APPENDIX 4

Effects of water diuresis on renal function in one sheep.

Previous studies have examined the effects of various diuretic agents on the renal function of sheep. Rabinowitz and his colleagues have determined the effects of urea, methylurea, amiloride, mannitol and ethacrynic acid on renal function of sheep (Rabinowitz & Gunther 1972a, Rabinowitz & Gunther 1972b, Rabinowitz & Gunther 1972c, Rabinowitz & Gunther 1975, Rabinowitz & Gunther 1978, Rabinowitz *et al* 1979). Studies from this laboratory (chapters 1 and 2) have examined the effects of sodium and potassium chloride and urea diuresis on renal function. Yet, despite its implications for animals grazing lush pastures (chapter 5) the effects of water diuresis has received relatively little study. Thornton (1970) studied some effects of water diuresis in cattle and Maloiy *et al* (1970) studied some of its effects in sheep and red deer.

This study was performed as a pilot experiment involving one animal, to investigate the renal responses to an intraruminal water load.

Methods

One merino ewe (BW 42.5 kg) was fed a ration consisting of 96 g of lucerne chaff and 704 g of oaten chaff at two hourly intervals using an automatic feeder. After the animal had been fed this ration for 12 days its ad libitum water consumption was measured over the following 5 days at 1180 mL/d.

The animal was then denied access to drinking water but was continuosly infused intraruminally with 1224 mL of water per day for five days. On the last day of this period a 24 hour urine sample was collected immediately followed by an ECV measurement using inulin, as described in chapter 2.

The animal was given 6050 mL of water via the infusion on the subsequent 5 days and the sampling procedures repeated. Analytical methods were as for those described in chapter 2.

Results

Results obtained are shown in the following table.

There was a substantial diuresis caused by the increased water infusion. Associated with this diuresis was an increased excretion of urea, sodium, calcium, and chloride. These changes were paralleled with changes in fractional excretion of the particular substance, because the GFR and the plasma levels were virtually constant. However plasma urea level did decrease slightly. The changes in excretion of phosphate may be an artifact as explained in chapter 2.

Discussion

The changes in renal excretion associated with water diuresis, are the result mostly of a change in residence time of glomerular filtrate in the kidney tubules. A reduction in the secretion of antidiuretic hormone also occurs with water loading. ADH reduces the water permeability of the collecting ducts of the kidney and hence prevents the building of a strong concentration gradient for the reabsorption of urea from the terminal collecting duct (Kokko & Rector 1972). NaCl transport from the ascending thick limb of Henlé's loop is also stimulated by ADH (Sasaki & Imai 1980). However the changes in excretion of electrolytes are probably more controlled by 'wash-out' than by hormonal influences (Atherton *et al* 1968).

A water diuresis in cattle caused by an intraruminal water infusion results in a substantial increase in the renal excretion and clearance of urea (Thornton 1970). Water loading of sheep with 3.0 L of water twice daily via a rumen fistula resulted in an increased excretion of urea and URAL (Maloiy *et al* 1970), however these changes may be related to N digestibility changes, rather than changes in renal function. Similar data were obtained from the sheep in the present study.

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Excretory data for one merino ewe infused continuously with two levels of water.

Water Intake (mL/d)	1224	6050
GFR (mL/min)	58.1	56.3
UFR (mL/min)	0.38	3.72
ECV (L)	7.6	7.5
PCV (%)	32	31
P_{prot} (g/L)	6.1	6.2
P_{urea} (mmol/L)	4.2	3.8
U_{urea} (μmol/min)	15.1	18.2
FE_{urea} (%)	6.2	8.5
P_{Na} (mmol/L)	139	138
U_{Na} (μmol/min)	81.2	90.1
FE_{Na} (%)	1.0	1.2
P_K (mmol/L)	4.1	4.1
U_K (μmol/min)	74.1	71.6
FE_K (%)	31.1	31.0
P_{Mg} (mmol/L)	1.42	1.43
U_{Mg} (μmol/L)	6.0	6.0
FE_{Mg} (%)	7.3	7.5
P_{Ca} (mmol/L)	2.42	2.41
U_{Ca} (μmol/min)	0.9	1.4
FE_{Ca} (%)	0.6	1.0
P_{Pi} (mmol/L)	1.73	1.81
U_{Pi} (μmol/min)	0.10	0.02
FE_{Pi} (%)	0.09	0.02
P_{Cl} (mmol/L)	112	113
U_{Cl} (μmol/min)	139	145
FE_{Cl} (%)	2.1	2.3

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APPENDIX 5

Variation in renal function over a 24 hour period.

Circadian variation in renal parameters has been known to occur in man and animals for some time. Sarelius & Greenaway (1975) determined the patterns of renal excretion of electrolytes in sheep given varied feeding regimes. They found that the intake of food was the most important factor determining changes in urine composition. However they did delineate the existence of several food independent rhythms of urine composition, but these were not clearly defined.

The present study was performed on two sheep to determine if any major variation in renal clearances of electrolytes and urea occurred throughout the day.

Methods

Two merino ewes (BW 41 and 38 kg) were housed in metabolism crates and fed a diet comprising of 96 g lucerne chaff plus 704 g of oaten chaff. This diet provided 16.91 MJ gross energy, 6.8 g N, 127 mmol Na, 112 mmol K, 86 mmol Ca, 55 mmol Mg and 30 mmol P per day. Each animal was fed the ration at 2 hourly intervals for 12 days prior to any experimentation. Following this period the bladder of each animal was catheterised in the afternoon and the following morning urine collection was commenced. A series of eight 3 hour clearance periods was determined for each animal.

Each animal was then given the ration ad libitum for 12 days and the experiment repeated. The food eaten during each period was recorded.

Analytical methods were as for those described in Chapter 2, with GFR determined as creatinine clearance.

Results

The pattern of food intake for the two feeding regimes are shown in Fig.1. The total intake for the 2 hourly fed animals was slightly greater than for the ad libitum fed animals (800 g/d and 787 g/d).

The patterns of renal excretion and GFR are shown in Figures 2-15.

During 2 hourly feeding there was no discernable changes in any parameter measured. However with ad libitum feeding there was a substantial variation in food intake with a higher amount being consumed during the period 09.00-18.00 h. During this period there was a substantial drop in both GFR and UFR. This corresponded with an increased excretion of Ca and glucose and a decreased excretion of osmoles. Urine osmolality was inversely related to UFR and was slightly increased during the above period. There were no major changes in the excretion of urea, URAL, Na, K, Mg or Pi.

Discussion

Scott (1975) showed that there is a period of low UFR after feeding in sheep that were meal fed. This low UFR was associated with the excretion of an acidic urine rich in Ca and Mg. In the present study the increase in food intake during the period 09.00-18.00 h probably accounts for the differences in U_{Ca}, UFR, GFR and $U_{glucose}$. These sheep were accustomed to feed refusal weigh backs daily, and the subsequent presentation of fresh food at 10.00-11.00 h. On previous occasions the sheep had been noted to eat heartily following the introduction of fresh food. This fact together with greater activity in the animal house is probably the reason for the increased food intake during this period.

