RENAL FUNCTION IN THE GRAZING SHEEP

1. Three experiments were conducted to examine the kidney function of sheep freely grazing high quality pastures. Urine, blood and rumen fluid were sampled every 2 h for periods of 24-48 hours, from sheep grazing lush green pastures.

2. The pattern of excretion of urea, electrolytes and water was recorded, together with the pattern of rumen ammonia concentration. In the latter two experiments GFR was determined by examining $Cr^{51}EDTA$ clearance from a constant intravenous infusion and from the clearance of endogenous creatinine.

3. In experiment 3 the animals were deprived of water for the first 20 h and then allowed free access to water. Water was available ad libitum in experiments 1 and 2.

4. Rumen ammonia level showed considerable variation with a distinct pattern emerging, which appeared to be related to food intake patterns.

5. There was a marked variation and cyclic phenomenon in the UFR and the excretion rate of most of the urinary constituents. Peaks in UFR occurred at approximately 1400 and 0200 h, whilst troughs in UFR occurred at approximately 1000 h and 2400 h. Many of the urine samples were hypotonic to plasma.

6. The composition of plasma remained relatively constant, despite large fluctuations in the excretion of urinary components. Urea excretion followed changes in UFR, as expected; the extent to which the excretion rate of electrolytes was caused by changes in the UFR is unknown. GFR was positively related to UFR and ERPF suggesting that a high water load to be excreted leads to an increased renal blood flow and GFR.

7. $Cr^{51}EDTA$ clearance did not correlate well with creatinine clearance in experiment 3, but was highly related to creatinine clearance in experiment 2. The possible reasons for this discrepancy are discussed.

The study of nutrition and physiology of the grazing sheep has obvious practical application yet relatively little work has been done on the grazing animal

relative to that performed on animals housed in pens or metabolism crates. McDonald (1968) outlined 13 major differences between hand-fed and grazing ruminants and much subsequent work has also shown substantial differences, for example, Corbett and Farrell (1970) noted that the maintenance energy requirements of grazing sheep were approximately 60 % greater than those for housed sheep.

During periods of growth pastures may contain in excess of 30 % crude protein on a dry matter basis (Johns 1955) and intakes of greater than 50 g of digestible N/d are common (Hogan 1965). Dietary electrolyte intakes, particularly of potassium of animals grazing green pastures are also likely to be well above requirements. On many pastures in temperate areas sheep can be deprived of drinking water indefinitely, without adverse effects on production (Brown and Lynch 1972). There are substantial changes in digesta flow and consequently in N entering the duodenum within any 24 hour period in freely grazing sheep (Corbett and Pickering 1983).

In view of these rhythmic flows of digesta and nutrients and the lack of need for water, this study was designed to investigate the haemodynamic aspects of kidney function and the excretion of nitrogen and electrolytes throughout the day, of sheep freely grazing dense stands of green pasture that provided abundant feed.

Methods

Three experiments were performed at Armidale N.S.W. (latitude 30° 36'S). Each utilized four rumen fistulated merino X border leicester ewes (50-56 kg BW experiment 1 and 2, 41-44 kg BW experiment 3). Experiments 1 and 3 also had an oesophageal fistulated sheep running with the 4 experimental animals.

The animals were allowed to graze each sward for at least 10 d before commencement of any of the experimental procedures. After this preliminary period 1 g of chromium sesquioxide (Cr_2O_3) was given *per os* to each animal at 12 h intervals (06.00 and 18.00 h) for 10 d.

On the afternoon of the ninth day the bladder of each animal was catheterised

(Folatex Balloon Paediatric Catheter, 8Ch, Euro-medical Industries, Sussex, England.). In experiments 2 and 3 one jugular vein of each animal was also catheterised (1.5 mm OD, medical grade PVC, Dural Plastics, N.S.W., Australia.) and filled with heparinised saline. A blood collection pack (500 mL Dry Pack, Tuta-Labs, Australia.) was strapped under the abdomen of each animal and this was then connected to the bladder catheter allowing urine to drain directly into the pack.

In experiments 2 and 3 a portable battery operated infusion pump (Corbett *et al* 1976) was mounted onto the back of each animal (see Plate 1).

Experiment 1 was performed in mid-December on animals grazing a stand of semi-mature phalaris (*Phalaris aquatica*) pasture. Water was freely available. Sampling commenced at 06.00 h. Every 2 h for 26 h a sample of blood, rumen fluid and the total volume of urine was collected.

Experiment 2 was performed in late April on animals grazing a stand of young paspalum (*Paspalum dilatum*) / ryegrass (*Lolium perenne*) / white clover (*Trifolium repens*) pasture. Water was freely available. A solution containing 0.5 MBq/mL of Cr^{51} ethylene diamine tetra acetate (Cr^{51}EDTA) was infused intravenously via the infusion pump and jugular catheter, at a rate of approximately 0.1 mL/min. Sampling was as in experiment 1.

Experiment 3 was performed in late October on animals grazing a dense stand of phalaris (*Phalaris aquatica*) with some lucerne (*Medicago sativa*). Water was witheld from 09.00 h on day 1 for 20h ie., until 05.00 h on day 2 when it was made freely available. Sampling of blood and rumen contents, and urine collections, started at 10.00 h on day 1 and the final sampling was at 06.00 h on day 3. Samples were mainly obtained at 2h intervals, but they were not taken at 01.00 or 03.00 h on day 3. The sheep were infused as in experiment 2 but with a solution containing 0.23 MBq/mL of I^{131} iodohippurate sodium and 0.63 MBq/mL of Cr^{51}EDTA at a rate of approximately 0.08 mL/min.

During this experiment behavioural observations were made every 20 min, to determine the times of feeding, resting and rumination.

Plate 1.

A crossbred ewe fitted with a portable infusion pump.

a = jugular catheter

b = infusion pump

c = infusate

d = urine collection bag

e = urine catheter

f = rumen fistula

Faecal samples were taken at periodic intervals throughout each experiment by gently inserting a finger into the rectum of the animals and removing several faecal pellets..

In vitro digestibility of oesophageal fistula extrusa was determined by the method of Tilley and Terry (1963). The dry matter intake was calculated from the concentration of Cr_2O_3 in the faeces and the in vitro digestibility.

The amount of N, energy, Na, K, Ca, Mg and P eaten was calculated from the dry matter intake and the concentration of these substances in the oesophageal fistula samples, after correction for salivary contamination. Salivary contamination was determined by feeding cut herbage of known composition, similar to that on offer to the sheep in the experiments, to an oesophageal fistulated sheep and subsequent determination of the composition of the extruded boli.

It took 15-20 min to obtain the samples which were immediately placed in ice. Within 15 min all samples had been appropriately processed and placed in storage at -20°C. The pH of urine was measured immediately. Rumen fluid samples were filtered by suction and the filtrate stored. Blood samples were divided into three, a small quantity for haematocrit determination, whole blood retained and the remainder was centrifuged for 10 min at 1500g to obtain plasma.

Analytical

Plasma and urine Mg, Pi, Ca, glucose, urea, uric acid plus allantoin (URAL), creatinine and plasma protein were measured with a Cobas-Bio centrifugal analyser (Hoffman La-Roche, Switzerland). Pierce kits (Pierce Chemical Co., Illinois, USA.) were used for Mg and Pi and Hoffman La-Roche kits for the remainder. In experiment 1 Mg and Ca were determined by atomic absorption spectrophotometry (GBC 900, GBC Scientific Equipment, Melbourne, Australia), using 1 % $LaCl_2$ as a diluent, Pi by the method of Lucenda-Conde and Prat (1957) and urea by the method of Momose *et al* (1965).

Plasma, urine and whole blood Na and K were determined by flame photometry (EEL 227, Evans Electroselenium, Essex, England.) and plasma and urine

Cl titrametrically (Radiometer CMT10, Copenhagen, Denmark.).

Whole blood Mg was measured by atomic absorption spectrophotometry. Whole blood adenosine triphosphate (ATP), 2,3-diphosphoglycerate (DPG) and glucose were determined according to Godwin *et al* (1983) and whole blood Ca, Pi and urea were determined on a supernatant prepared by adding equal volumes of whole blood to 8 % trichloroacetic acid, then analysing as for plasma. The red blood cell values for all these substances were calculated from the packed cell volume (PCV) and the plasma level.

Faeces and oesophageal extrusa were digested according to Cresser and Parsons (1979). Total N was determined by steam distillation and titration, and Na, K, and Mg by atomic absorption spectrophotometry. Ca and P were determined as for plasma.

The Cr content of faeces was determined by atomic absorption spectrophotometry following the digestion procedure of Christian and Coup (1954).

Ammonia concentration was measured in rumen fluid after protein precipitation with ethanol, by distillation and titration. No correction was made for urinary ammonia when urea and URAL were measured using the enzymatic methods on the Cobas-Bio. However as urine pH was always alkaline, urine ammonia levels were assumed to be very low.

Osmolality of rumen fluid, urine and plasma were determined either by freezing point depression (Fiske Assoc., Connecticut, USA) or with a vapour pressure osmometer (Wescor 5100B,UT.USA.).

PCV was estimated by a micromethod (Hawksley and Sons, Sussex, England.).

Cr^{51} and I^{131} were counted on an automated gamma emission spectrometer (Gammamatic, Kontron, Sydney, Australia.).

There was negligible interference between the two isotopes. Each was counted to 10,000 counts, with urine samples being diluted to reduce coincident counting.

