifteen of the animals were on nitrogen-free nutrition and the rest received a casein infusion at 1–1·2 times the maintenance N requirement (taken as 435 mg casein-N/kg $W^{0.75}$ per d). The animals were given a constant energy input at 1 to 1·5 times maintenance requirement (taken as 450 kJ metabolizable energy (ME)/kg $W^{0.75}$ per d).

Cattle. Six steers (Friesian × Hereford, 220–352 kg) received nutrient inputs varying from zero (fasting) to maintenance requirement (450 kJ ME and 435 mg casein-N/kg $W^{0.75}$ per d). One cow (Friesian, 468 kg) was given an N input of 1100–1300 mg casein-N/kg $W^{0.75}$ per d. Her daily milk N output was 400–700 mg N/kg $W^{0.75}$ per d. Three calves (Friesian × Hereford, 23- to 24-d-old, 40–49 kg) were fed on a commercial milk-substitute (Volac Ltd, Royston, Herts) at 345 g dry matter (DM)/d. This level of feeding was approximately the maintenance requirement for energy and protein.

Pigs. Four growing pigs (Large White, 48–50 kg) were given about 1·7 kg/d of a semi-synthetic diet containing (relative proportions) 0·30 maize starch, 0·30 glucose, 0·20 sucrose, 0·04 vegetable oil, 0·06 cellulose, 0·04 casein plus synthetic amino acids and 0·06 mixed vitamins (Wang, 1988).

Plasma enzyme activity. Plasma from normally fed animals was used for the examination of xanthine oxidase and uricase (*EC* 1.7.3.3) activities. Three lactating cows, two sheep and two pigs were randomly chosen from those available at the Institute.

Measurements and chemical analyses

Urinary purine derivatives. Urine (24 h; 168 h for calves) was collected into sulphuric acid so that the final pH was between 2 and 3. The urine was sampled and stored at 4°. The samples were normally analysed within 1 week. Allantoin was measured by the AutoAnalyzer method of Pentz (1969) with some modifications. Uric acid was determined using the phosphotungstic acid method adapted for an AutoAnalyzer (Technicon Instruments Co., 1979). The content of xanthine plus hypoxanthine was measured as uric acid after treatment of the urine with xanthine oxidase. A detailed description of the methods has been given (Chen, 1989).

Plasma xanthine oxidase and uricase. Jugular blood samples were collected into heparinized tubes. The plasma samples obtained were used within 1 h. These samples were incubated with xanthine and the production of uric acid with time was monitored. Since uricase can remove the uric acid produced, it was also necessary to examine the uricase activity in the plasma. The plasma samples were, therefore, also incubated with uric acid.

Incubation with xanthine. 0·20 M-potassium dihydrogen phosphate buffer (pH 7·4; 3 ml) and 4 ml plasma were mixed in a test-tube. One ml xanthine solution (prepared by

dissolving 192·1 μmol xanthine and 214·6 μmol L-histidine in 50 ml 0·01 M-sodium hydroxide) was then added. After mixing, a 1 ml sample was taken immediately. The remaining solution was incubated at 37° and sampled at 1 h intervals for 7 h. The presence of L-histidine in the incubation system removed the possible inhibition by excess substrate of the activity of xanthine oxidase (Muraoka, 1962a, b).

Incubation with uric acid. Three ml 0·67 M-glycine buffer (pH 9·3) and 4 ml plasma were mixed in a test-tube. One ml uric acid solution (357 μmol/l) was added. The contents were well mixed and a 1 ml sample of the mixture was taken immediately. The reaction mixture was then incubated at 37° and sampled at 1 h intervals for 7 h.

All samples of the incubation mixtures were immediately pipetted into 10 ml centrifuge tubes containing 0·5 ml trichloroacetic acid solution (100 g/l) and centrifuged at 2000 g for 10 min. The supernatant fractions were analysed for uric acid using the same method as for the urine samples. The samples containing glycine buffer (for examination of uricase activity) were analysed against the uric acid standards containing the same quantities of glycine.

