The effects on glucose metabolism of feeding a high-urea diet to sheep

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1. Sheep were given either a basal diet of 107 g crude protein (nitrogen × 6·25)/kg or the same diet to which urea was added to increase the crude protein content to 221 g/kg. Isotope-dilution techniques with [U-^{14}C]glucose and [2-^{3}H]glucose were used to measure various criteria of glucose metabolism. The plasma concentrations of urea and potassium were determined. The sheep were then given the alternative diet and the experiment was repeated.

2. Plasma K concentrations were decreased on feeding urea ($P < 0{\cdot}05$).

3. Plasma glucose concentrations were reduced on the urea treatment ($P < 0{\cdot}05$), while glucose space and metabolic clearance rate were not significantly reduced ($P > 0{\cdot}05$).

4. Some implications for the feeding of non-protein-N to ruminants are discussed.

The problems of feeding non-protein-nitrogen to ruminants are well known and have been excellently reviewed (see, for example, Chalupa, 1968; Oltjen, 1969). Morris & Payne (1970) confirmed that urea poisoning was synonymous with hyperammonaemia, and it is well known that uncontrolled usage can lead eventually to coma and death. Even at sublethal blood ammonia concentrations there are indications of alterations in intermediary metabolism (Lewis & Buttery, 1972; Soar, Buttery & Lewis, 1973). Despite extensive work, no complete explanation has yet been offered for the toxicity of ammonia, although various suggestions have been made. Among these are that depletion of 2-oxoglutarate by the action of glutamate dehydrogenase (*EC* 1.4.1.3) leads to derangements in the tricarboxylic acid cycle. The conversion of large amounts of 2-oxoglutarate to glutamate is also linked with a disturbance of the redox state of the pyridine nucleotides. Katunuma, Okada & Nishi (1967) have shown that high concentrations of ammonia directly affect isocitrate dehydrogenase (*EC* 1.1.1.42)), again leading to a decrease in 2-oxoglutarate concentration. Soar *et al.* (1973) investigated the effect of ammonia on glucose metabolism in sheep by infusing ammonium acetate for a period of several hours and monitoring the plasma glucose concentrations. They found an increase commensurate with increasing blood ammonia concentrations. Alterations in several blood metabolites were noted before there was any outward manifestation of toxicity. It should be noted, however, that as this was an 'acute' experiment, continued to the point of collapse of the sheep, stress factors could be masking the basic biochemical adaptations.

Tracer techniques offer the opportunity of studying glucose kinetics without unduly disturbing the animal's endocrine system. This approach has been used for studying the effect of diet (Ford, 1965; Judson, Anderson, Luick & Leng, 1968; Judson & Leng, 1968; Ulyatt, Whitelaw & Watson, 1970; Evans & Buchanan-Smith, 1975) and also to show how starvation and pregnancy affect glucose synthesis (Annison, Brown, Leng, Lindsay & West, 1967; Steel & Leng, 1968).

In the present study sheep were given a conventional basal diet and a similar diet supplemented with urea. After a period of adaptation the glucose kinetics were studied by isotope-dilution methods. Rations were offered twice daily to mimic normal feeding habits and to present an appreciable ammonia challenge.

Table 1. *Composition (g/kg) of the experimental diets*

Ingredient	Diet: Basal	Diet: Urea-supplemented
Grass meal	200	195
Ground oats	775	742
Urea	—	38
Mineral and vitamin supplement*	25	25
Crude protein (nitrogen × 6·25) content (g/kg)	107	221
Gross energy (MJ/kg)	18·3	18·2

* Wrightman Sheep; Frank Wright (Feed Supplements) Ltd, Ashbourne, Derbyshire.

EXPERIMENTAL

Animals and diets

Eight sheep were allocated to four 2 × 2 Latin squares in a random fashion. Half the sheep were given a basal diet containing grass meal and ground oats and having a crude protein (N × 6·25) content of 107 g/kg. The remaining sheep were given a similar diet but supplemented with 40 g urea/kg to bring the crude protein content to 221 g/kg (see Table 1).