Organic matter of faeces and oesophageal fistula samples was obtained by ashing for 2 h at 400°C followed by 12 h at 600°C. The energy content of faeces

and oesophageal extrusa was estimated by bomb calorimetry (Gallenkamp Ser 330, Sussex, England.).

Urine pH was measured with a glass electrode (Radiometer, Copenhagen, Denmark.).

Total and constituent volatile fatty acid (VFA) levels of rumen fluid were measured by gas chromatography (Packard Model 427, Packard Instruments Pty. Ltd., Darlinghurst, Australia.).

Glomerular filtration rate (GFR) was determined from the clearances of endogenous creatinine and Cr^{51}EDTA, both of which have been validated for this purpose in sheep (Stacy and Thorburn 1966, Bishara and Bray 1978, Valtonen *et al* 1982, Appendix 1). Effective renal plasma flow (ERPF) was calculated from the renal clearance of I^{131} iodohippurate sodium using Rabinowitz *et al* (1971) correction for plasma protein binding of para-aminohippurate.

Statistical

Regression analysis with repeated measures was employed to produce the equations presented in Table 4 (Davidson and Toporek 1981). To obtain sufficient data for statistical analysis for cyclicity would have required collection of samples for at least 4d, a procedure that would have produced profound effects on animal behaviour, hence data are presented on the basis of visual appraisal for patterns occurring in each experiment. Values presented are means ± standard error of the mean.

Results

Data presented on figures are mean values for the four animals at each sampling. The standard errors of these values have been omitted to enhance clarity. The range of standard errors for each parameter are listed on the figures, together with a mean daily value for each experiment .

The intake, digestibility and composition of the food in each experiment are indicated in Table 1.

Rumen NH_3 - Rumen NH_3 values were maximal at approximately 09.00 h

and decreased throughout the day with a second peak occurring at approximately 21.00 h (see Fig. 1). The variation in rumen ammonia levels in experiment 3 during the period of water deprivation were somewhat smaller but after access to water was allowed the patterns noted in experiments 1 and 2 reappeared.

Rumen total VFA - Rumen total VFA (measured only in experiment 3) followed very similar patterns to that of ammonia (Fig. 2). Constituent VFA levels were proportionately constant. The approximate proportion of the total VFA were as follows: acetate 61 %,propionate 25 %, butyrate 11 %, isobutyrate 1%, isovalerate 1%, valerate 1%.

Rumen osmolality - Rumen osmolality was high when drinking water was restricted, but was otherwise uniform. The values were generally higher than those for plasma osmolality. (Fig. 3)

Blood values and PCV - During the 24 h with the animals provided with or without drinking water the plasma and RBC concentration of all the constituents measured and PCV remained remarkably constant with no consistent patterns being noted. Values for these parameters are presented as daily means with standard errors in Table 2. However, when water was allowed in the second 24 h of experiment 3 there was a substantial decrease in the P_{urea} and P_{Mg} levels and a concurrent increase in RBC_{urea}, RBC_{Mg} and RBC_K concentration following rehydration (see Fig. 4). The mean values of these parameters during the restricted and ad libitum water periods were all significantly different except for RBC_{Mg} and RBC_K.

Urine Flow Rate - The UFR showed a distinct cyclic phenomena, which was interrupted during water deprivation (experiment 3), but returned upon rehydration (Fig. 5). Peaks in UFR occurred at approximately 1400 and 0200 h, whilst UFR was lowest at 1000 and 2400 h.

Glomerular Filtration Rate and Effective Renal Plasma Flow - The GFR values measured by Cr^{51}EDTA clearance in experiment 2 followed closely the pattern of UFR (Table 4) and showed wide variation. In experiment 3 (restricted water period) the Cr^{51}EDTA clearance values were unrelated to any parameter

including UFR (Table 4) and creatinine clearance. The appropriateness of this marker as a measure of GFR in this experiment is discussed later. Creatinine clearance values in experiment 3 however did follow a similar pattern to that found for UFR (Table 4). ERPF data were significantly correlated with creatinine clearance data during the whole of experiment 3 ($r = 0.63$, $P < 0.05$), but not with $Cr^{51}EDTA$ clearances during the restricted water period ($r = -0.15$,NS). They were however correlated with UFR during the ad libitum water period ($r = 0.54$, $P > 0.05$). Creatinine clearance was used for the calculation of all fractional excretion values.

Urine Excretion Data (Tables 3 & 4) - All values are expressed as daily means (restricted and ad libitum water period means in experiment 3) with standard errors. All urine constituents followed a similar pattern to that of UFR except for Mg, Pi and URAL. U_{Mg} remained relatively constant during the three experiments, whereas U_{Pi} and U_{URAL} were both very low and below detection limits in many animals. Data for these two constituents are therefore not presented. Table 4 shows the linear regression equations for each constituent and UFR. Urine osmolality was significantly negatively related to the UFR. The urinary excretion of urea, K, Cl, osmoles and the UFR, GFR and ERPF were all significantly greater during the ad libitum water period of experiment 3 ($P > 0.05$).

Fractional Excretion Data (Table 5) - The fractional excretion of urea and electrolytes remained relatively constant. All values were less than unity.

Animal Behaviour (Fig. 6) - Analysis of the behavioural data in experiment 3 indicates that the major grazing periods were 07.00-10.15 h and 14.30-17.00 h. Rumination appeared to occur mostly at night between 18.00 and 01.00 h.

Discussion

Penning the sheep every 2h was a disturbing influence. Two persons but no dog, were involved and the animals were herded into a pen without much difficulty in 3 minutes. After sampling was completed on a sheep, chosen at random, it was released immediately. The sheep quickly resumed their pre-sampling activities.

Total food intake and rumination times were possibly affected. The length of time between samplings was specifically chosen to obtain renal data at short enough intervals so as not to miss any major changes and at the same time to limit as far as possible the effect of sampling on the measured renal parameters.

The pastures on offer to the animals in this study were particularly different to the foods fed in the preceding chapters. The bulk of the feed constituents was water. The dry matter fraction had a very high N content, with a large proportion of this N, because it had not been denatured through drying or senescent processes, in a soluble form and hence readily available to the rumen microbiota (Bryant 1964).

Earlier studies in this laboratory (McIntyre 1970) showed that as N intake increased the excretion of urea increased linearly, and the plasma urea level plateaued at about 11 mmol/L. A subsequent study (Chapter 1.) revealed that the renal mechanisms responsible for this constancy of plasma urea level during high N intakes were the diuretic effects of both urea and electrolytes. Preliminary data (Appendix 4, M. Ullyat personal comm.) indicated that P_{urea} level of sheep grazing high quality pastures rarely exceeds 11 mmol/L. Brown and Lynch (1972) also noted that sheep grazing pastures at Armidale, N.S.W., with and without drinking water for up to 22 months, showed no differences in their P_{urea} level.

Even with digestible N intakes as high as those in the present study, the plasma urea level still remained at or below 11 mmol/L. By far the major determinant of the excretion of urea in the present experiments was the UFR (U_{urea} = 27.3UFR + 350, $r^2 = 0.82$, $P < 0.05$), although multiple regression of UFR, P_{urea} and GFR from data obtained in experiments 2 and 3, yielded the equation:

$$U_{urea} = 35.2UFR + 21.6P_{urea} + 0.35GFR + 106.6$$

which accounted for 89 % of the variance of U_{urea} ($r^2 = 0.89$, $P < 0.01$).

If drinking water is freely available to animals fed dry roughages, the major determinant of UFR variation is the intake of food (see appendix 5). The osmolality of many urine samples in the present study, particularly in experiment 2, were below 300 mmol/kg indicating that the animals were in a state of water diuresis.

Even the animals in experiment 3, when water was restricted, only produced a urine with a maximum osmolality of 1535 mmol/kg, well below that obtained for severely dehydrated sheep (Macfarlane *et al* 1956). Grab samples of the pasture on offer, revealed a moisture content of approximately 85 %, which indicated that the involuntary intake of water in food was of the order of 5-6 L/day. A heavy dew was also noted during the collections at night, which would further add to the water consumption if grazing occurred during these periods.

It was shown in chapter 1 that the plasma urea level can be controlled by electrolyte or urea stimulation of water intake and the consequent osmotic diuresis.

The maintenance of plasma urea at a concentration below 11 mmol/L, the plateau achieved by McIntyre (1970) and in chapter 1., in the animals grazing these high quality pastures appears to be achieved by a water diuresis inherent from the high intake of dietary water. A sheep fed a low protein roughage and infused intraruminally with water showed a drop in P_{urea} associated with a rise in U_{urea} (See Appendix 4). In this animal there was no change in GFR despite a 10 fold increase in UFR. The present data showed that the GFR (creatinine clearance) was closely related to the UFR (Table 4).

U_{urea}, U_K, U_{Na}, U_{Cl} and U_{Ca} all varied markedly, yet the fractional excretion of these substances remained virtually constant, despite large variations in GFR. The ERPF was linearly related to the GFR, producing a relatively constant filtration fraction. The renal mechanisms allowing these wide changes in excretion of urea and electrolytes appears simply to be the result of increased filtered loads of these substances, because the plasma levels of all constituents remained relatively constant.