RESULTS
Endogenous excretion of purine derivatives

Sheep. The results from animals on protein-free and maintenance protein infusion are grouped separately. The mean daily excretions of allantoin, uric acid and xanthine plus hypoxanthine are shown in Table 1. The mean excretion of total purine derivatives in the twenty-nine lambs was 168 (SE 5·0) μmol/kg $W^{0.75}$ per d, the relative contributions from allantoin, uric acid and xanthine plus hypoxanthine being 0·55, 0·31 and 0·14 respectively. There did not appear to be differences between the two groups of animals. The variation (coefficient of variation (CV)) between animals was 17 and 16% for the protein-free and protein infusion groups respectively. The within-animal variation (between daily measurements of the same animal) averaged 17 (SE 2·1) and 6 (SE 1·1) % for the two groups of lambs respectively.

Cattle. In cattle, xanthine and hypoxanthine were not detectable in urine and thus total purine derivatives included only allantoin and uric acid. The mean excretions of the individual animals (steers, the cow and calves) are listed in Table 2. There was no difference between the preruminant calves and the mature animals when the measurements were expressed on the basis of metabolic weight ($W^{0.75}$). The mean excretion of total purine derivatives was 514 (SE 20·6) μmol/kg $W^{0.75}$ per d, with relative proportions of 0·82 allantoin and 0·18 uric acid. The between-animal variation (CV) of the total excretion was 12%.

Pigs. The endogenous purine derivative excretions of the four pigs are presented in Table 3. Total purine derivative excretion averaged 166 (SE 2·6) μmol/kg $W^{0.75}$ per d. The proportions of allantoin, uric acid and xanthine plus hypoxanthine were 0·81, 0·13 and 0·06 respectively. In the four animals observed the between-animal variation was small (3%).

Plasma xanthine oxidase and uricase

Xanthine oxidase. Fig. 1 shows the formation of uric acid from xanthine at different incubation times. There was an increase in uric acid concentration when xanthine was incubated with the cow's plasma, whereas essentially no uric acid was produced with the plasma of either the sheep or pigs.

The mean production of uric acid (Y; μmol) in the 8 ml incubation mixtures containing plasma samples from the three cows were fitted into a mono-exponential function of incubation time (t; h) using the maximum likelihood programme (MLP) of Ross (1987):

$$Y = 1·12 \text{ (SE 0·035)} - 1·04 \text{ (SE 0·032)} \, e^{-0·262 \text{(SE 0·018)} \, t} \text{ (residual SD (RSD) 0·013)},$$

Table 1. *The endogenous purine derivative excretion ($\mu mol/kg$ live weight $(W)^{0.75}$ per d) by lambs nourished by intragastric infusion**

(Mean values with their standard errors)

Protein input†	No. of animals	W (kg)	X+H Mean	SE	Uric acid Mean	SE
Nitrogen free	15	31–69	23	2·1	47	3·0
1–1·2 times maintenance‡	14	29–57	25	1·9	55	3·5
All sheep	29	31–69	24	1·4	51	2·4

Protein input†	No. of animals	W (kg)	Allantoin Mean	SE	Total purine derivatives Mean	SE
Nitrogen free	15	31–69	96	4·9	167	7·3
1–1·2 times maintenance‡	14	29–57	89	4·8	169	7·1
All sheep	29	31 69	93	3·4	168	5·0

X + H, xanthine plus hypoxanthine.

* The values given in this table include those provisionally reported by Fujihara *et al.* (1987 *a*).

† For details of procedures, see p. 122.

‡ Maintenance requirement for N was taken as 435 mg casein-N/kg $W^{0.75}$ per d (Agricultural Research Council, 1984).

Table 2. *The endogenous purine derivative* excretion ($\mu mol/kg$ live weight $(W)^{0.75}$ per d) by steers and a cow nourished by intragastric infusion, and calves given a milk substitute†*

(Mean values with their standard errors)

Animal	W (kg)	Period of measurement (d)	Uric acid Mean	SE	Allantoin Mean	SE	Total purine derivatives Mean	SE
Steer								
A	248	10	89	11	468	41	557	51
B	220	19	71	5	500	10	571	11
C	329	5	61	2	342	6	401	6
D	268	5	71	4	500	42	571	46
E	352	11	95	8	373	22	469	23
F	285	12	107	4	411	17	518	18
Cow	468	46	89	2	424	6	513	7
Calf								
A	44	‡	95	—	348	—	443	—
B	40	‡	119	—	494	—	613	—
C	49	‡	155	—	329	—	484	—
Mean of all cattle			95	9	419	22	514	21

* Xanthine and hypoxanthine excretions were negligible in cattle urine.

† For details of procedures, see p. 122.