After 2 weeks on these diets isotope-dilution experiments were performed to evaluate various criteria of glucose metabolism. The sheep were then given the alternative diet for a further 2 weeks and the isotope-dilution experiments repeated.

The sheep were kept in individual metabolism crates and allowed water *ad lib*. They were fed 400 g twice daily, and were trained to consume the rations within 15 min. On the day before an experiment the sheep were weighed and each was prepared with a catheter in the jugular vein. On the day of the experiment the sheep were given their first 400 g ration at 07.30 hours. At 09.00 hours a single injection of mixed [2-^{3}H]glucose (150 μCi) and [U-^{14}C]glucose (100 μCi) was given. Fourteen blood samples were taken in a 12 h period, the second feed being given at 16.30 hours. The heparinized blood samples were centrifuged and the plasma stored at $-15°$ until it was analysed.

Analytical methods

The plasma was deproteinized according to the method of Somogyi (1945). Plasma glucose concentration was measured using a glucose oxidase test kit (Boehringer GmbH, Mannheim, W. Germany). The radioactivity content of the glucose, after isolation as the penta-acetate derivative (Jones, 1965), was determined using a liquid-scintillation counter (Model SL 33; Intertechnique, Plaisir, France). Values for ^{3}H:^{14}C were calculated using an external-standard channels-ratio method.

Also determined were the concentrations of urea and K in the plasma sample taken at 14.30 hours. Urea was determined using Varley's (1967) method. K was assayed by flame photometry (Corning-Eel; Evans Electroselenium Ltd, Halstead, Essex).

Calculations

The four Latin squares were considered to be 'tied by periods' in the analyses of variance of the results.

The dilution of the dose of labelled glucose *v.* period after injection was plotted on semilogarithmic co-ordinates (see Fig. 1). The radioactivity content was expressed as % dose/l and not in terms of specific activity because of the fluctuations in plasma glucose