Cr^{51}EDTA has been used as a GFR marker by many workers since its introduction by Stacy and Thorburn in 1966. It appears to be very similar to creatinine and inulin as a glomerular marker in sheep (see appendix 1). Non-isotopic CrEDTA has also been used widely as a non absorbable marker in digestion studies in sheep. However Dobson *et al* (1976) showed that when rumen

osmotic pressure was increased above about 320 mmol/kg, the absorption of this marker was considerable, due to a higher permeability of the rumen epithelium. If the marker is present in the blood in a higher concentration than in the rumen fluid, as in the present study, one would expect considerable leakage of the marker from blood to rumen (the direction of the concentration gradient). Many of the rumen fluid samples in the present study had an osmolality greater than 320 mmol/kg. Corbett (1981) noted that as much as 10 % of Cr^{51}EDTA infused into the rumen can be recovered in the urine. The lack of significant correlation of Cr^{51}EDTA clearance with creatinine clearance during the restricted water period in experiment 3 is possibly explained by the leakage of the marker from the blood into the gastrointestinal tract. The amount of creatinine that may leak across the gastrointestinal tract under hypertonic conditions is unknown. In Chapter 2, when animals were infused intraruminally with NaCl osmolality of the rumen contents increased up to a mean of 312mmol/kg with individual values reaching as high as 328mmol/kg. Under these conditions inulin and creatinine clearance still correlated well. This fact coupled with the correlation of creatinine clearance with ERPF suggests that the creatinine clearance values are valid.

Corbett and Pickering (1983) measured the flow rate of digesta to the duodenum of grazing sheep. The present study found a pattern of rumen ammonia concentration similar to the filtrate fraction of digesta flow noted by Corbett and Pickering, with a short delay period of about 1 h.

Digesta flows are greatest about 3 hours after rumination (Harris and Phillipson (1962). Rumination with its concurrent loss of plasma liquid would be expected to reduce UFR and decrease urine pH (Scott 1975). The major period of rumination in experiment 3 was between 18.00 and 01.00 h. During this period in experiments 1 and 2 UFR was substantially decreased, whilst in experiment 3 this time period was associated with an increased UFR when water access was allowed and a relatively unchanged UFR when water was denied. However as the water content of the pasture was relatively high (ca. 85 %) the salivary flow rate was likely to have been low.

Interestingly there was no relationship between rumen NH_3 and P_{urea} as has been shown in numerous previous experiments (Weston 1973). This lack of relationship may be partly because of reduced salivary urea input into the rumen (because of reduced salivary flow rate on this type of diet) and the added control of plasma urea by the water diuresis.

The sheep tended to feed shortly after sunrise. The rapid fermentation of this kind of herbage results in a peak of rumen NH_3 and VFA shortly after the feeding period (Christian and Williams 1966). These peaks also occurred in the present experiment. The inherent high intake of dietary water associated with the intake of forage, increased the flow rate of urine and consequently the excretion of urea. However, no significant relationship between rumen NH_3 and U_{urea} was obtained.

The shift of urea into cells following rehydration in experiment 3 (Fig. 4) is an interesting phenomena. Havassy *et al* (1973) noted that the distribution of urea between intracellular and extracellular fluids appears to be dependent on the metabolic and hydration status of the animal. They speculated that the mechanism was an evolutionary adaptation to conserve urea during times of excess.

The concurrent changes in cellular and plasma levels of Mg, K and urea suggest that a change in cell membrane permeability was likely to have occurred. Plasma protein and PCV were virtually unaltered, indicating that a fluid shift was not responsible for the phenomenon. Urea is transported into red blood cells by a facilitated diffusion process (Brahm 1983), whilst K is thought to enter the cell via active transport processes involving Na-K-ATPase.

ADH is known to influence the permeability of various tissues to urea and the activity of Na-K-ATPase. Following rehydration the circulating ADH level would almost certainly have been lowered, but the changes induced by this hormone in other tissues, would suggest the occurrence of changes in RBC_{urea} and RBC_K levels opposite to those observed. The phenomenon thus remains unexplained.

The redistribution of Mg between intracellular and extracellular compart-

ments has been noted before during changes in metabolic status (Martens and Rayssiguier 1980).

It could be thought that fresh herbage containing about 85 % water, would cause an increased rumen turnover rate because of the high intake of water associated with the dry matter fraction. However, infusion of water alone does not produce this effect (Harrison *et al* 1975). It is apparently an osmotic pressure phenomenon. The pastures contained large amounts of electrolytes, especially K, and it has been shown that a direct linear relationship exists between the duodenal flow of non-ammonia N and the intake of K (Macrae 1976).

The possibility of increasing pasture K content by fertiliser application, as a means to increase rumen turnover rate, and hence the flow of bypass protein to the duodenum, remains. However an increase in dietary K is likely to increase the excretion of urea in the urine, through its diuretic effects (chapter 1).

It must be concluded that the renal function of the grazing animal under conditions of lush green pasture are likely to be very different to those obtained for animals housed and fed dry roughages. The major determinant of the excretion of urea and electrolytes appears to be the UFR which is very high due to the inherent high intake and absorption of dietary water.

Table 1. The dietary intakes per day and apparent digestibility of dietary components in experiments 1, 2 and 3 (apparent digestibility coefficients for each component are listed in parentheses).

Experiment / Constituent	1		2		3	
DM (g)	820	(0.68)	897*	(0.75)	902	(0.72)
OM (g)	735	(0.72)	725†	(0.75)	740	(0.71)
Na (mmol)	92	(0.84)	86*	(0.88)	93	(0.86)
K (mmol)	321	(0.81)	370*	(0.91)	406	(0.81)
Ca (mmol)	120	(0.18)	183*	(0.21)	146	(0.18)
Mg (mmol)	101	(0.21)	164*	(0.18)	144	(0.19)
P (mmol)	74	(0.18)	65*	(0.21)	78	(0.17)
N (g)	29	(0.71)	36*	(0.75)	33	(0.72)
Gross Energy (MJ)	17.2	(0.62)	17.6*	(0.71)	18.8	(0.69)

† - The OM intake for experiment 2 was calculated using Lambourne and Reardon (1963) regression equation which relates faecal N and Cr_2O_3 concentration with OM intake. The values derived for experiments 1 and 3 using this equation were 728 and 738 g respectively which compare favourably with the observed values.

* - These values were obtained from analysis of grab samples of herbage on offer and the calculated OM intake value.

Table 2. Blood constituents levels and PCV of freely grazing sheep (daily means ± SE)

Constituent	Experiment 1	Experiment 2	Experiment 3 Water Restricted	Experiment 3 Water Ad Libitum
P_{urea} (mmol/L)	7.1 ± 1.2	9.5 ± 1.8	6.6 ± 0.4	5.6 ± 0.2*
P_{URAL} (mmol/L)	-	-	0.11 ± 0.03	0.10 ± 0.03
$P_{creatinine}$ (mmol/L)	-	1.6 ± 0.21	1.6 ± 0.20	1.6 ± 0.19
P_{Na}	144 ± 1.2	147 ± 2.8	146 ± 3.2	147 ± 4.2
P_{K} (mmol/L)	4.1 ± 0.18	4.4 ± 0.21	4.3 ± 0.19	4.4 ± 0.24
P_{Cl} (mmol/L)	110 ± 2.1	111 ± 2.4	110 ± 4.3	110 ± 4.0
P_{Ca} (mmol/L)	2.35 ± 0.09	2.38 ± 0.12	2.38 ± 0.18	2.35 ± 0.24
P_{Mg} (mmol/L)	1.52 ± 0.08	1.63 ± 0.14	1.31 ± 0.35	1.10 ± 0.15*
P_{Pi} (mmol/L)	1.60 ± 0.18	1.81 ± 0.15	1.60 ± 0.22	1.69 ± 0.25
$P_{osmolality}$ (mmol/L)	-	306 ± 4.8	305 ± 4.3	305 ± 5.2
$P_{glucose}$ (mmol/L)	-	3.5 ± 0.3	3.5 ± 0.4	3.6 ± 0.4
$P_{protein}$ (g/dL)	-	6.2 ± 0.41	6.5 ± 0.28	6.5 ± 0.32
PCV (%)	33.2 ± 1.2	34.2 ± 0.9	33.4 ± 1.8	33.0 ± 1.9
RBC_{DPG} (µmol/L)	-	-	11.4 ± 1.8	9.8 ± 2.6
RBC_{ATP} (µmol/L)	-	-	182 ± 14.6	153 ± 15.2
$RBC_{glucose}$ (mmol/L)	-	-	0.22 ± 0.04	0.22 ± 0.06
RBC_{K} (mmol/L)	-	-	22.3 ± 1.4	23.4 ± 1.6
RBC_{Pi} (mmol/L)	-	-	1.54 ± 0.18	1.39 ± 0.21
RBC_{Mg} (mmol/L)	-	-	1.59 ± 0.52	1.64 ± 0.62
RBC_{urea} (mmol/L)	-	-	3.3 ± 0.6	4.3 ± 0.4*

*Significantly different from water restricted period experiment 3 ($P < 0.05$)

Table 3. Excretion of urinary components during experiments 1, 2 and 3.