‡ Single measurement from 7 d bulked urine.

Table 3. *The endogenous purine derivative excretion ($\mu mol/kg$ live weight $(W)^{0.75}$ per d) by growing pigs fed on a semi-synthetic purine-free diet**

(Mean values with their standard errors)

Animal	W (kg)	Period of measurement (d)	X + H		Uric acid		Allantoin		Total purine derivatives	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	47·5	10	8·9	0·4	20·2	0·8	138	7·4	167	8·0
B	49·0	10	8·3	0·6	20·2	0·9	135	5·8	163	9·5
C	50·0	9	8·3	0·6	24·5	2·2	128	6·4	161	7·0
D	47·5	9	12·5	0·8	24·1	0·7	136	8·0	173	9·3
Mean			9·5	1·0	22·3	1·2	134	2·2	166	2·6

X + H, xanthine plus hypoxanthine.
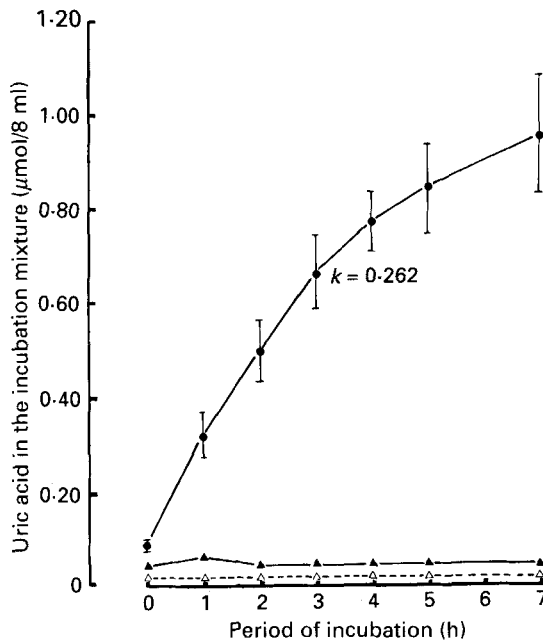* For details of procedures, see p. 122.

Fig. 1. Uric acid produced from the incubation *in vitro* of xanthine with plasma from three cows (●), two sheep (▲) or two pigs (△). For details of procedures, see p. 123. Values are means, and standard deviations represented by vertical bars. k is the fractional rate (proportion/h) for production of uric acid.

which indicates that a maximum of 1·04 μmol uric acid could be produced from the xanthine in the 8 ml mixture at a fractional rate of 0·262 /h. In the first hour of incubation 0·272 μmol uric acid was produced. This gives an activity of 1·13 μmol/min per l plasma ($0·272 \div 60 \div 4 \times 1000 = 1·13$).

Uricase. Fig. 2 shows the changes in the content of uric acid in the incubation mixtures where uric acid was added initially. There was no metabolism of uric acid with cow or pig plasma, indicating the absence of uricase. However, with sheep plasma, there was a slight decrease in uric acid content of about 0·007 μmol/h. This is equivalent to an activity of 29 nmol/min per l plasma.
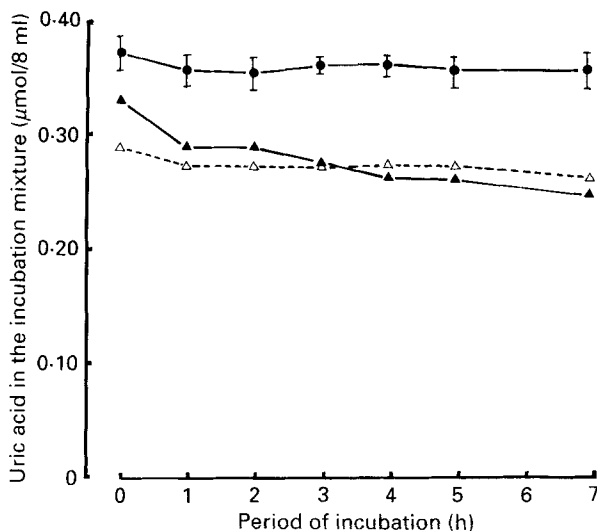
Fig. 2. Loss of uric acid during incubation with plasma from three cows (●), two sheep (▲) or two pigs (△). For details of procedures, see p. 123. Values are means, and standard deviations represented by vertical bars.