There appears to little rhythmic changes in the renal parameters measured apart from food induced changes. For sheep fed at 2 hourly intervals a representative sample of renal function can probably be gained during any three hour period of the day. Allowance for differences in food intake should occur when examining renal function in sheep fed ad libitum.

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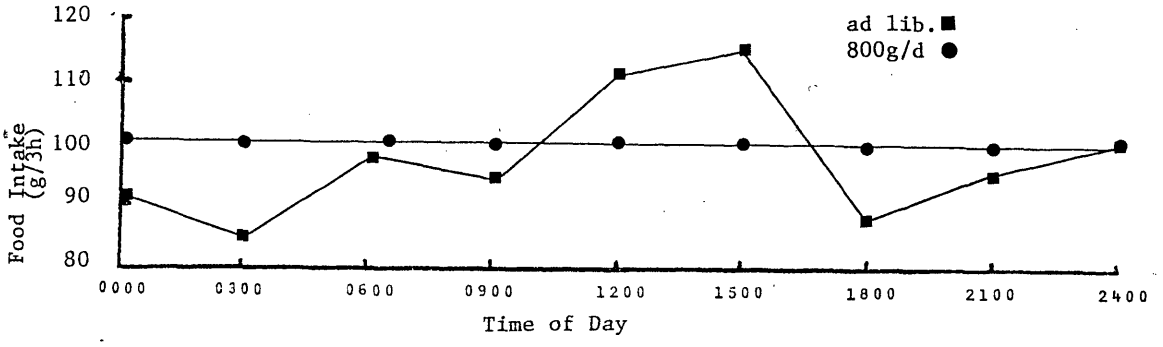

Fig. 1. Variation in food intake

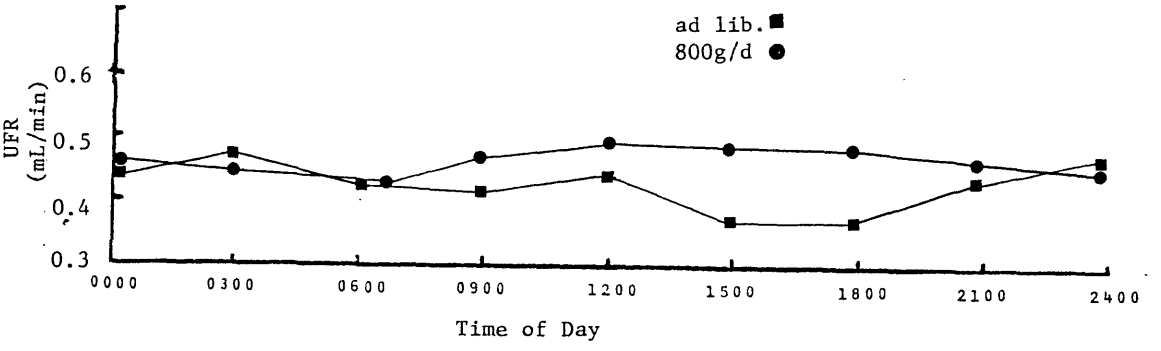

Fig.2. Variation in UFR.

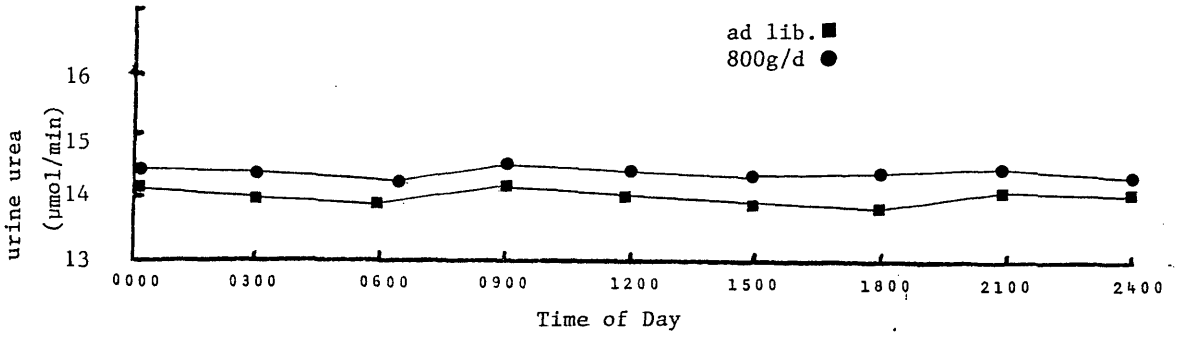

Fig. 3. Variation in urine urea excretion.