Component	Experiment 1	Experiment 2	Experiment 3 Water Restricted	Experiment 3 Water Ad Libitum
Urea (μmol/min)	348 ± 25	387 ± 22	325 ± 18	360 ± 45*
URAL (μmol/min)	-	-	8.2 ± 1.4	8.3 ± 1.5
Na (μmol/min)	122 ± 45	74 ± 31	84 ± 17	99 ± 25
K (μmol/min)	163 ± 28	181 ± 45	148 ± 40	175 ± 44*
Cl (μmol/min)	158 ± 35	172 ± 37	156 ± 30	170 ± 38*
Ca (μmol/min)	1.8 ± 0.6	1.6 ± 0.5	1.2 ± 0.5	1.5 ± 0.6
Mg (μmol/min)	8.9 ± 0.3	10.4 ± 0.3	6.4 ± 0.6	6.3 ± 0.5
Pi (μmol/min)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.02
Osmolality (mmol/kg)	421 ± 156	365 ± 102	711 ± 104	524 ± 124*
Osmoles (μmol/min)	926 ± 189	840 ± 165	640 ± 104	838 ± 192*
pH	8.1 ± 0.1	8.1 ± 0.1	8.1 ± 0.3	8.0 ± 0.1
UFR (mL/min)	2.2 ± 1.9	2.3 ± 1.7	0.9 ± 0.4	1.6 ± 1.1*
GFR ($C_{creatinine}$) (mL/min)	-	64.2 ± 16.2	60.7 ± 9.2	72.1 ± 16.8*
GFR ($C_{Cr51EDTA}$) (mL/min)	-	62.5 ± 17.2	65.1 ± 8.4	75 ± 24.1*
ERPF (mL/min)	-	=	476 ± 25	492 ± 32*

*Significantly different from water restricted period experiment 3 ($P < 0.05$)

Table 4. Linear regression equations and statistical significance of the relationship between the excretion of urinary components and UFR (y = urinary component, x = UFR (ml/min)).

Component	Experiment	Equation	r^2	Significance
Urea (µmol/min)	1	y = 26.2x + 290	0.86	P < 0.01
	2	y = 28.4x + 322	0.74	P < 0.01
	3	y = 26.8x + 320	0.64	P < 0.05
URAL (µmol/min)	3	-	0.12	NS
Na (µmol/min)	1	y = 42.5x + 28.5	0.78	P < 0.01
	2	y = 46.2x - 32.3	0.72	P < 0.01
	3	y = 29.2x - 59.0	0.64	P < 0.05
K (µmol/min)	1	y = 14.6x + 130.9	0.66	P < 0.05
	2	y = 12.4x + 152.5	0.64	P < 0.05
	3	y = 6.2x + 154.6	0.61	P < 0.05
Cl (µmol/min)	1	y = 43.4x + 62.5	0.81	P < 0.01
	2	y = 38.4x + 83.7	0.74	P < 0.01
	3	y = 15.2x + 142.8	0.72	P < 0.01
Ca (µmol/min)	1	y = 1.8x - 2.16	0.65	P < 0.05
	2	y = 0.8x - 0.24	0.52	P < 0.05
	3	y = 0.4x + 0.92	0.50	P < 0.05
Mg (µmol/min)	1	-	0.11	NS
	2	-	-0.24	NS
	3	-	0.14	NS
Pi (µmol/min)	1	-	-0.12	NS
	2	-	-0.08	NS
	3	-	0.21	NS
Osmoles (µmol/min)	1	y = 102.0x + 701.6	0.55	P < 0.05
	2	y = 99.7x + 610.7	0.68	P < 0.05
	3	y = 42.4x + 771.1	0.79	P < 0.01
Osmolality (mmol/kg)	1	y = -25.6x + 477.3	-0.85	P < 0.01
	2	y = -19.8x + 410.5	-0.88	P < 0.01
	3	y = -48.3x + 743.0	-0.64	P < 0.05
pH	1	-	-0.22	NS
	2	-	-0.14	NS
	3	-	-0.20	NS
GFR (ml/min)	2	y = 21.6x + 15.7	0.88	P < 0.01
creatinine	3	y = 12.4x + 40.5	0.82	P < 0.01
GFR (ml/min)	2	y = 19.6x + 19.1	0.75	P < 0.01
$Cr^{51}EDTA$	3	-	0.09	NS
ERPF (ml/min)	3	y = 15.0x + 452	0.48	P < 0.05

NS = not significant

Table 5. Fractional excretion of urinary components in experiment 2 and 3. (Creatinine clearance was used as the measure for GFR)

Component	Experiment 2	Experiment 3 Water Restricted	Experiment 3 Water Ad Libitum
Urea	0.76 ± 0.08	0.81 ± 0.08	0.89 ± 0.09
Na	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.003
K	0.64 ± 0.05	0.75 ± 0.04	0.63 ± 0.06
Cl	0.02 ± 0.002	0.03 ± 0.002	0.03 ± 0.004
Ca	0.01 ± 0.000	0.01 ± 0.001	0.01 ± 0.001
Mg	0.10 ± 0.008	0.1 ± 0.004	0.08 ± 0.005
Pi	0.00 ± 0.000	0.0 ± 0.000	0.00 ± 0.000
FF*	-	0.12 ± 0.005	0.10 ± 0.007

*FF = Filtration Fraction

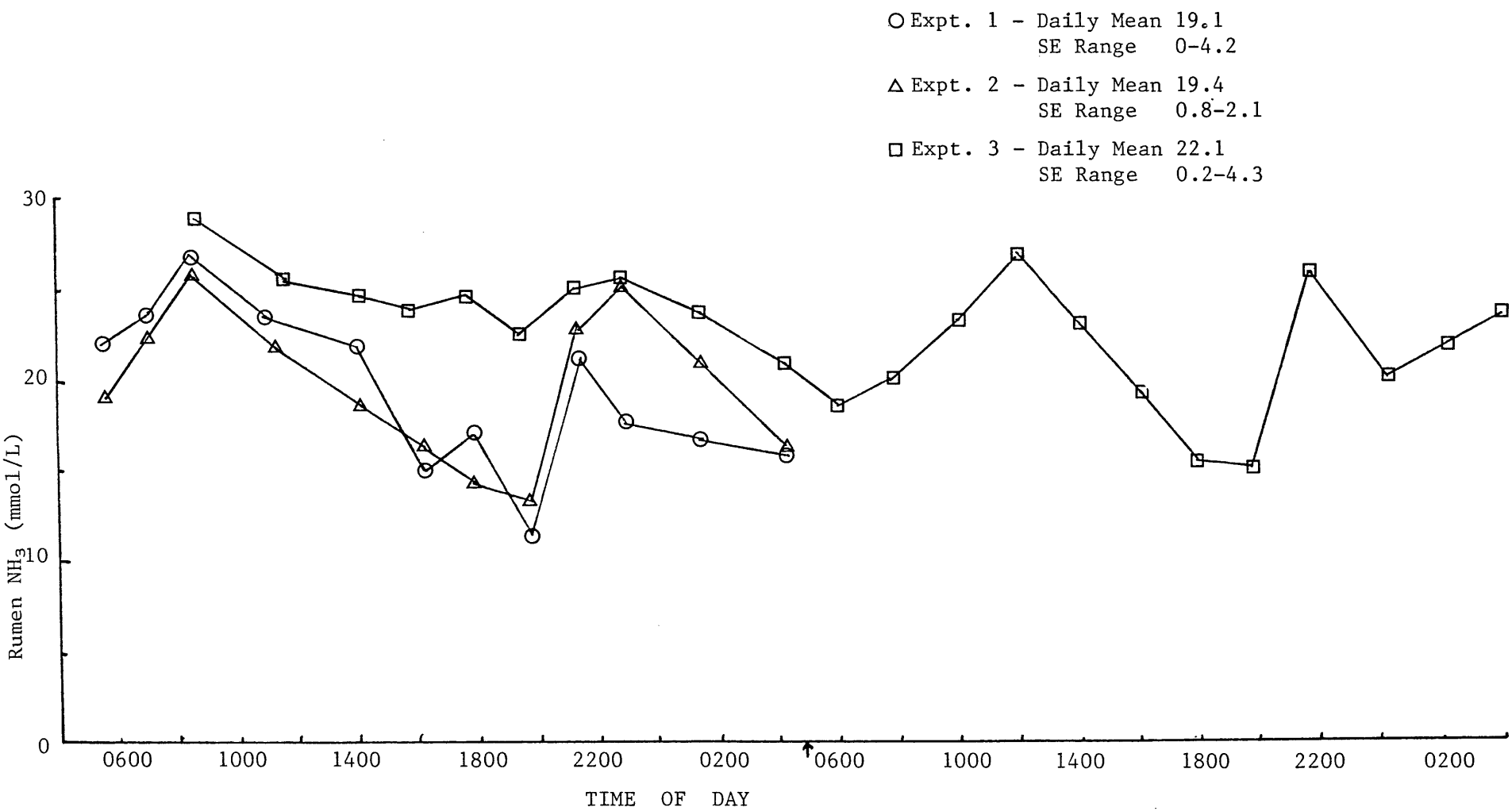

Fig. 1 - Variation in rumen ammonia level during Experiments 1-3
(arrow indicates time of access to water)