Plasma uric acid. The uric acid contents in the original plasma of the three cows averaged 25·9 (SD 0·5) μmol/l. The mean values for the two sheep and for the two pigs were 9·0 and 5·6 μmol/l respectively.

DISCUSSION
Endogenous excretion of purine derivatives

Although there was a considerable variation between individual animals (12% for cattle and 17% for sheep), the difference between species of animals was distinctive. Cattle excreted three times as much purine derivatives as sheep and pigs, but there did not seem to be differences between sheep and pigs. When the values were combined within species, the endogenous excretion of purine derivatives could be described as:

$$\text{Sheep } (n\ 29)\text{: } Y = 142\ W^{0.80(\text{SE } 0.177)}\ (\text{RSD } 0.0782,\ r\ 0.75),$$

$$\text{Cattle } (n\ 10)\text{: } Y = 543\ W^{0.74(\text{SE } 0.049)}\ (\text{RSD } 0.0602,\ r\ 0.98),$$

where Y is the amount of derivative excreted daily (μmol).

The exponents of 0·80 and 0·74 do not differ significantly from each other, or from that of 0·75 commonly used to describe 'metabolic' weight, therefore 0·75 has been used in the present report. The results also suggest that there was no difference between cattle in different physiological states (preruminant calves, steers and the lactating cow), although this point should be confirmed as Sibanda *et al.* (1982) noted a higher endogenous allantoin excretion in two cows than in two steers.

The endogenous excretion by sheep observed in the present study agreed well with the 164 μmol total purine derivatives/kg $W^{0.75}$ per d provisionally reported by Fujihara *et al.* (1987*a*), and therefore the values were combined in the present report. The within-animal variation referred to previously (p. 123) for the total excretion in sheep was based on observations in which the nutrient supply to each animal was relatively constant. Table 1 showed that when sheep were grouped on the basis of those given protein or those on N-

free nutrition, no difference was detected. However, subsequent work has demonstrated small within-animal changes in endogenous excretion related to changes in protein supply, and possibly to nutrient status (Chen, 1989).

The steers which provided the values given in Table 2 were from other experiments. Their nutrition ranged from fasting to maintenance supply for energy and protein. There was evidence (Chen, 1989) that excretion was about one-third greater during the first 3 d of fasting, but the data were not sufficiently systematic for a full analysis. This does not detract from the fact of there being differences between animal species, but does mean that with the cattle, some of the within-animal variation was due to changes in nutrient status.

Plasma xanthine oxidase and uricase

With the reservation that the number of animals was small, the results of the present study showed xanthine oxidase to be absent from sheep and pig plasma, but present at a considerable concentration in cattle plasma. These observations are qualitatively in agreement with the findings of Al-Khalidi & Chaglassian (1965). However, the xanthine oxidase activity found in plasma of cows as reported here (1·13 μmol/min per l) was higher than that reported by these authors (0·142 μmol/min per l). This may have been due to the method adopted in the present study in which the use of L-histidine removed the feed-back inhibition of xanthine oxidase by excess xanthine. An additional possibility could have been differences between breeds of animals. We have been unable to find any report in the literature of measurements of uricase activity in blood of farm animals. Given the limited number of animals, the results of the work reported here also indicate uricase to be absent from blood of both cows and pigs, but present at a very low level in the blood of sheep.

The markedly different xanthine oxidase activities and absence of uricase or low uricase activities in the blood of these animal species could explain why uric acid concentrations in cow's plasma were considerably higher than those in pig and sheep plasma.

Possible link between the xanthine oxidase activity and the urinary excretion of purine derivatives

Cattle had a high activity of blood xanthine oxidase and a much greater urinary excretion of purine derivatives (514 μmol/kg $W^{0·75}$ per d) than sheep and pigs (168 and 166 μmol/kg $W^{0·75}$ per d respectively), which both lack xanthine oxidase in the blood and had similar magnitudes of endogenous excretion of purine derivatives.

Humans excrete uric acid as the end-product of purine degradation, and also lack xanthine oxidase in the blood (Al-Khalidi & Chaglassian, 1965). Measurements of endogenous uric acid excretion reported in the literature (xanthine and hypoxanthine were not recorded) averaged 131 (SE 4·5, n 11) μmol/kg $W^{0·75}$ per d (Folin *et al.* 1924; Buzard *et al.* 1952; Wyngaarden & Stetten, 1953), which is close to the values for sheep and pigs reported here. Simmonds *et al.* (1973) also noted the similarity in the endogenous purine derivative excretion by pigs and humans.