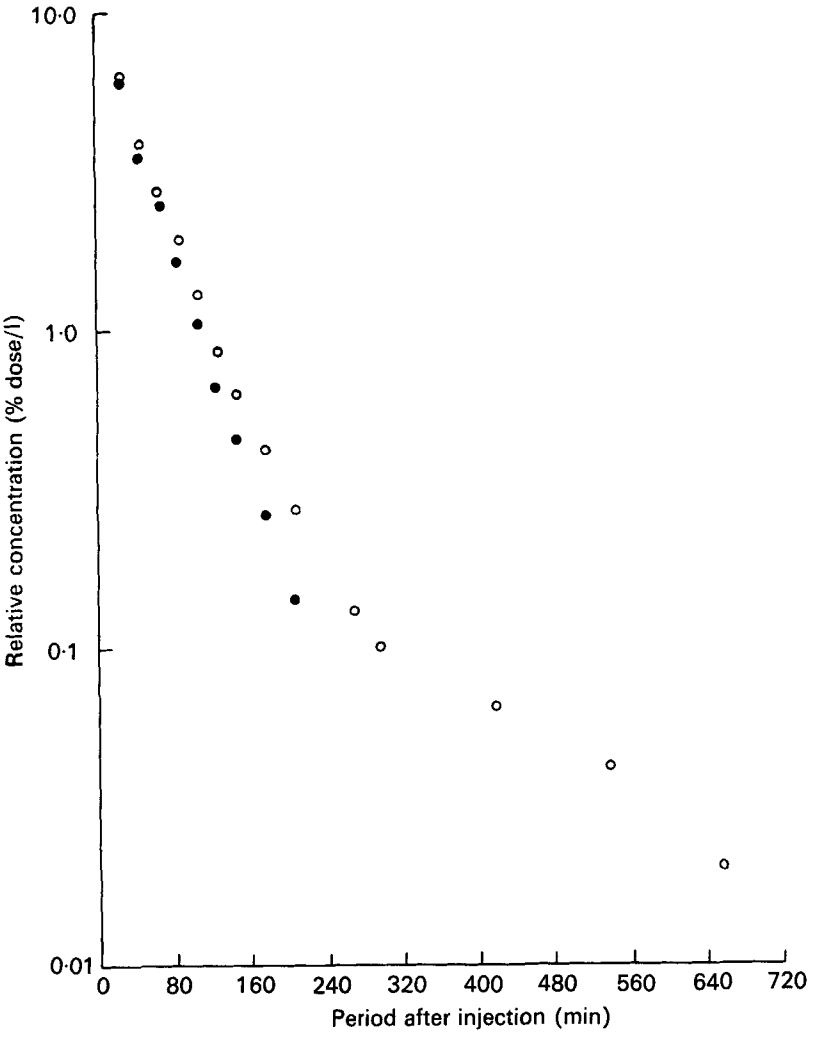

Fig. 1. Relationship between the relative concentration (% dose/l) of labelled glucose in plasma and the period after a single injection of mixed [2-^{3}H]glucose (150 μCi) (●) and [U-^{14}C]glucose (100 μCi) (○). The coefficient of determination was 0·998. Results presented are those for sheep no. 84 given a urea-supplemented diet (for details of composition, see Table 1).

concentration. This approach is justified because variation in plasma glucose concentration appeared to be random, that is, there was no systematic change with the period after injection.

The dilution curves were fitted using a least-squares method using a computer (Model 1906 A; I.C.L., Berks; based in the Cripps Computing Centre, Nottingham University).

A single exponential was fitted to the tritium values for the first 3·5 h (Judson & Leng, 1972). Two exponential terms were found to be necessary to fit the ^{14}C values for the 12 h period.

The curves fitted were of the form:

$$C_T = P_1 e^{-q_1 t},$$

$$Cc = (P_1 - P_2) e^{-q_1 t} + P_2 e^{-q_2 t},$$

Table 2. *Effect of urea supplementation on the criteria of glucose metabolism in sheep given the basal diet or a similar diet with added urea*†

(Mean values for eight sheep/diet except plasma glucose where the values are for fourteen observations for each animal on each diet)

Sheep no.	Body-wt (kg)	Plasma glucose concentration (mmol/l)	Glucose space (l)	Metabolic clearance rate (ml/min)		Coefficient of determination
				^{14}C	^{3}H	R^3
			Basal diet			
82	41·5	4·1	6·1	91	109	0·991
83	39·0	4·2	8·0	134	160	0·990
84	37·0	3·9	8·6	149	167	0·999
89	33·5	3·9	8·7	135	159	0·997
86	34·5	4·3	9·7	159	187	0·997
88	37·5	4·1	8·7	127	151	0·997
92	37·0	4·3	6·3	76	102	0·987
93	37·0	3·6	7·5	126	151	0·997
Mean	37·1	4·1	7·9	125	148	
			Urea-supplemented diet			
82	38·0	3·8	8·2	125	143	0·999
83	35·5	3·6	7·3	72	89	0·993
84	35·0	3·9	8·7	169	194	0·998
89	31·5	3·6	6·7	95	113	0·991
86	35·5	3·9	6·6	168	200	0·988
88	38·5	3·4	7·1	109	136	0·994
92	35·5	3·9	8·2	117	138	0·998
93	36·0	3·2	5·9	83	103	0·991
Mean	35·7	3·7	7·3	117	139	
SE of difference	0·39*	0·07*	0·69	14·7	15·9	

Statistical significance of difference between diets: $*P < 0·05$.
† For details of composition see Table 1.

where C_T is the relative concentration of ^{3}H (% dose/l), Cc is the relative concentration of ^{14}C (% dose/l), P_1P_2 are the coefficients of exponential terms, q_1q_2 are the exponents of exponential terms, t is period after injection (min).

The programme constrained the initial rate constant, q_1, and the zero time intercept, P_1, of the two functions to be the same.

This method of analysis yielded values for metabolic clearance rates (MCR) and a value for glucose space (G_s). G_s and MCR for [^{14}C]glucose were calculated according to the method described by White, Steel, Leng & Luick (1969) for multi-exponential functions.

$$G_s = \frac{100}{\sum P_i} = \frac{100}{P_1}, \quad \text{MCR } (^{14}C) = \frac{G_s}{\sum \frac{P_i'}{q_i}}$$

where $P_i' = P_i/\sum P_i$.