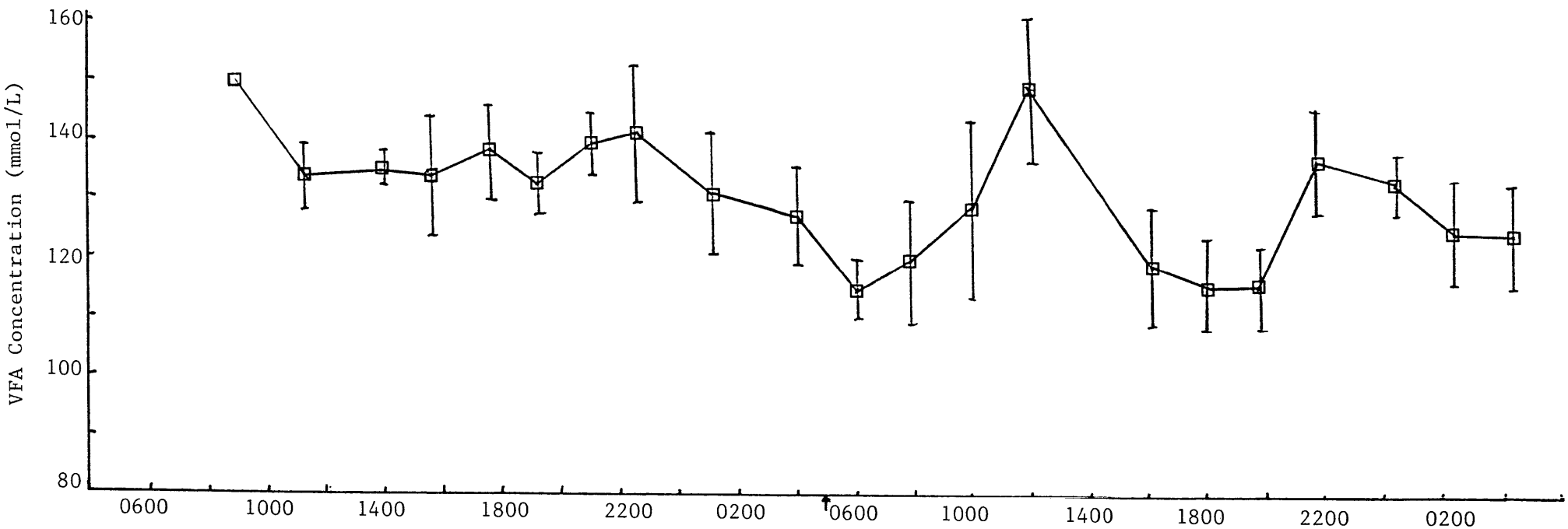

Fig. 2 - Variation in rumen total VFA during Experiment 3
(arrow indicates time of access to water)

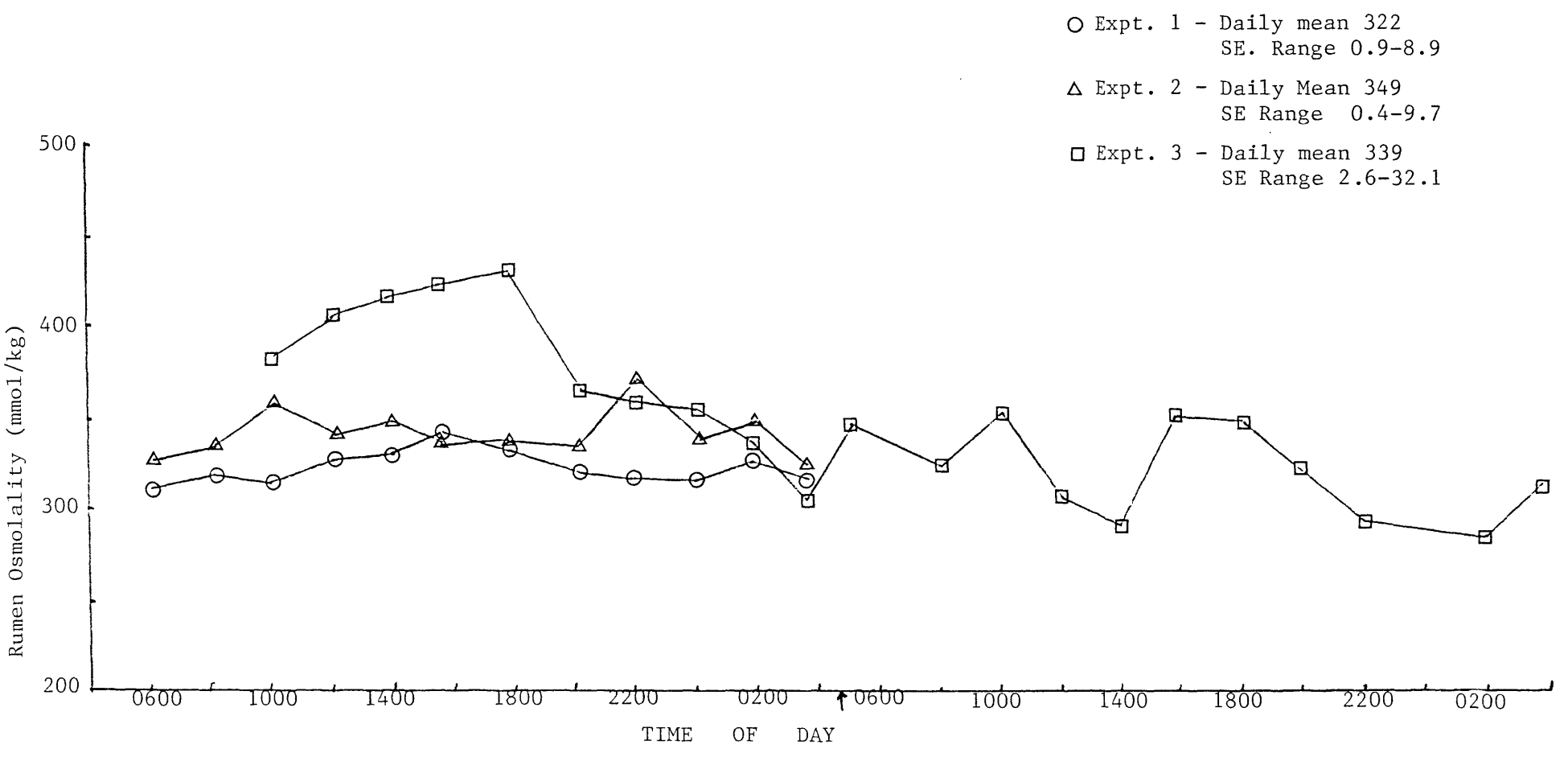

Fig. 3 - Variation in rumen osmolality during experiments 1, 2 and 3
(arrow indicates time of access to water)

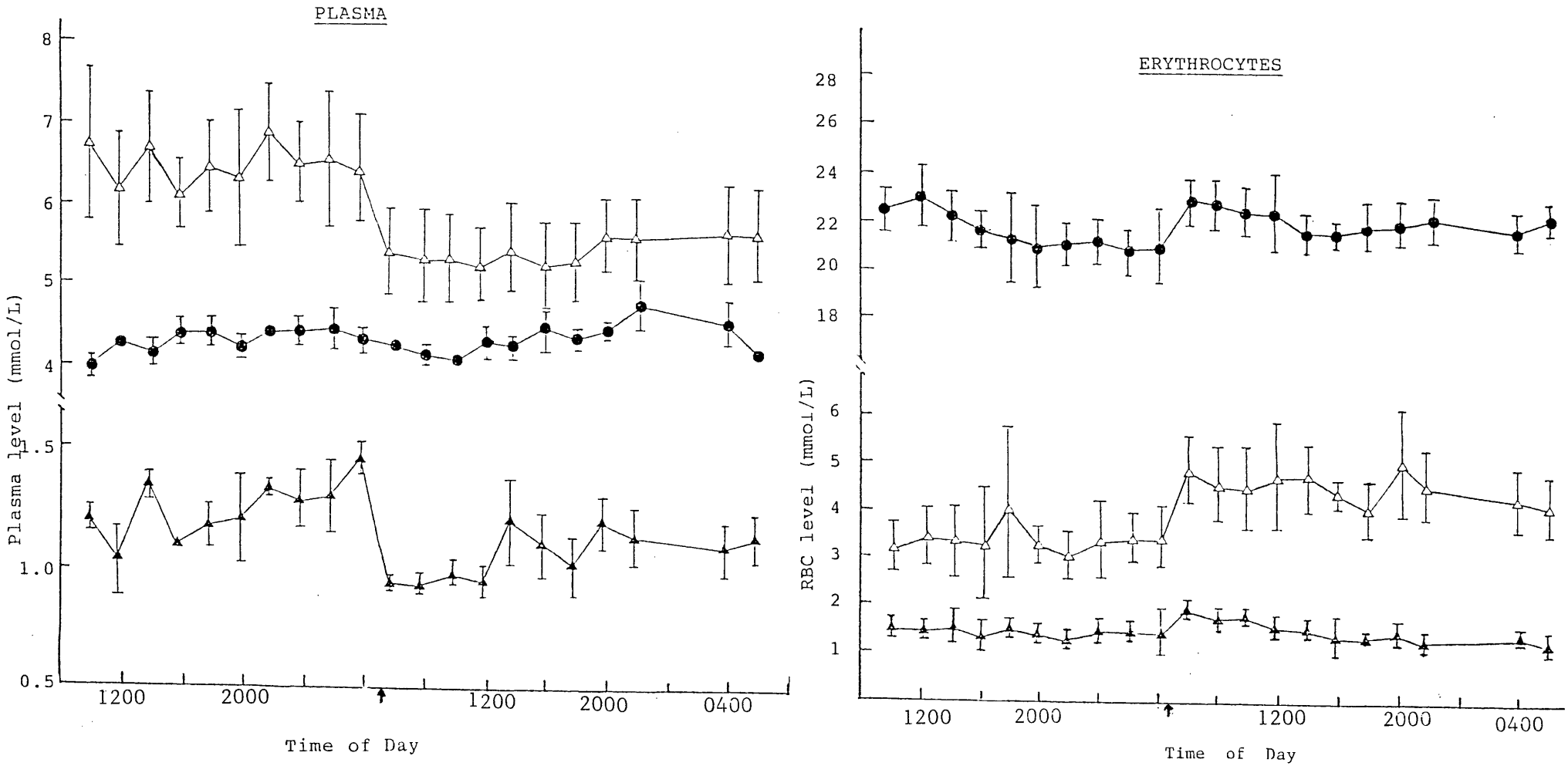

Fig. 4 - The effect of rehydration on plasma and RBC urea, Mg and K levels (△urea, ▲Mg, ●K, arrow indicates time of access to water)