The endogenous purine derivatives excreted in urine originate from the turnover of tissue nucleic acids. Of the purine nucleosides and free bases released from the degradation of nucleic acids (nucleotides), most are salvaged for the re-synthesis of new nucleotides. There is a substrate cycle involving the nucleosides and free bases (Metzler, 1977) which enables a more efficient salvage of the intermediary products of nucleic acid degradation. Xanthine oxidase diverts hypoxanthine away from this cycle to form xanthine and uric acid which can be subsequently oxidized in the presence of uricase to form allantoin. Uric acid and allantoin are not salvageable, and although xanthine may be salvaged by hypoxanthine-guanine phosphoribosyltransferase to form xanthosine monophosphate (Gots, 1971), this

reaction is slow due to the low affinity for xanthine by the enzyme (Hitchings, 1978). Therefore, xanthine oxidase is probably the key enzyme which defines the loss of purine derivatives in the urine.

If it is assumed that the rates of nucleic acid turnover are similar in sheep and cattle, then the threefold difference in the excretion of endogenous purine derivatives between these species should be due to differences in salvage efficiency. The marked differences in plasma xanthine oxidase activities of cattle and sheep demonstrated here, and in plasma and other tissues reported by Al-Khalidi & Chaglassian (1965) support this idea (xanthine oxidase activities in most tissues of cattle were 100 times higher than those of sheep).

Although the plasma activities may simply reflect the activities of other tissues, most of the differences in salvage between cattle and sheep, pigs and man could theoretically be attributed to blood xanthine oxidase activity. A 468 kg cow with 0·031 litres plasma/kg (Frandson, 1981) and a plasma xanthine oxidase activity of 1·1 μmol/min per l, would have the potential capacity for the conversion of hypoxanthine or xanthine to other purine derivatives of 34 mmol/d. This is equivalent to 0·64 of the daily excretion measured (Table 2). The remainder (185 μmol/kg $W^{0.75}$ per d), which in this case would be produced by the other tissues, is similar to the measured excretions by sheep and pigs (Tables 1 and 3).

The species difference in endogenous purine derivative excretion between cattle, sheep and pigs is also expressed in the forms of the derivatives excreted. The xanthine plus hypoxanthine fraction accounted for a considerable proportion of the total excretion in urine of sheep and pigs. This was negligible in cattle urine, which is again in accord with the differences in the plasma xanthine oxidase activities of these animals. The proportion of allantoin in urine of both cattle and pigs was higher than that of sheep. Since uricase in the blood of all three species of animals was absent, or present in only trace amounts, conversion of uric acid to allantoin took place in other tissues. Presumably tissue uricase activities are higher in cattle and pigs than in sheep.

In conclusion, there are significant differences between cattle and sheep in the magnitude and the form of the endogenous purine derivatives excreted in urine. This relates to the difference in profiles of xanthine oxidase activities between these species of animals, with blood xanthine oxidase being a quantitatively more important factor than the corresponding differences in other tissues.

REFERENCES

Agricultural Research Council (1984). *The Nutrient Requirements of Ruminant Livestock*, Supplement no. 1. Slough: Commonwealth Agricultural Bureaux.

Al-Khalidi, U. A. S. & Chaglassian, T. H. (1965). The species distribution of xanthine oxidase. *Biochemical Journal* 97, 318–320.

Antoniewicz, A. M. & Pisulewski, P. M. (1982). Measurement of endogenous allantoin excretion in sheep urine. *Journal of Agricultural Science, Cambridge* 98, 221–223.

Buzard, J., Bishop, C. & Talbott, J. H. (1952). Recovery in humans of intravenously injected isotopic uric acid. *Journal of Biological Chemistry* 196, 179–184.

Chen, X. B. (1989). Excretion of purine derivatives by sheep and cattle and its use for the estimation of absorbed microbial protein. PhD Thesis, University of Aberdeen.

Folin, O., Berglund, H. & Derick, C. (1924). The uric acid problem. An experimental study on animals and man, including gouty subjects. *Journal of Biological Chemistry* 60, 361–479.

Frandson, R. D. (1981). *Anatomy and Physiology of Farm Animals*, 3rd ed., p. 249. Philadelphia: Lea & Febiger.