MCR for [^{3}H]glucose was calculated as described by Judson & Leng (1972) for a single exponential function.

$$\text{MCR } (^{3}H) = G_s q_1.$$

The total entry rate (ER) and irreversible loss rate (IL) for glucose were obtained by multiplying MCR (^{3}H) and MCR (^{14}C) respectively by the mean plasma glucose concentration. Pool size was similarly obtained by multiplying glucose space by mean plasma

Table 3. *Plasma urea and plasma potassium concentrations (mmol/l) of sheep given the basal diet and a similar diet with added urea*†

(Mean values for eight sheep/diet)

Diet	Plasma urea	Plasma potassium
Basal	3·3	4·8
Urea-supplemented	10·3	4·3
SE of difference	0·53***	0·17*

Statistical significance of difference between diets: $*P < 0{\cdot}05$, $***P < 0{\cdot}005$.
† For details of composition see Table 1.

glucose concentration. The difference between total ER and IL for glucose provides an estimate of the extent of glucose resynthesis (RC) (Judson & Leng, 1972):

$$RC = ER - IL.$$

RESULTS

The theoretical model described above accounted for 99% of the variation in the dilution curves, as can be seen from the determination coefficients (R^2) presented in Table 2. The criteria of glucose metabolism calculated from the model are also shown in Table 2. Expressing the values on a per kg body-weight basis had no effect on the pattern of the results.

The plasma urea concentration reflected the urea content of the diet (see Table 3). On the basal diet values ranged from 2·3–5·3 mmol/l (mean 3·3 mmol/l), whereas on the urea-supplemented diet the range was 9·2–11·8 mmol/l (mean 10·3 mmol/l). Increased blood urea concentrations have previously been shown to correlate with elevated blood ammonia concentrations (see for example, Morris & Payne, 1970).

Plasma K was found to be decreased on the urea diet ($P < 0{\cdot}05$) (see Table 2). The K concentrations found for the basal diet ranged from 4·5–5·1 mmol/l (mean 4·8 mmol/l) and for the urea-supplemented diet the range was 3·5–4·8 mmol/l (mean 4·3 mmol/l).

Plasma glucose concentration varied considerably over the period of the experiment, presumably because the animals were fed twice daily. The coefficient of variation on the basal diet was 5–15 (mean 10) and on the urea-supplemented diet was 4–13 (mean 7). The mean plasma glucose concentration (fourteen observations) showed a decrease ($P < 0{\cdot}05$) on the urea-supplemented diet. Other criteria of glucose metabolism showed no significant 'diet' effects. However, not only was the range of values on each diet very large but also the response of the animals to the diets varied considerably (see Table 3).

DISCUSSION

The increased plasma urea concentrations found in the sheep on the urea-supplemented diet are related to the increased activity of the urea-cycle enzymes. A variety of conditions has previously been shown to affect positively these enzymes: increased protein intake (Schimke, 1962*a*; Morris & Payne, 1970) starvation (Schimke, 1962*b*), cortisone treatment (Schimke, 1963), glucagon treatment and alloxan diabetes (McLean & Novello, 1965) and increased ammonia load (Soar *et al.* 1973).

Administration of urea has been shown to increase urinary K elimination (Juhász, Szegedi & Keresztes, 1975). Juhász *et al.* (1975) suggest that increased blood ammonia concentrations inhibit the transport of K from the extracellular space into the cells and

promote the release of K from the cells by diffusion. This could be an explanation for the depletion in plasma K observed on the urea-supplemented diet.