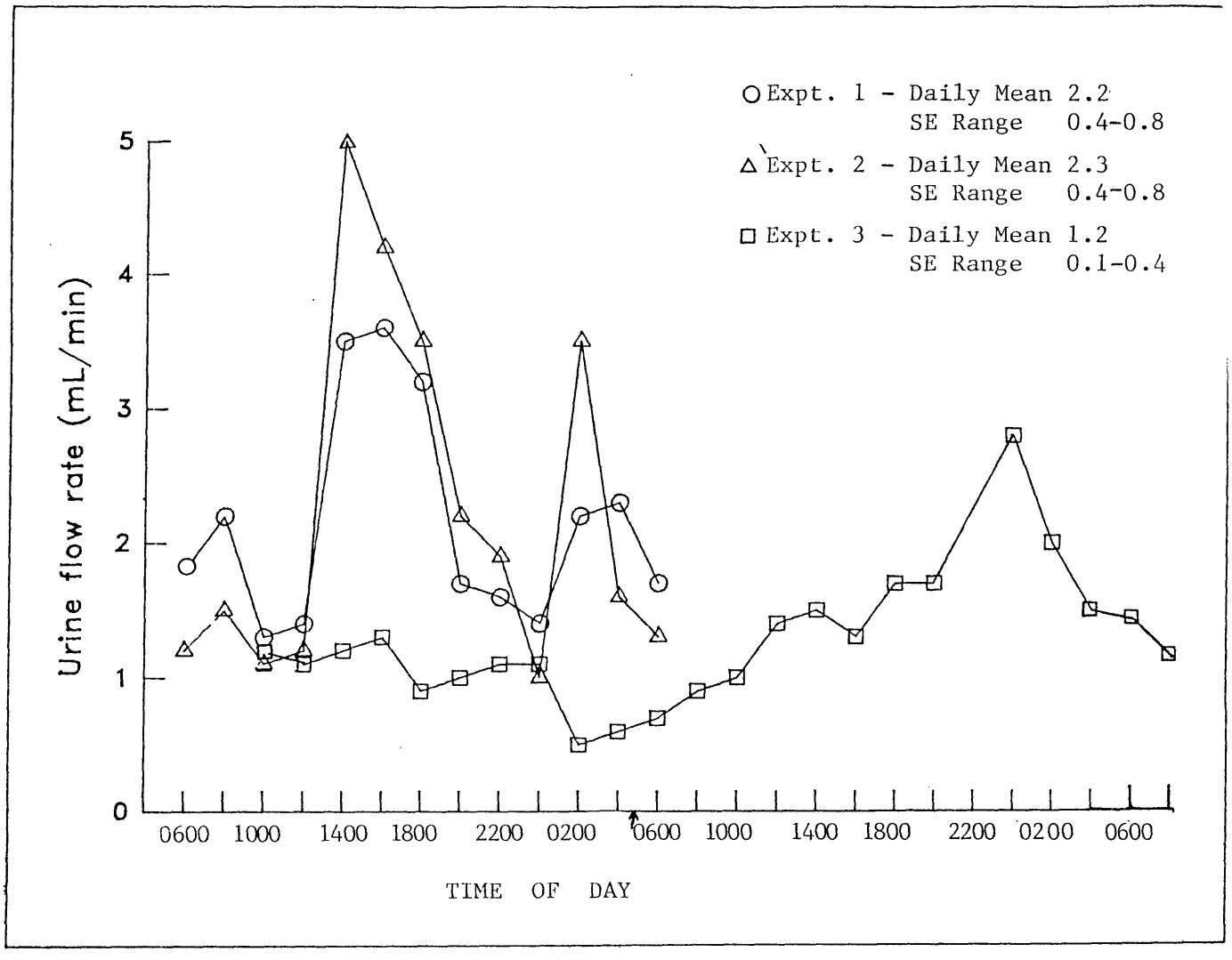

Fig. 5 - Variation in Urine Flow Rate in experiments 1 , 2 and 3.

(arrow indicates time of access to water)

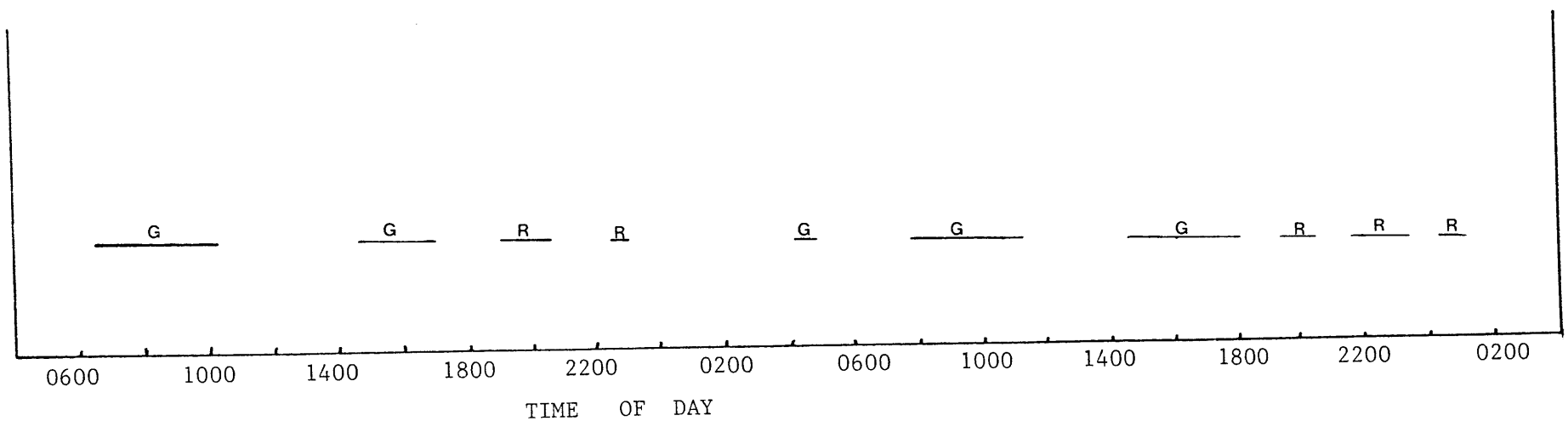

Fig. 6 - Major Behavioural Patterns in Experiment 3
(R = rumination, G = grazing)

THE RENAL EFFECTS OF HEAD-OUT WATER IMMERSION IN SHEEP

1. Four merino ewes were stood in an immersion tank and renal functional measurements taken over a total of eleven 15 min clearance periods. After 3 control measurements, water was added to the tank and 4 immersed measurements were made. The water was rapidly drained and a further 4 clearance measurements were taken.

2. The plasma level of all constituents was not altered except for $P_{protein}$ which was substantially decreased during the immersion period. PCV was unaltered throughout the experiment.

3. The excretion and fractional excretion of Na, K and Ca and GFR were all enhanced during the initial stages of immersion, but returned to near control values in the later stages.

4. The UFR and the C_{H_2O} were increased during all stages of immersion and returned to control values after immersion. The U_{urea} and FE_{urea} were both markedly enhanced during immersion, and also returned to control values 15 min post-immersion.

5. It is concluded that the renal response of sheep to water immersion is similar to that obtained in man, although the return to control values of U_{Na}, U_K and GFR after 30 min of immersion whilst UFR and U_{urea} remained elevated, suggests several controlling mechanisms are operating for these variables in sheep during immersion.

An increase in ECV is normally accompanied by marked renal changes, particularly diuresis and natriuresis.

Although it is customary to talk of ECV volume control, it is the blood volume control which is the critical function. Indeed ECV is only controlled through blood volume control and the shift of fluids between the interstitial and vascular compartments.

The compliance of the interstitial space is virtually identical to that of the vascular space and hence provided that the balance of Starling's forces at the

capillary bed are unaltered, changes in plasma and ECV will be mutual (Gauer *et al* 1970).

The present experiment was designed to investigate some of the renal effects of head-out water immersion in sheep, with a view to examining the effects of blood volume expansion without changes in ECV.

Head-out water immersion in man results in a striking natriuresis and diuresis. This effect is thought to be due to a redistribution of the body fluids, resulting in an increased central blood volume. McCally (1964) found a decrease in haemoglobin and haematocrit (PCV) levels of about 4.5 % 25 min after water immersion in man. However, Epstein *et al* (1972, 1973) failed to show any change in PCV of subjects immersed in the seated posture. It has been suggested that the changes in PCV are due to a shift of interstitial fluid into plasma (Crane and Harris 1974), or alternatively a shift of blood of low haematocrit from small venules in the periphery to the circulating blood volume (Epstein 1978).

Methods

Four shorn adult merino ewes (42 ± 1.2 kg BW) were fed oaten chaff and commercial sheep nuts (Fielders, Tamworth, N.S.W.) ad libitum and allowed free access to water for several weeks prior to the experiment. Each animal was fitted with bilateral jugular catheters and a urinary bladder catheter draining into a blood collection pack (Tuta Laboratories, Lane Cove, N.S.W.) strapped under the animal. The pack was readily drained of urine by suction with a syringe connected to an evacuating tube.

Each animal was stood in an immersion tank at approximately 20.30 h. A total of eleven 15 min clearance periods was begun 30 min later. The first three were baseline measurements, then water at 37°C was rapidly added to the tank and four 'immersed' clearance measurements were begun. At the end of the seventh clearance period the water was rapidly drained and four post-immersion clearance periods were commenced.