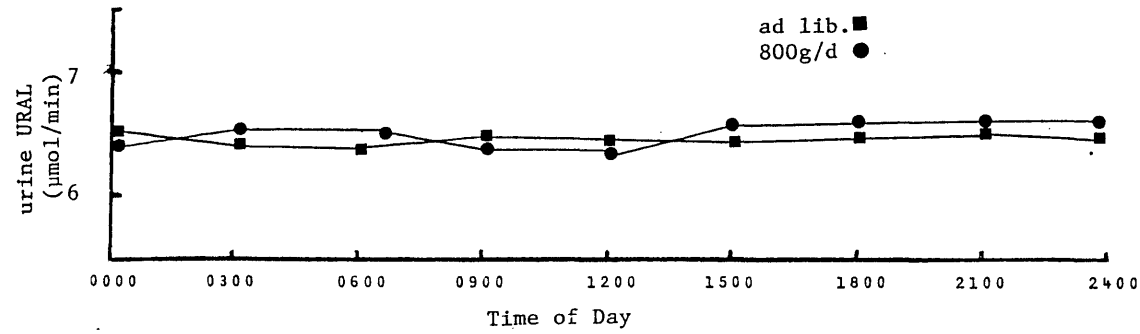

Fig. 4. Variation in urine URAL

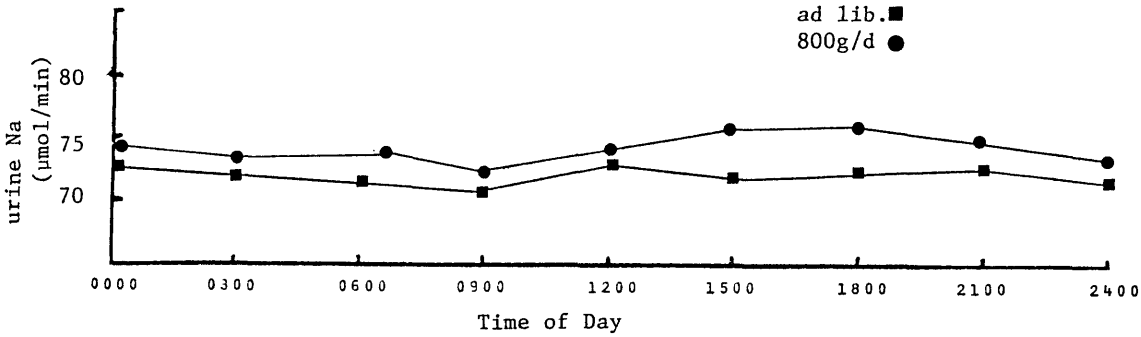

Fig. 5. Variation in Na exretion

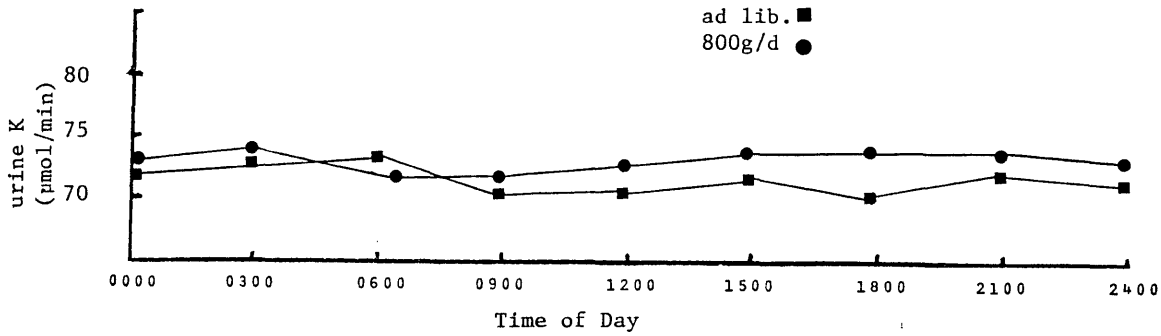

Fig. 6. Variation in K excretion.

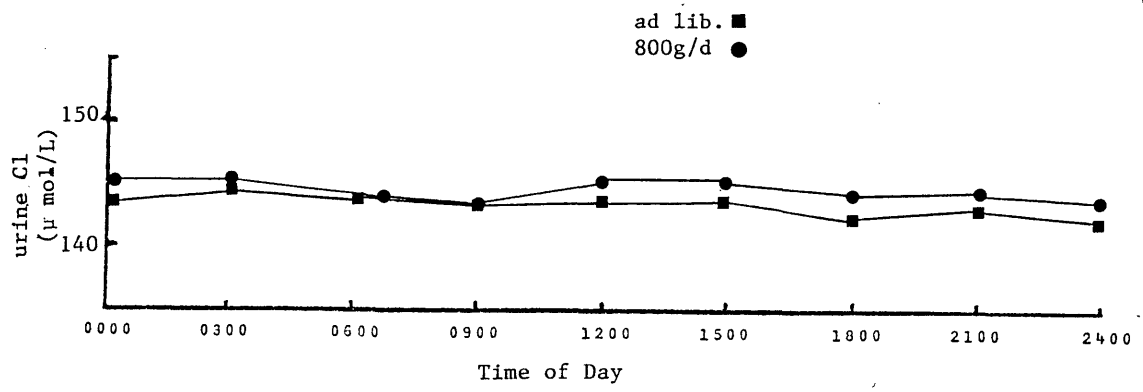

Fig. 7. Variation in chloride excretion.

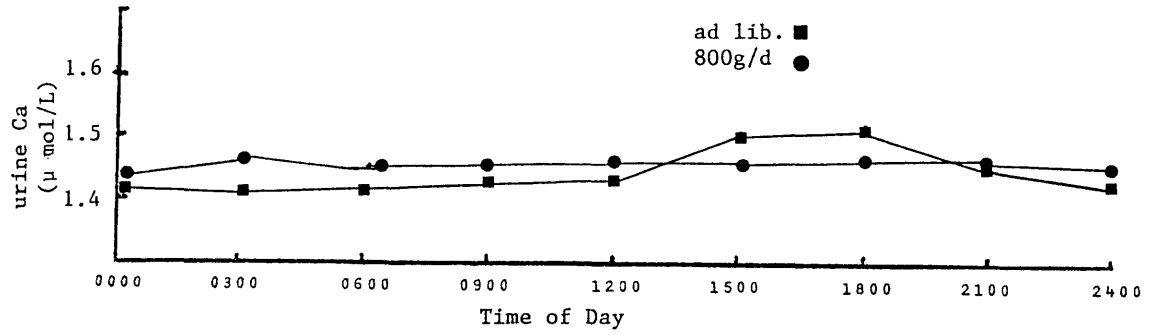

Fig. 8. Variation in Ca excretion.

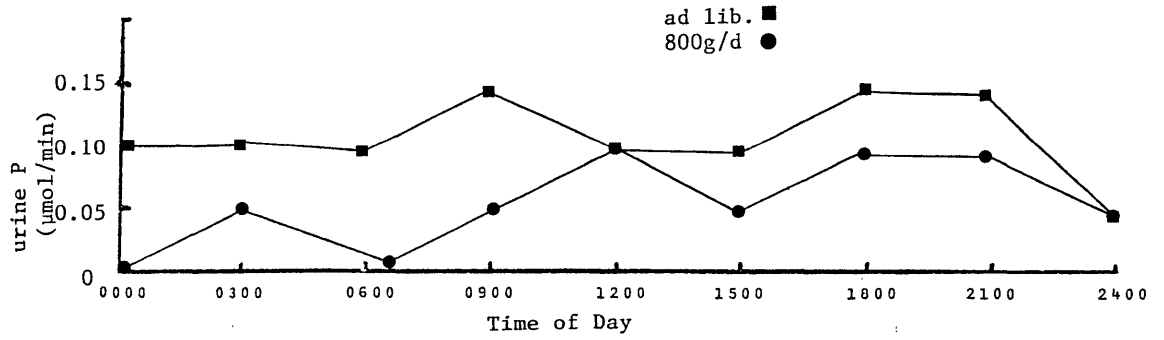

Fig. 9. Variation in phosphorus excretion.

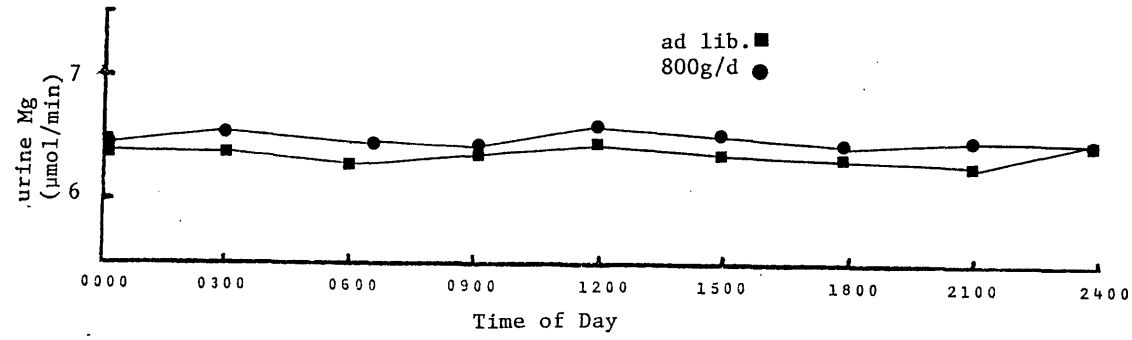

Fig. 10. Variation in Mg excretion.