Alterations in blood glucose concentrations under various conditions associated with ammonia toxicity have been reported on many occasions in the literature (Chalupa & Opliga, 1969; Prior, Clifford, Hogue & Visek, 1970; Clifford, Prior, Hintz, Brown & Visek, 1972; Prior, Milner & Visek, 1972; Soar *et al.* 1973; Prior, 1976). In some cases an increase was found, in others a decrease. Prior *et al.* (1972) fed purified diets containing either soya-bean meal or urea to ram lambs. Rations were available twice daily for 2 h and blood samples were taken after the morning feed. As with the results presented here a significantly lower blood glucose concentration was observed in the urea-fed lambs. However, when sampling blood before feeding (Prior, Milner & Visek, 1970) or when rations were supplied at 2 hourly intervals (Prior, 1976) no significant alterations in blood glucose were detected. Chalupa & Opliger (1969) found increased blood glucose concentrations when sampling 3 h after an oral dose of 0·33 g urea/kg. Soar *et al.* (1973) also found increased glucose concentrations after intravenous ammonium acetate infusions. Obviously the method of administration of the ammonia load plays an important part in the metabolic response, endocrine function and blood glucose levels being closely related. Supplementing diets with urea does not appear to reduce propionic acid concentrations in the rumen, at least as judged by supplementing semi-purified diets with urea (M. C. Leonard, P. J. Buttery & D. Lewis, unpublished observations).

The observed variability in plasma glucose concentration in the period of the experiment was clearly related to the twice daily feeding regimen. This regimen was chosen to mimic conventional feeding habits and also to present an adequate ammonia challenge to induce an upset in glucose metabolism. That this was achieved can be seen from the reduction in plasma glucose and K levels. Although there was some fluctuation in plasma glucose concentrations, they did not change throughout the day in any systematic way. This justified the particular isotope-dilution technique we used. There was no significant effect of diet on the criteria calculated. It is possible, however, that there may be differences in response of different animals. The responses of sheep nos. 82, 84, 86, 92 (Table 3) were different from the other four with regard to the values for MCR. Judson *et al.* (1968), also found that the variation within each of three groups of sheep fed different rations was too great to detect significant differences in the mean ER values for glucose. Prior *et al.* (1972) when feeding sheep twice daily found evidence to suggest that gluconeogenesis was slower in urea-fed lambs than in lambs fed soya-bean meal. However, in later work (Prior, 1976) where glucose turnover rates were determined by primed infusion techniques and food was supplied at 2 hourly intervals, turnover rate was not found to be significantly affected by the dietary N source. Prior (1976) suggested that the 'frequent-feeding' regimen might account for the lack of significant differences.

It would therefore appear that although frequent feeding results in the achievement of steady-state conditions, insufficient challenge is presented to upset carbohydrate metabolism. However, twice daily feeding in turn presents problems arising from the lack of steady-state, leading to difficulties in interpreting the results. Evans & Buchanan-Smith (1975) used a single injection of [2-^{3}H]glucose to evaluate ER for glucose in sheep fed twice daily. They were satisfied that even with a mean variation in plasma glucose concentration of 9·2 % (range 3·8–20·5 %) in the 3 h experiment, they were justified in using the results. They found that substitution of concentrate for roughage might increase ER for glucose during a short period after eating.

The use of [2-^{3}H]glucose as a tracer for glucose metabolism was suggested by Katz & Dunn (1967). Tritium was shown to disappear more rapidly than ^{14}C from circulating glucose and this difference was thought to be due to the rapid loss of tritium to water,

and hence the minimal recycling of the label to the glucose pool. Judson & Leng (1972) combined the use of [2-^{3}H]glucose with [U-^{14}C]glucose in order to measure total ER and also to obtain an estimate of the extent of glucose resynthesis. When expressed as a percentage of IL of glucose, resynthesis was found to vary between 13–22 % depending on the method of analysis. This compares with values from the present study of 20 mg/min (23 %) on the basal diet and 16 mg/min (21 %) on the urea-supplemented diet. This difference was not significant; the range on the basal diet was 15–33 mg/min and on the urea-supplemented diet was 11–31 mg/min.

In the present experiment while there were no significant differences ($P > 0{\cdot}05$) between the values for MCR as a result of feeding urea, plasma glucose concentration was significantly lowered ($P < 0{\cdot}05$). It is therefore possible that there was a reduction in IL for glucose [MCR (^{14}C) × glucose concentration] and total ER [MCR (^{3}H) × glucose concentration] with urea feeding. The most likely reason for such a reduction would be a reduction in gluconeogenesis.