At the beginning of the first clearance period 20 mL of warm 20 % (w/v) in-

ulin solution were injected into one jugular catheter and at the commencement of the fifth clearance period 20 mL of warm Cr^{51}EDTA in 0.9 % NaCl (0.2 MBq/mL) were injected into the same catheter. In two sheep this procedure was reversed with the Cr^{51}EDTA being given at the first and inulin at the fifth period. Blood samples for Cr^{51}EDTA and inulin concentration measurements were taken from the contralateral catheter, prior to injection and at approximately 2, 5, 10 and 20 min after injection, as well as at the beginning of each clearance period. ECV was calculated by the following formulae (Poulsen *et al* 1977).

$$ECV = Q \int_0^\infty tC_t dt / (\int_0^\infty C_t dt) \quad and \quad TC = Q / \int_0^\infty C_t dt$$

Then by substitution of the second equation into the first,

$$ECV = (TC) \int_0^\infty tC_t dt / Q$$

where Q = total amount of marker injected

C_t = concentration of marker at time t

TC = total plasma clearance of marker

Assuming TC approximates glomerular filtration rate (GFR), then from substitution of individual clearance period GFR values, determined by endogenous creatinine clearance, and summation of definite integrals for each clearance period, a valid estimate of ECV can be obtained despite changes in GFR. In normal sheep calculation of ECV using Poulsen *et al* (1977) formulae following simultaneous injection of Cr^{51}EDTA and inulin gives substantially different values for the two markers (see appendix 2). For this reason, the values for ECV determined using Cr^{51}EDTA were reduced by 24 % to allow for this difference in distribution space.

Analytical

$Cr^{51}EDTA$ activity was determined using an automated gamma emission spectrometer (Gammamatic, Kontron, Sydney, N.S.W.), inulin concentration by colourimetry (Bacon and Bell 1948), Na and K by flame photometry (EEL 227, Evans Electroselenium, Essex, England), osmolality by freezing point depression (Fiske Assoc. Connecticut, U.S.A.), Cl by titration (CMT 10 Chloride Titrator, Radiometer, Copenhagen, Denmark), PCV using the microhaematocrit method (Hawksley and Sons, Sussex, England), and Ca, urea, uric acid plus allantoin (URAL), glucose, creatinine and plasma protein using Roche kits and Mg and inorganic phosphate (Pi) using Pierce kits (Pierce Chemical Co., Rockford,Il, U.S.A.) on a Cobas-Bio centrifugal analyser (Hoffman-La Roche, Switzerland).

Statistics

As each 'immersed' and 'post-immersed' clearance period was performed without allowing time for a steady physiological state to occur in the animals, data were analysed by comparing each of these clearance periods with that of the mean 'pre-immersion' period. These comparisons were made using paired t-tests with Bonferroni probability adjustments (Dixon 1981). Data are presented as means $\pm$ standard errors of the means.

Results

The effects of water immersion on renal haemodynamics and water, glucose and N excretion are shown in Table 1.

An increased GFR was apparent after the first 30 min of immersion. However it returned to normal during the second half of the immersion period.

The UFR increased about 5 fold during immersion and returned to the pre-immersion value after about 15 min post immersion. Similarly free water clearance also increased substantially during water immersion.

The PCV was unaltered but the $P_{protein}$ level was substantially reduced during the immersion phase and for the first clearance period following immersion. ECV was the same at the two measured times.

U_{urea} and FE_{urea} both increased substantially during immersion,with the FE_{urea} being greatest during the second half of immersion. U_{URAL} was unaltered throughout the experiment, but FE_{URAL} was markedly increased during the post-immersion period.

The excretion of electrolytes during the experiment are given in Table 2 and their plasma levels in Table 3.

Na excretion showed no change until 15 min after immersion but then a three-fold increase occurred, followed by a return to normal values during the final immersion period; K and Cl excretion showed a similar trend.

U_{Ca} excretion increased about four-fold during the second and third 'immersed' clearance periods. The remaining times it showed a normal pattern. FE_{Ca} was also elevated only during the same clearance periods. The excretion of Mg and Pi were not altered. However, as the plasma level of Mg was not varied (Table 3.), the FE_{Mg} was inversely related to the changes in GFR.

Glucose excretion and $FE_{glucose}$ were decreased during the early phase of immersion, but then increased during the latter half of immersion.

Discussion

The return to normal of the GFR during the latter stages of immersion is in agreement with the findings of Epstein *et al* (1972, 1973), who showed a rise in GFR restricted to the early stages of immersion in man.

The natriuresis of immersion appeared to be dissociated from the changes in GFR and UFR. Similarly UFR and GFR tended to be dissociated, indicating that independent controlling mechanisms were probably operating for these variables during water immersion. An acute expansion of the ECV of sheep using a rapid intravascular infusion of isotonic saline (hence presumably an increase in blood volume) has no effect on GFR. However it substantially alters UFR and Na excretion (Szanyiova *et al* 1980).

The return to near normal GFR and U_{Na} values whilst the animals were still immersed is unexplained, but may be the result of a large numbers of arterio-

venous anastomoses under the skin of sheep (Hales 1974), compensating for the compression of capillary blood.

The fall in $P_{protein}$ level and the unchanged ECV suggest that there was a shift of interstitial fluid into the plasma. The static PCV values, although more variable during and after the immersion phase, may be the result of a splenic contraction increase (Dooley and Williams 1975), compensating for a dilutional fall.

The increased free water clearance is indicative of a substantial 'water' diuresis. Water immersion in man is associated with a decrease in ADH secretion (Epstein *et al* 1975) and the diuresis can be substantially abolished by ADH administration (Gauer *et al* 1970, Epstein *et al* 1981).

The natriuresis of water immersion is thought to be the result of increased plasma activity of natriuretic hormone (Epstein *et al* 1978), which is normally released as a result of blood volume expansion (De Wardener 1982).

U_{Ca} normally parallels U_{Na} (Walser 1961) and this relationship appears to remain true during water immersion.

Epstein *et al* (1974) found an increase in urine pH with water immersion in man, associated with a large increase in bicarbonate excretion. In the present experiment there was no change in urinary pH, probably because the sheep normally has an alkaline urine, and at the baseline pHs in the present experiment increased bicarbonate excretion would show little effect on urine pH.

The U_{urea} and FE_{urea} were determined by the urine flow rate, as has been shown in many previous studies. The FE_{urea} exceeded 100 % during some stages of water immersion and if true, indicates that the sheep kidney is capable of urea secretion. Kawamura and Kokko (1976) found active secretion of urea in the pars recta of the rabbit kidney and Nawaz and Shah (1984) have shown FE_{urea} of greater than 100 % in a large number of normal grazing sheep.

Interestingly the excretion of URAL showed a different pattern to that of urea and perhaps this is because the sheep kidney normally excretes uric acid by tubular secretion (Chesley *et al* 1978).

Water immersion is a non-invasive means of altering the blood volume without altering either the ECV or Na or water balance. Many studies have suggested that expansion of the ECV by, for example, increased Na intake, is responsible for many of the changes in renal function noted in chapters 1 and 2 of this thesis. However, it would appear from the present study that the plasma volume component of the ECV is the controlling or controlled factor leading to many of these changes.

Because qualitatively similar data to those previously obtained for man were obtained in the present study, the sheep may become an interesting model for future studies of the physiology of water immersion.

Table 1. The effects of water immersion on renal haemodynamics and N and glucose excretion.

Clearance period	GFR (mL/min)	UFR (mL/min)	C_{H_2O} (mL/min)	PCV (%)	$P_{protein}$ (g/dL)	ECV (L)
Pre-immersion						
1	40.1 ± 2.1	0.81 ± 0.09	-4.21 ± 0.12	36 ± 0.9	4.01 ± 0.08	7.5 ± 0.2
2	43.5 ± 2.3	0.64 ± 0.10	-4.84 ± 0.14	35 ± 0.2	3.95 ± 0.02	---
3	44.3 ± 2.1	0.73 ± 0.12	-4.62 ± 0.11	36 ± 0.8	3.96 ± 0.03	---
Immersion						
4	64.1 ± 2.1*	1.20 ± 0.19*	-4.42 ± 0.18	34 ± 1.9	3.69 ± 0.01*	---
5	69.8 ± 5.1*	3.93 ± 0.31*	-0.96 ± 0.21*	36 ± 1.5	3.49 ± 0.02*	7.4 ± 0.4
6	41.5 ± 3.2	4.20 ± 0.32*	-1.38 ± 0.61*	35 ± 2.5	3.40 ± 0.04*	---
7	47.6 ± 3.1	4.17 ± 0.32*	-2.46 ± 0.43*	35 ± 2.0	3.61 ± 0.03*	---
Post-immersion						
8	41.0 ± 2.2	2.67 ± 0.28*	-0.62 ± 0.41*	34 ± 2.3	3.71 ± 0.06*	---
9	39.8 ± 2.4	0.97 ± 0.20	-3.06 ± 0.18*	35 ± 2.1	4.21 ± 0.10	---
10	38.4 ± 2.8	0.52 ± 0.21	-3.15 ± 0.31	35 ± 0.2	4.18 ± 0.06	---
11	35.4 ± 3.1	0.51 ± 0.21	-4.49 ± 0.42	34 ± 0.4	4.09 ± 0.08	---