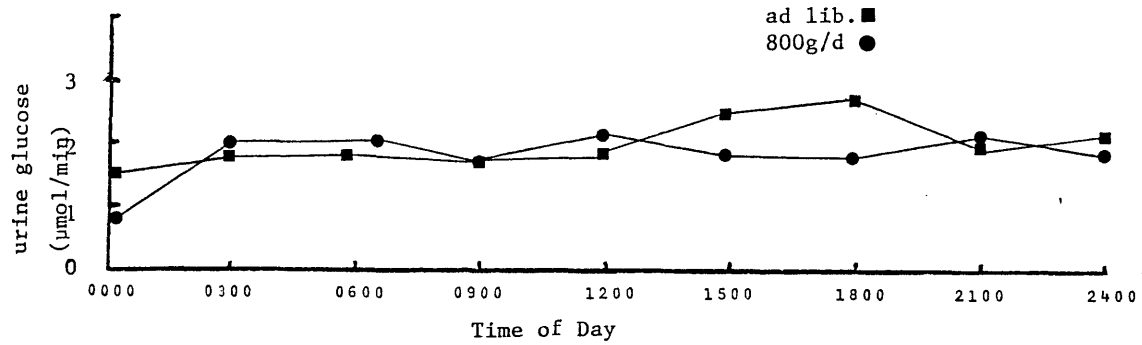

Fig. 11. Variation in glucose excretion.

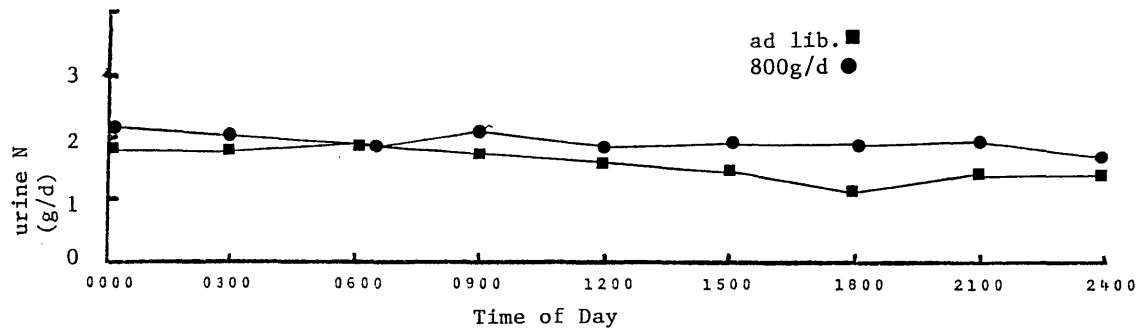

Fig. 12. Variation in N excretion.

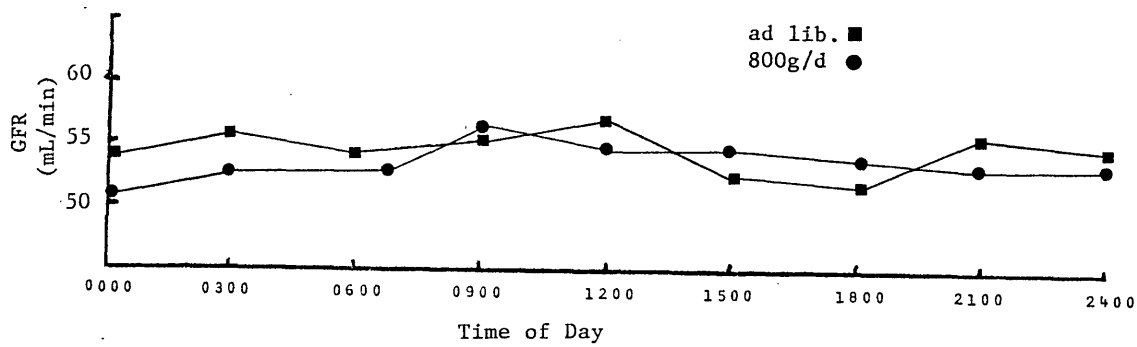

Fig. 13. Variation in GFR.

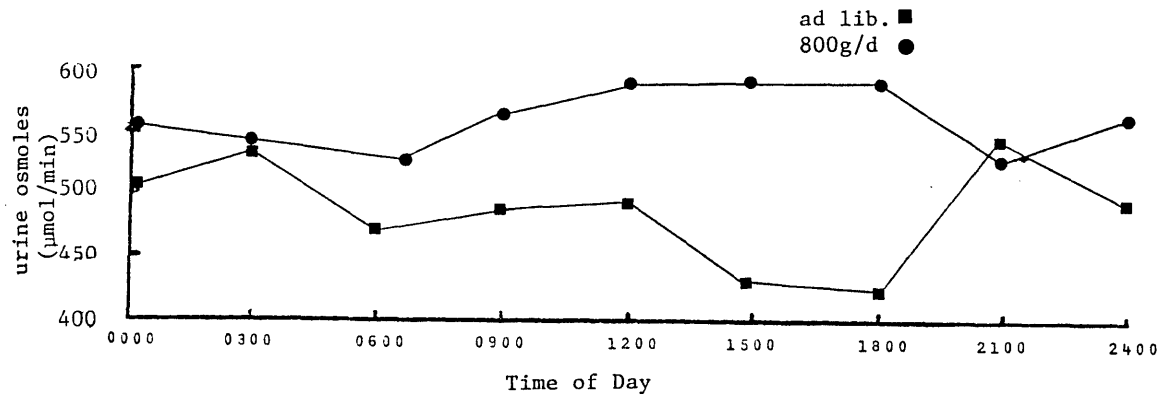

Fig. 14. Variation in the excretion of osmoles

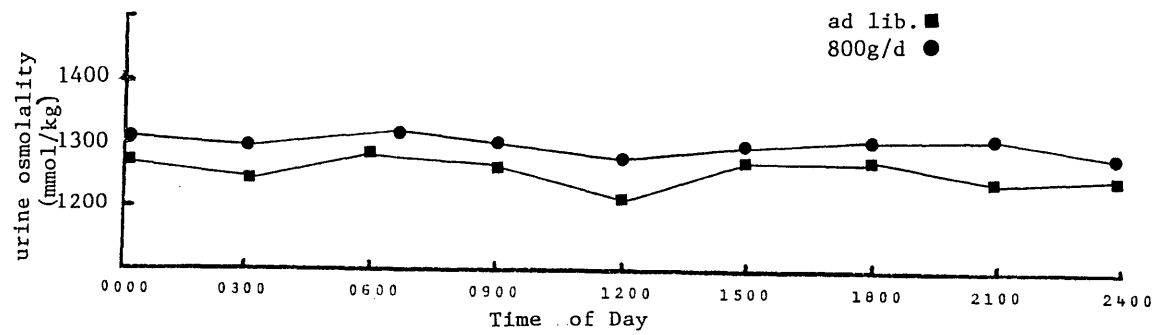

Fig. 15. Variation in urine osmolality.