Although it has not been possible to show that urea-supplemented diets have a consistent effect on glucose clearance rates, it is possible that some derrangements in intermediary metabolism have occurred. In particular, plasma glucose and K concentrations were reduced. These observations suggest that care should be exercised when including large amounts of urea in ruminant rations, especially for the high-yielding lactating dairy cow where glucose output is very important. The results also give encouragement for the development of slow release forms of non-protein-N to reduce the ammonia challenge associated with diets supplemented with non-protein-N.

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REFERENCES

Annison, E. F., Brown, R. E., Leng, R. A., Lindsay, D. B. & West, C. E. (1967). *Biochem. J.* **104**, 135.
Chalupa, W. (1968). *J. Anim. Sci.* **27**, 207.
Chalupa, W. & Opliger, P. (1969). *J. Anim. Sci.* **29**, 154.
Clifford, A. J., Prior, R. L., Hintz, H. F., Brown, P. R. & Visek, W. J. (1972). *Proc. Soc. exp. Biol. Med.* **140**, 1447.
Evans, E. & Buchanan-Smith, J. G. (1975). *Br. J. Nutr.* **33**, 33.
Ford, E. J. H. (1965). *J. agric. Sci.*, Camb. **65**, 41.
Jones, G. B. (1965). *Analyt. Biochem.* **12**, 249.
Judson, G. J., Anderson, E., Luick, J. R. & Leng, R. A. (1968). *Br. J. Nutr.* **22**, 69.
Judson, G. J. & Leng, R. A. (1968). *Proc. Aust. Soc. Anim. Prod.* **7**, 354.
Judson, G. J. & Leng, R. A. (1972). *Aust. J. biol. Sci.* **25**, 1313.
Juhász, B., Szegedi, B. & Keresztes, M. (1975). *Tracer Studies on Non-protein Nitrogen for Ruminants.* Vol. 2, p. 123. Vienna: International Atomic Energy Authority.
Katunuma, N., Okada, M. & Nishi, Y. (1967). *Adv. Enzym. Reg.* **4**, 317.
Katz, J. & Dunn, A. (1967). *Biochemistry, Easton* **6**, 1.
Lewis, D. & Buttery, P. J. (1972). In *Production Disease in Farm Animals*, p. 201 [J. M. Payne, K. G. Hibbit and B. F. Sansom, editors]. London: Ballière Tindall.
McLean, P. & Novello, F. (1965). *Biochem. J.* **94**, 410.
Morris, J. G. & Payne, E. (1970). *J. agric. Sci., Camb.* **74**, 259.
Oltjen, R. R. (1969). *J. Anim. Sci.* **28**, 673.
Prior, R. L. (1976). *J. Anim. Sci.* **42**, 160.
Prior, R. L., Clifford, A. J., Hogue, D. E. & Visek, W. J. (1970). *J. Nutr.* **100**, 438.
Prior, R. L., Milner, J. A. & Visek, W. J. (1972). *J. Nutr.* **102**, 1223.
Schimke, R. T. (1962*a*). *J. biol. Chem.* **237**, 459.
Schimke, R. T. (1962*b*). *J. biol. Chem.* **237**, 1921.
Schimke, R. T. (1963). *J. biol. Chem.* **238**, 1012.
Soar, J. B., Buttery, P. J. & Lewis, D. (1973). *Proc. Nutr. Soc.* **32**, 77A.

Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
Steel, J. W. & Leng, R. A. (1968). *Proc. Aust. Soc. Anim. Prod.* **7**, 342.
Ulyatt, M. J., Whitelaw, F. G. & Watson, F. G. (1970). *J. agric. Sci., Camb.* **75**, 565.
Varley, H. (1967). *Practical Clinical Biochemistry*, 4th ed., p. 161. London: W. Heinemann.
White, R. G., Steel, J. W., Leng, R. A. & Luick, J. R. (1969). *Biochem. J.* **114**, 203.

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