Fujihara, T., Chen, X. B., Ørskov, E. R. & Hovell, F. D. DeB. (1987 a). The possible use of purine derivatives in urine to estimate rumen microbial protein production. *Proceeding of the 5th International Symposium on*

Protein Metabolism and Nutrition, European Association for Animal Production Publication no. 35, pp. 2.S. 17–18. Rostock: Wilhelm-Pieck-University.

Fujihara, T., Ørskov, E. R. & Reeds, P. J. (1987*b*). The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. *Journal of Agricultural Science, Cambridge* **109**, 7–12.

Giesecke, D., Stangassinger, M. & Tiemeyer, W. (1984). Nucleic acid digestion and urinary purine metabolites in sheep nourished by intragastric infusions. *Canadian Journal of Animal Science* **64**, Suppl., 144–145.

Gots, J. S. (1971). Regulation of purine and pyrimidine metabolism. In *Metabolic Regulation*, vol. 5, pp. 225–255 [H. J. Vogel, editor]. New York and London: Academic Press.

Hitchings, G. H. (1978). Uric acid: chemistry and synthesis. In *Uric Acid*, pp. 1–20 [W. N. Kelly and J. M. Weiner, editors]. Berlin, Heidelberg and New York: Springer-Verlag.

Hovell, F. D. DeB., Ørskov, E. R., Kyle, D. J. & MacLeod, N. A. (1987). Undernutrition in sheep. Nitrogen repletion by N-depleted sheep. *British Journal of Nutrition* **57**, 77–88.

Laurent, F. & Vignon, B. (1983). Factors of variations in urinary excretion of allantoin in sheep and goats. *Archiv für Tierernährung* **33**, 671–681.

Metzler, D. E. (1977). *Biochemistry: The Chemical Reactions of Living Cells*, p. 881. London: Academic Press.

Muraoka, S. (1962*a*). Studies on xanthine oxidase. I: Activation of milk xanthine oxidase by histamine. *Biochimica et Biophysica Acta* **73**, 17–26.

Muraoka, S. (1962*b*). Studies on xanthine oxidase. II. Mechanism of substrate inhibition and its reversal by histamine. *Biochimica et Biophysica Acta* **73**, 27–38.

Ørskov, E. R., Grubb, D. A., Wenham, G. & Corrigall, W. (1979). The sustenance of growing and fattening ruminants by intragastric infusion of volatile fatty acids and protein. *British Journal of Nutrition* **41**, 553–558.

Pentz, E. I. (1969). Adaptation of the Rimini-Schryver reaction for the measurement of allantoin in urine to the autoanalyzer: allantoin and taurine excretion following neutral irradiation. *Analytical Biochemistry* **27**, 333–342.

Ross, G. J. S. (1987). *MLP User Manual*. Rothamsted: Rothamsted Experimental Station.

Rys, R., Antoniewicz, A., & Maciejewicz, J. (1975). Allantoin in urine as an index of microbial protein in the rumen. In *Tracer Studies on Non-protein Nitrogen for Ruminants*, pp. 95–98. Vienna: IAEA.

Sibanda, S., Topps, J. H., Storm, E. & Ørskov E. R. (1982). The excretion of allantoin by ruminants in relation to protein entering the abomasum. *Proceedings of the Nutrition Society* **41**, 75A.

Simmonds, H. A., Hatfield, P. J., Cameron, S., Jones, A. S. & Cadenhead, A. (1973). Metabolic studies of purine metabolism in the pig during the oral administration of guanine and allopurinol. *Biochemical Pharmacology* **22**, 2525–2551.

Technicon Instruments Co. (1979). *Uric acid. Technicon Method* no. SD4-0013FM9. Tarrytown, NY: Technicon Instruments Co.

Topps, J. H. & Elliott, R. C. (1965). Relationships between concentrations of ruminal nucleic acid and excretion of purine derivatives by sheep. *Nature* **205**, 498–499.

Walker, D. M. & Faichney, G. J. (1964). Nitrogen balance studies with the milk fed lamb. 2. The partition of urinary nitrogen and sulphur. *British Journal of Nutrition* **18**, 201–215.

Wang, T.-C. (1988). The ideal protein for growing pigs. PhD Thesis, University of Aberdeen.

Wyngaarden, J. B. & Stetten, D. Jr. (1953). Uricolysis in normal man. *Journal of Biological Chemistry* **203**, 9–21.