APPENDIX 6

Plasma urea and electrolyte levels of Poll Dorset sheep grazing a lush 'new growth' pasture.

A flock of 38 mixed sex stud poll dorset sheep at 'Alton Park Stud' Dubbo N.S.W. was freely grazing a fresh growth of pasture resulting from drought breaking rains some 4 weeks previous. The pasture consisted of mostly Trefoil Medic (*Medicago polymorpha*) and barley grass (*Hordeum vulgare*) at a height of about 6 cm with the occasional dry standing plant of *Sporobolis elongatis* and 3 awned spear grass (*Stipa arundinacea*). The latter were not eaten by the sheep.

The animals were yarded quietly and quickly (<5min) and blood samples were taken by jugular venepuncture immediately. The samples were centrifuged and the plasma frozen for later analysis.

Results from plasma analysis are shown in Table 1.

Grab samples of the 'green' pasture on offer were analysed to yield the results shown in Table 2.

Despite an almost assured very high N intake, all sheep had plasma urea levels below 12 mmol/L, the approxinmate plateau level achieved by McIntyre (1970) and in chapter 1 of this thesis. The level of urea was slightly higher for the ewe hoggets and the dry ewes possibly because their N requirement per unit body weight would be less.

There was no significant differences in the electrolyte levels between the groups of animals except for Pi which was substantially higher for the lambs. Plasma Pi level in animals is an age dependent variable, being higher for younger animals (Sestoft 1979).

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Table 1. Plasma urea and electrolyte levels of dorset sheep (mmol/L).

Age and Sex	Urea	Na	K	Cl	Ca	Mg	Pi
2 y.o. ram	10.1	139	4.3	110	2.39	1.41	1.92
2-4 y.o. dry ewes	11.4 ± 0.2a	140 ± 1.3	4.3 ± 0.2	111 ± 0.8	2.43 ± 0.04	1.26 ± 0.18	1.72 ± 0.09a
2-4 y.o. lact. ewes	9.9 ± 0.6b	137 ± 1.8	4.0 ± 0.3	109 ± 0.4	2.31 ± 0.08	1.27 ± 0.14	1.65 ± 0.09a
1 y.o. ewe hoggets	11.2 ± 0.4a	139 ± 1.3	4.4 ± 0.2	111 ± 0.3	2.42 ± 0.02	1.65 ± 0.36	1.84 ± 0.21a
0.5 y.o. lambs (weaned)	10.2 ± 0.8b	142 ± 2.1	4.2 ± 0.1	112 ± 0.8	2.38 ± 0.08	1.50 ± 0.21	2.14 ± 0.06b

means with different subscripts are significantly different ($P < 0.01$)

Table 2. The nitrogen and electrolyte content of the herbage on offer.

Constituent	Concentration (as a % of dry matter)
Dry Matter (% of total)	10.8
Organic Matter	89.1
N	3.87
Na	0.27
K	3.05
Ca	0.64
Mg	0.29
P	0.49

APPENDIX 7

Losses of plasma and urinary constituents with storage.

Eight urine and blood samples were collected from sheep being infused intravenously with inulin and PAH. The blood samples were taken by jugular venepuncture into heparinised syringes and the urine samples were collected from indwelling urinary catheters. The samples were transferred to new sterile plastic containers, but the collection procedures were not aseptically performed.

The blood was centrifuged immediately and an aliquot of the plasma and the urine was analysed immediately. The remaining plasma was divided into two containers (A and B). The remaining urine was divided into 4 containers (A, B, C and D). Urines A and B were untreated and samples C and D were acidified to pH 4-5 with H_2SO_4. The samples were all then stored at –20°C. Every 14 days plasma sample B and urine samples B and D were thawed at room temperature, reanalysed and refrozen. After 3 months all samples were thawed and reanalysed. Analyses were for urine total N, urine ammonia, urea, URAL, creatinine, plasma protein, glucose, inulin, PAH, Na, K, Ca, Mg, Pi, and Cl all using the methods described in Chapter 2.

The results are shown in Tables 1 - 14.

Storage of plasma samples at –20°C appears to be satisfactory for periods of at least 3 months. However thawing and refreezing more than twice will probably result in abberrant results in the analysis of most substances measured. The consistency of the increase in the concentration of all the substances with time suggests that the phenomena is caused by evaporative loss of water from the samples.

The loss of urea and the increased NH_3 level after the first thawing of the urine samples indicates that ureolysis occurs in the untreated samples. However the total loss of N from the sample was negligible, even though the pH was relatively high (about a mean value of 8.1). Glucose disappeared from the urine samples after the first thawing. Acidification reduced the loss of urea and glucose

such that only minor amounts were lost.

The electrolyte levels were not substantially altered with time. The lack of an evaporative concentration could be explained by the relatively larger volume to surface area ratio of the urine sample containers compared to the plasma sample containers.

Recommendations

1. All plasma samples should be analysed at or before the initial thawing.

2. All urine samples should be acidified before freezing if N constituents are to be measured.

Table 1. The effects of storage on the concentration of N in urine and protein in plasma (g/dL)

Plasma A	5.1 ± 0.5	---	---	---	---	---	5.1 ± 0.6
Plasma B	5.1 ± 0.5	5.2 ± 0.6	5.4 ± 0.8	5.5 ± 0.6	5.5 ± 0.6	5.7 ± 0.7	5.8 ± 0.8
Urine A	10.2 ± 0.4	---	---	---	---	---	10.2 ± 0.6
Urine B	10.2 ± 0.4	10.3 ± 0.5	10.1 ± 0.6	9.9 ± 0.5	9.9 ± 0.7	9.9 ± 0.7	9.8 ± 0.8
Urine C	10.3 ± 0.5	---	---	---	---	---	10.2 ± 0.7
Urine D	10.3 ± 0.5	10.2 ± 0.5	10.2 ± 0.6	10.3 ± 0.7	10.3 ± 0.4	10.1 ± 0.4	10.1 ± 0.5

Table 2. The effects of storage on the urea concentration of plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	5.2 ± 0.9	---	---	---	---	---	5.2 ± 1.0
Plasma B	5.2 ± 0.9	5.3 ± 0.8	5.5 ± 0.8	5.5 ± 0.6	5.7 ± 0.5	5.7 ± 0.7	5.8 ± 0.8
Urine A	524 ± 36	---	---	---	---	---	529 ± 45
Urine B	524 ± 36	521 ± 34	515 ± 31	509 ± 62	508 ± 63	500 ± 67*	501 ± 68*
Urine C	527 ± 38	---	---	---	---	---	525 ± 48
Urine D	528 ± 41	522 ± 48	526 ± 52	519 ± 55	523 ± 36	520 ± 44	522 ± 40

* significantly different from week 0 ($P<0.01$).

Table 3. The effects of storage on the URAL concentration of plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	0.12 ± 0.10	---	---	---	---	---	0.12 ± 0.09
Plasma B	0.12 ± 0.10	0.12 ± 0.11	0.12 ± 0.10	0.14 ± 0.10	0.15 ± 0.12	0.15 ± 0.13	0.16 ± 0.12
Urine A	15.2 ± 2.2	---	---	---	---	---	15.3 ± 2.0
Urine B	15.2 ± 2.2	15.3 ± 2.1	15.4 ± 2.3	15.0 ± 3.5	15.3 ± 3.2	15.3 ± 3.9	15.1 ± 3.0
Urine C	15.2 ± 2.8	---	---	---	---	---	15.4 ± 1.6
Urine D	15.2 ± 2.8	15.6 ± 3.5	15.4 ± 2.5	15.8 ± 3.2	15.1 ± 2.1	14.9 ± 2.2	15.0 ± 2.5

Table 4. The effects of storage on the concentration of urine ammonia (mmoL/L).

Week	0	2	4	6	8	10	12
Urine A	5.9 ± 0.10	---	---	---	---	---	6.1 ± 0.11
Urine B	5.9 ± 0.10	5.5 ± 0.10	5.6 ± 0.11	6.8 ± 0.19	7.2 ± 0.22	7.1 ± 0.20	7.8 ± 0.24*
Urine C	6.1 ± 0.12	---	---	---	---	---	6.2 ± 0.19
Urine D	6.1 ± 0.12	6.2 ± 0.16	6.2 ± 0.16	6.3 ± 0.14	6.1 ± 0.21	6.0 ± 0.22	6.4 ± 0.25

* significantly different from week 0 ($P<0.01$).

Table 5. The effects of storage on the Na concentration of plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	142 ± 2.1	---	---	---	---	---	143 ± 1.9
Plasma B	142 ± 2.1	142 ± 2.1	143 ± 2.2	144 ± 2.2	145 ± 2.3	145 ± 2.5	147 ± 1.9*
Urine A	184 ± 9.2	---	---	---	---	---	183 ± 9.6
Urine B	184 ± 9.2	183 ± 9.8	184 ± 11.2	183 ± 9.6	184 ± 10.7	183 ± 9.5	185 ± 8.3
Urine C	183 ± 10.0	---	---	---	---	---	184 ± 11.2
Urine D	183 ± 10.0	184 ± 8.9	184 ± 7.8	184 ± 11.1	186 ± 9.8	185 ± 9.8	185 ± 9.9

* significantly different from week 0 ($P<0.01$).

Table 6. The effects of storage on the K concentration of plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	4.3 ± 0.05	---	---	---	---	---	4.3 ± 0.04
Plasma B	4.3 ± 0.05	4.4 ± 0.05	4.4 ± 0.04	4.5 ± 0.05	4.5 ± 0.06	4.6 ± 0.08*	4.6 ± 0.05*
Urine A	311 ± 24	---	---	---	---	---	315 ± 26
Urine B	311 ± 24	316 ± 31	318 ± 25	311 ± 30	311 ± 24	304 ± 25	314 ± 27
Urine C	319 ± 26	---	---	---	---	---	310 ± 32
Urine D	319 ± 26	315 ± 34	315 ± 25	302 ± 39	315 ± 26	309 ± 34	316 ± 34

* significantly different from week 0 ($P<0.01$).

Table 7. The effects of storage on the concentration of Cl in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	111 ± 0.1	---	---	---	---	---	111 ± 0.2
Plasma B	111 ± 0.1	112 ± 0.1	112 ± 0.2	113 ± 0.2	115 ± 0.2*	116 ± 0.3*	115 ± 0.2*
Urine A	527 ± 26	---	---	---	---	---	531 ± 22
Urine B	527 ± 26	526 ± 28	533 ± 32	521 ± 21	532 ± 25	520 ± 31	522 ± 26
Urine C	524 ± 28	---	---	---	---	---	531 ± 25
Urine D	524 ± 28	518 ± 28	520 ± 29	528 ± 32	531 ± 31	524 ± 26	521 ± 22

* significantly different from week 0 ($P<0.01$).

Table 8. The effects of storage on the concentration of Ca in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	2.4 ± 0.02	---	---	---	---	---	2.4 ± 0.02
Plasma B	2.4 ± 0.02	2.4 ± 0.02	2.4 ± 0.03	2.5 ± 0.02*	2.5 ± 0.02*	2.5 ± 0.03*	2.5 ± 0.02*
Urine A	2.5 ± 0.4	---	---	---	---	---	2.6 ± 0.4
Urine B	2.5 ± 0.4	2.6 ± 0.4	2.4 ± 0.2	2.5 ± 0.5	2.6 ± 0.4	2.5 ± 0.5	2.5 ± 0.4
Urine C	2.5 ± 0.4	---	---	---	---	---	2.5 ± 0.5
Urine D	2.5 ± 0.4	2.5 ± 0.4	2.5 ± 0.5	2.6 ± 0.5	2.3 ± 0.4	2.4 ± 0.5	2.6 ± 0.3

* significantly different from week 0 ($P<0.01$).

Table 9. The effects of storage on the concentration of Mg in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	1.4 ± 0.08	---	---	---	---	---	1.5 ± 0.08
Plasma B	1.4 ± 0.08	1.4 ± 0.08	1.5 ± 0.08	1.5 ± 0.06	1.6 ± 0.06*	1.6 ± 0.05*	1.5 ± 0.07
Urine A	19 ± 3	---	---	---	---	---	18 ± 2
Urine B	19 ± 3	18 ± 2	19 ± 3	18 ± 3	19 ± 3	20 ± 3	19 ± 3
Urine C	19 ± 3	---	---	---	---	---	19 ± 2
Urine D	19 ± 3	19 ± 3	21 ± 4	20 ± 3	19 ± 4	19 ± 3	19 ± 3

* significantly different from week 0 ($P<0.01$).

Table 10. The effects of storage on the concentration of Pi in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	1.8 ± 0.2	---	---	---	---	---	1.8 ± 0.3
Plasma B	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	2.1 ± 0.3*	2.0 ± 0.3
Urine A	0.09 ± 0.05	---	---	---	---	---	0.09 ± 0.05
Urine B	0.09 ± 0.05	0.09 ± 0.05	0.09 ± 0.05	0.07 ± 0.06	0.07 ± 0.05	0.08 ± 0.06	0.08 ± 0.05
Urine C	0.09 ± 0.05	---	---	---	---	---	0.08 ± 0.05
Urine D	0.09 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	0.08 ± 0.06	0.08 ± 0.06	0.08 ± 0.06	0.08 ± 0.06

* significantly different from week 0 ($P<0.01$).

Table 11. The effects of storage on the concentration of glucose in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	3.8 ± 0.2	---	---	---	---	---	3.6 ± 0.3
Plasma B	3.8 ± 0.2	3.9 ± 0.3	3.9 ± 0.1	4.1 ± 0.3*	4.1 ± 0.2*	4.1 ± 0.2*	4.1 ± 0.2*
Urine A	2.8 ± 0.8	---	---	---	---	---	2.4 ± 0.5
Urine B	2.8 ± 0.8	2.4 ± 0.6	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
Urine C	2.7 ± 0.8	---	---	---	---	---	2.6 ± 0.9
Urine D	2.7 ± 0.8	2.6 ± 0.8	2.6 ± 0.8	2.6 ± 0.6	2.5 ± 0.6	2.5 ± 0.7	2.5 ± 0.6

* significantly different from week 0 (P<0.01).

Table 12. The effects of storage on the concentration of inulin in plasma and urine (g/L).

Week	0	2	4	6	8	10	12
Plasma A	0.31 ± 0.02	---	---	---	---	---	0.32 ± 0.01
Plasma B	0.31 ± 0.02	0.31 ± 0.01	0.32 ± 0.04	0.33 ± 0.02	0.33 ± 0.04	0.34 ± 0.02*	0.34 ± 0.02*
Urine A	15.2 ± 0.6	---	---	---	---	---	15.2 ± 0.5
Urine B	15.2 ± 0.6	15.2 ± 0.6	15.3 ± 0.7	15.2 ± 0.5	15.1 ± 0.2	15.3 ± 0.7	15.3 ± 0.6
Urine C	15.2 ± 0.6	---	---	---	---	---	15.1 ± 0.5
Urine D	15.2 ± 0.6	15.3 ± 0.2	15.2 ± 0.2	15.3 ± 0.3	15.2 ± 0.2	15.4 ± 0.3	15.3 ± 0.3

* significantly different from week 0 (P<0.01).

Table 13. The effects of storage on the concentration of PAH in plasma and urine (g/L).

Week	0	2	4	6	8	10	12
Plasma A	0.03 ± 0.002	---	---	---	---	---	0.03 ± 0.001
Plasma B	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.001	0.04 ± 0.002*	0.04 ± 0.001*	0.04 ± 0.002*	0.04 ± 0.002*
Urine A	7.69 ± 0.12	---	---	---	---	---	7.65 ± 0.15
Urine B	7.69 ± 0.12	7.65 ± 0.15	7.75 ± 0.18	7.79 ± 0.12	7.76 ± 0.25	7.78 ± 0.31	7.88 ± 0.26*
Urine C	7.75 ± 0.15	---	---	---	---	---	7.64 ± 0.21
Urine D	7.75 ± 0.15	7.62 ± 0.12	7.76 ± 0.31	7.81 ± 0.21	7.79 ± 0.22	7.74 ± 0.19	7.65 ± 0.22

* significantly different from week 0 (P<0.01).

Table 14. The effects of storage on the concentration of creatinine in plasma and urine (mmol/L).

Week	0	2	4	6	8	10	12
Plasma A	2.01 ± 0.08	---	---	---	---	---	2.02 ± 0.10
Plasma B	2.01 ± 0.08	2.02 ± 0.08	2.06 ± 0.10	2.08 ± 0.10	2.18 ± 0.19*	2.21 ± 0.21*	2.20 ± 0.21*
Urine A	42.6 ± 1.2	---	---	---	---	---	42.3 ± 1.2
Urine B	42.6 ± 1.2	42.8 ± 1.8	43.8 ± 3.5	44.1 ± 4.2*	44.1 ± 4.1*	44.0 ± 4.0*	44.8 ± 2.8*
Urine C	43.1 ± 1.2	---	---	---	---	---	44.2 ± 2.2
Urine D	43.1 ± 1.2	44.6 ± 5.1	43.2 ± 4.1	44.1 ± 3.9	44.1 ± 3.5	43.8 ± 3.1	43.0 ± 3.2

* significantly different from week 0 ($P<0.01$).

APPENDIX 8

On sampling urine samples.

On many occasions the existence of a cloudy suspension that settled with time was noted in sheep urine samples. Godwin & Williams (1982) subjected an amorphous urinary sediment to infra-red spectral analysis which showed compatability with a uric acid-carbonato-apatite mixture, with a probable mucoprotein matrix.

Because the suspension settles and in view of the possible uneven distribution of urinary constituents that would result from settlement the concentration of various urinary components was measured in the different fractions of settled out and mixed urine samples.

Methods

Five urine samples were collected from indwelling catheters in sheep receiving intravenous infusions of inulin and PAH. The urine was acidified (pH 4-5) with H_2SO_4 and allowed to stand overnight at 5°C in plastic sample containers. The following morning a 'precipitated' fraction had settled to the base of each sample.

Three aliquots of each sample were taken for analysis. The first sample (A) was taken from the sedimented fraction. The second (B) from the 'supernatant' fraction and the third from the middle of the container after thorough mixing (C). The analytical methods were as for those described in chapter 2.

Results

There was an obvious disparity in the distribution of N constituents between the aliquots. The sedimented material (A) contained more N, NH_3 and URAL than the 'supernatant' fraction (B). Electrolyte and urea distribution across the aliquots appeared to be even. One would have expected to find Mg, Ca and Pi in higher concentrations in fraction (A) if the sediment contained an apatite mixture

as suggested by Godwin & Williams (1982). However as the Pi content of the urine in the present study was negligible the content of apatite was unlikely to be of significance. The suspension was probably uric acid precipitated by the acidification and cooling of the samples.

Recommendation

All urine samples should be thoroughly mixed before analysis.

References

Godwin,I.R. & Williams,V.J. (1982) *Aust.J.Agric.Res.* **33**,843

APPENDIX 9

The effects of pH and inorganic phosphate level on red blood cell metabolism in vitro.

Blood samples from 8 merino sheep (7 ewes and 1 wether) of LK phenotype (Agar *et al* 1972) and 4 human volunteers were collected into heparinised tubes. The red cells were washed twice with 150 mmol/L of NaCl and suspended in the following 6 buffers:

Buffer	pH	Pi (mmol/L)
B1	7.4	1
B2	7.4	15
B3	7.4	30
B4	8.2	1
B5	8.2	15
B6	8.2	30

Each buffer contained 50 mmol/L Tris, 8 mmol/L glucose and was adjusted to pH using HCl and adjusted to 300 mmol/kg osmolality with NaCl.

Red cell suspensions with PCVs of about 30 % were incubated in a stirring water bath at 37°C. Aliquots were taken at 0, 1, 2 and 3 h and immediately precipitated with equal volumes of 8 % TCA for the analysis of glucose and lactate by the method of Godwin *et al* (1983).

Data for the sheep and human red blood cells are shown in Table 1. and Table 2., respectively.

Glucose consumption and lactate production correlated well in both species ($r^2 = 0.964, y = 0.412x + 0.140$ and $r^2 = 0.996$, $y = 0.700x - 0.624$; where y =glucose consumption and x =lactate production for sheep and human respectively).

In sheep the glucose consumption and lactate production were only stimulated by Pi level to 15 mmol/L. Further Pi increase to 30 mmol/L had no

significant effect on these parameters. An increase in pH resulted in an increase in glucose consumption and lactate production at every Pi level.

In the human RBCs an increase in pH resulted in increased RBC metabolism at every Pi level and increasing the Pi level increased glucose consumption and lactate production at the lower pH, but at the higher pH, Pi only increased RBC metabolism up to 15 mmol/L.

The values obtained for the sheep were substantially lower than those for man.

References

Agar,N.S., Evans,J.V. & Roberts,J. (1972) *Anim.Breed.Abstr.* **40**,407

Godwin,I.R., Agar,N.S. & Roberts,J. (1983) *Clin.Chem.* **29**,1855

Table 1. Effects of different buffers on glucose consumption and lactate production (μmol/hr/mLRBC) in sheep RBC.

Buffer	pH	Pi	glucose	lactate
B1	7.4	1	0.47±0.089a	0.92±0.144a
B2	7.4	15	0.64±0.090b	1.20±0.159b
B3	7.4	30	0.64±0.092b	1.31±0.168bc
B4	8.2	1	0.65±0.071b	1.40±0.144c
B5	8.2	15	0.80±0.050c	1.69±0.109d
B6	8.2	30	0.87±0.093c	1.85±0.214e

means with different subscripts are significantly different ($P<0.01$)

Table 2. Effects of different buffers on glucose consumption and lactate production (μmol/hr/mLRBC) in human RBC.

Buffer	pH	Pi	glucose	lactate
B1	7.4	1	0.48±0.215a	1.52±0.177a
B2	7.4	15	0.89±0.257b	2.18±0.382b
B3	7.4	30	1.63±0.008c	3.19±0.162c
B4	8.2	1	1.65±0.235c	3.36±0.342c
B5	8.2	15	3.04±0.275d	5.32±0.424d
B6	8.2	30	3.32±0.229d	5.52±0.742d

means with different subscripts are significantly different ($P<0.01$)

APPENDIX 10

Comparison of an ion-selective electrode and steam distillation as methods of ammonia analysis.

Samples of urine and Kjeldahl digests of faeces were compared for ammonia content using a standard distillation procedure with subsequent titration and an ammonia gas-sensing electrode (Orion 95-10, Orion Research Inc., Mass. U.S.A.).

Methods

Distillation method - 1 mL of Kjeldahl digest or urine was distilled with 5 mL of 10 % NaOH. The distillate was titrated against 0.01N acid to a constant colour change using phenolpthalein/phenol red indicator. Blank values were determined from distillation of a 1 mL sample containing no ammonia.

Electrode method - The electrode was connected to a millivolt meter and immersed in a solution containing approximately 10^{-4}M ammonium chloride and 10M NaOH, continuously stirred at a constant rate using a magnetic stirrer. 10M NaOH was used both for pH adjustment and as a total ionic strength adjusting buffer (TISAB). 2 mL samples were placed in 20 mL plastic vials and just prior to analysis 8 mL of 10 % NaOH was added to the vial. The electrode and a magnetic spin bar were then placed in the vial and the potential recorded. 1.1 mL of 0.01M ammonium chloride was then added and the potential again recorded.

The concentration of the sample was determined using the following formula:-

$$C_x = Cst \frac{Vst}{Vx}\left[\frac{1}{1 - 10^{\Delta E/S}\left(\frac{Vst}{Vx} + 1\right)}\right]$$

Where:

C_{st} = concentration of the standard (0.01 M)

V_{st} = volume of standard (1.1 mL)

V_x = volume of sample + TISAB (10 mL)

ΔE = change in electrical potential

S = electrode slope determined from daily calibration (usually 57 mV).

Results

A plot of the values obtained for the two methods is shown in Fig.1.

A typical electrode calibration curve is shown in Fig.2.

Discussion

The two methods of analysis correlated well and the two readings can be considered identical.

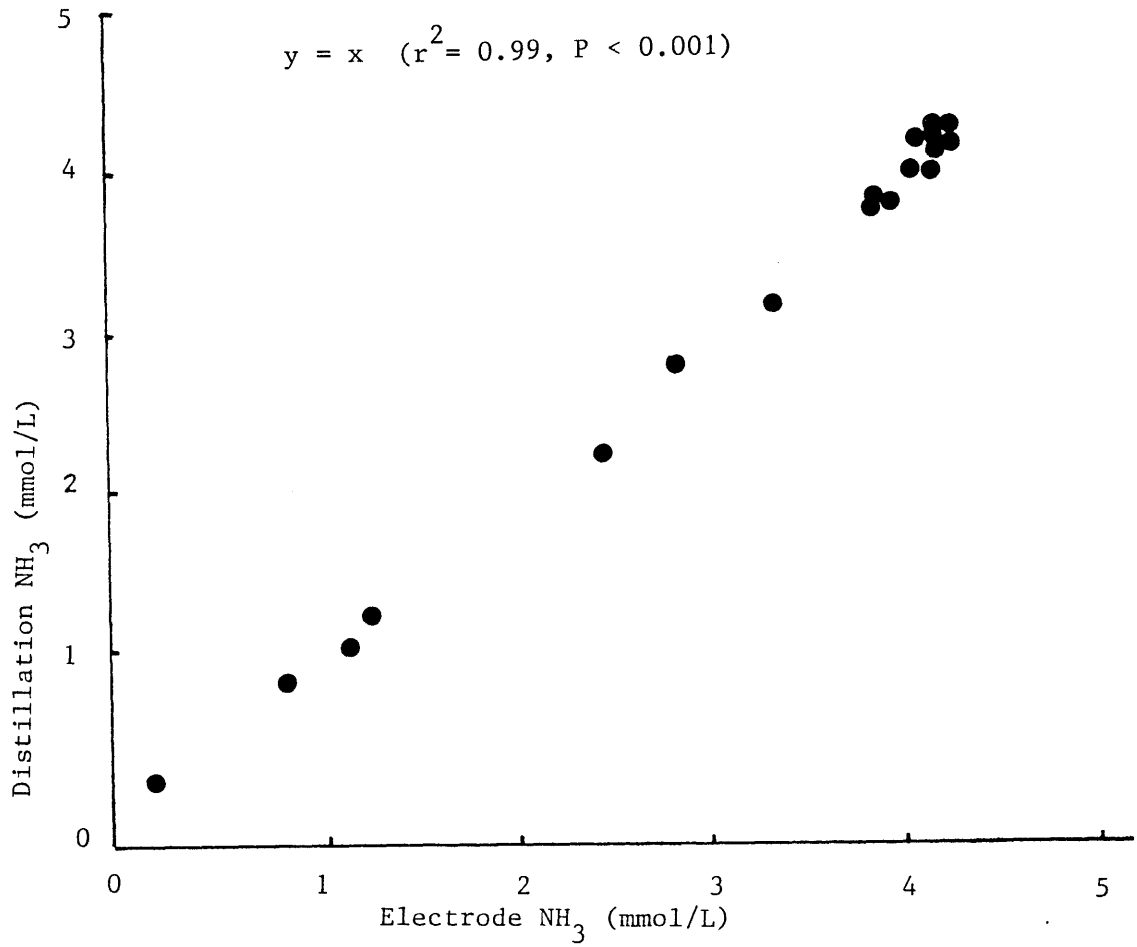

Fig. 1. Comparison of electrode and distillation methods of NH_3 analysis.

Fig 2. Calibration curve for ammonia electrode