Clearance period	U_{urea} (μmol/min)	FE_{urea} (%)	U_{URAL} (μmol/min)	FE_{URAL} (%)	$U_{glucose}$ (μmol/min)	$FE_{glucose}$ (%)
Pre-immersion						
1	177 ± 14	55 ± 5.2	6.2 ± 0.3	129 ± 12.3	1.50 ± 0.21	1.01 ± 0.05
2	168 ± 18	48 ± 4.2	6.1 ± 0.5	140 ± 15.2	1.56 ± 0.19	0.97 ± 0.03
3	211 ± 18	60 ± 6.8	6.5 ± 0.6	113 ± 14.1	1.58 ± 0.16	0.94 ± 0.06
Immersion						
4	331 ± 26*	63 ± 2.6*	6.1 ± 0.5	106 ± 12.0	1.06 ± 0.12*	0.42 ± 0.06*
5	360 ± 12*	63 ± 4.2*	6.5 ± 0.8	93 ± 11.7	1.18 ± 0.11*	0.44 ± 0.08*
6	446 ± 21*	126 ± 11.9*	5.9 ± 1.1	118 ± 15.2	1.72 ± 0.13*	1.15 ± 0.04*
7	471 ± 26*	115 ± 10.0*	6.0 ± 0.8	115 ± 10.7	1.85 ± 0.14*	1.05 ± 0.05
Post-immersion						
8	297 ± 14*	88 ± 6.4*	6.2 ± 0.7	168 ± 19.8	1.60 ± 0.13	1.00 ± 0.04
9	152 ± 9	48 ± 6.7	6.1 ± 0.6	153 ± 18.5	1.46 ± 0.10	1.02 ± 0.06
10	114 ± 13*	38 ± 4.6*	6.3 ± 0.7	164 ± 16.5	1.46 ± 0.18	1.02 ± 0.03
11	144 ± 14	50 ± 2.8	6.4 ± 0.5	164 ± 17.0	1.37 ± 0.16	1.02 ± 0.04

* significantly different from the mean pre-immersion value ($P < 0.01$)

Table 2. The effects of water immersion on the excretion of electrolytes.

Clearance period	U_{Na} (μmol/min)	FE_{Na} (%)	U_{K} (μmol/min)	FE_{K} (%)	U_{Cl} (μmol/min)	FE_{Cl} (%)
Pre-immersion						
1	137 ± 12	2.4 ± 0.1	233 ± 21	135 ± 5.2	321 ± 19	7.2 ± 0.6
2	164 ± 14	2.7 ± 0.3	228 ± 19	131 ± 4.3	361 ± 18	7.5 ± 0.6
3	110 ± 15	1.8 ± 0.2	241 ± 31	127 ± 2.9	346 ± 21	7.1 ± 0.7
Immersion						
4	189 ± 21	2.1 ± 0.1	306 ± 32*	116 ± 3.7	402 ± 31	5.8 ± 0.5
5	581 ± 101*	5.8 ± 0.4*	465 ± 46*	167 ± 6.6*	1010 ± 102*	12.9 ± 1.3*
6	592 ± 96*	9.9 ± 1.2*	725 ± 69*	397 ± 21.2*	1110 ± 123*	24.1 ± 3.9*
7	127 ± 36	1.9 ± 0.2	248 ± 39	124 ± 5.8	306 ± 51	5.8 ± 0.8
Post-immersion						
8	161 ± 19	2.8 ± 0.3	239 ± 36	139 ± 6.2	344 ± 56	7.7 ± 0.6
9	101 ± 15	1.8 ± 0.2	286 ± 26	180 ± 5.1*	381 ± 32	8.6 ± 0.8
10	98 ± 23	1.8 ± 0.1	301 ± 41	187 ± 4.8*	316 ± 28	8.0 ± 0.6
11	75 ± 22*	1.5 ± 0.1*	316 ± 44	198 ± 5.6*	307 ± 17	7.9 ± 0.4

Clearance period	U_{Ca} (μmol/min)	FE_{Ca} (%)	U_{Mg} (μmol/min	FE_{Mg} (%)	U_{PI} (μmol/min	FE_{PI} (%)
Pre-immersion						
1	2.41 ± 0.41	2.5 ± 0.1	7.3 ± 0.3	12.0 ± 1.1	0.01 ± 0.00	0.01 ± 0.00
2	2.42 ± 1.63	2.3 ± 1.1	7.5 ± 0.5	11.8 ± 2.1	0.02 ± 0.01	0.03 ± 0.01
3	2.18 ± 0.81	2.1 ± 0.2	7.6 ± 0.5	12.3 ± 2.3	0.00 ± 0.00	0.00 ± 0.00
Immersion						
4	2.62 ± 1.09	1.7 ± 0.8	7.4 ± 0.6	7.8 ± 1.2*	0.02 ± 0.01	0.02 ± 0.01
5	8.14 ± 2.22*	4.8 ± 0.6*	7.8 ± 0.5	7.9 ± 0.9*	0.01 ± 0.00	0.01 ± 0.01
6	10.08 ± 3.67*	10.2 ± 1.5*	7.1 ± 0.7	12.3 ± 1.2	0.00 ± 0.00	0.00 ± 0.00
7	2.99 ± 1.00	2.6 ± 0.8	7.0 ± 0.3	10.5 ± 1.0	0.00 ± 0.00	0.00 ± 0.00
Post-immersion						
8	2.46 ± 0.43	2.5 ± 0.2	6.4 ± 0.8	11.4 ± 0.6	0.01 ± 0.01	0.01 ± 0.01
9	2.31 ± 0.33	2.4 ± 0.3	7.2 ± 0.6	12.3 ± 1.1	0.02 ± 0.01	0.03 ± 0.01
10	1.94 ± 0.23	2.1 ± 0.2	8.1 ± 1.1	15.1 ± 3.8	0.02 ± 0.00	0.03 ± 0.01
11	1.90 ± 0.72	2.2 ± 0.3	7.4 ± 0.8	14.8 ± 2.1	0.01 ± 0.01	0.02 ± 0.01

* significantly different from mean pre-immersion value ($P < 0.01$)

Table 3. The effects of water immersion on plasma urea, URAL, glucose and electrolyte levels (mmol/L).

Clearance period	P_{urea}	P_{URAL}	P_{Na}	P_K	P_{Cl}	P_{Ca}	P_{Mg}	P_{Pi}	$P_{glucose}$
Pre-immersion									
1	8.0 ± 0.9	0.12 ± 0.01	144 ± 2.5	4.3 ± 0.2	111 ± 0.9	2.41 ± 0.08	1.52 ± 0.19	1.81 ± 0.10	3.7 ± 0.3
2	8.1 ± 0.9	0.10 ± 0.02	140 ± 2.1	4.0 ± 0.3	111 ± 0.1	2.40 ± 0.10	1.46 ± 0.08	1.75 ± 0.11	3.7 ± 0.3
3	7.9 ± 1.0	0.13 ± 0.02	141 ± 2.0	4.3 ± 0.1	110 ± 0.3	2.39 ± 0.10	1.39 ± 0.02	1.63 ± 0.08	3.8 ± 0.1
Immersion									
4	8.2 ± 0.8	0.09 ± 0.02	142 ± 1.9	4.1 ± 0.2	109 ± 1.6	2.45 ± 0.11	1.48 ± 0.04	1.72 ± 0.05	3.9 ± 0.3
5	8.2 ± 0.6	0.10 ± 0.03	144 ± 2.1	4.0 ± 0.2	112 ± 0.2	2.42 ± 0.02	1.42 ± 0.02	1.69 ± 0.02	3.8 ± 0.2
6	8.5 ± 0.8	0.12 ± 0.01	144 ± 2.4	4.4 ± 0.1	111 ± 0.2	2.38 ± 0.08	1.39 ± 0.06	1.72 ± 0.06	3.6 ± 0.4
7	8.6 ± 1.1	0.11 ± 0.02	142 ± 2.1	4.2 ± 0.3	110 ± 0.1	2.39 ± 0.09	1.40 ± 0.05	1.59 ± 0.21	3.7 ± 0.3
Post-immersion									
8	8.2 ± 0.5	0.09 ± 0.01	141 ± 1.8	4.2 ± 0.2	109 ± 0.4	2.40 ± 0.07	1.37 ± 0.08	1.72 ± 0.10	3.9 ± 0.2
9	8.0 ± 0.4	0.10 ± 0.04	140 ± 1.5	4.0 ± 0.3	111 ± 0.0	2.42 ± 0.02	1.47 ± 0.01	1.64 ± 0.09	3.6 ± 0.2
10	7.9 ± 0.6	0.10 ± 0.01	139 ± 1.6	4.2 ± 0.2	113 ± 0.5	2.44 ± 0.05	1.40 ± 0.10	1.82 ± 0.12	3.7 ± 0.3
11	8.1 ± 0.5	0.11 ± 0.04	141 ± 1.5	4.5 ± 0.4	110 ± 0.1	2.40 ± 0.01	1.41 ± 0.11	1.80 ± 0.16	3.8 ± 0.3

SUMMARY AND CONCLUSIONS

A series of experiments was conducted basically to determine how the kidney controls the plasma urea level and urea excretion rate during various physiological manoeuvres.

Early studies in this laboratory (McIntyre 1970) showed that the plasma urea level of sheep fed dry roughage rations plateaus at about 11 mmol/L and urinary urea excretion maintains a linear increase with increasing dietary N intake.

Inherent in McIntyre's dietary regime was an increase in food intake with increasing N intake. The first experiment reported in this thesis, examined the effects of increased food intake on parameters of kidney function. This experiment showed that the increased food intake increased U_{urea}, GFR, UFR and ECV. A second experiment was then conducted to delineate which factors in the increased intake of food were responsible for the above changes.

Sheep were fed a basal low protein roughage ration and infused intraruminally with increasing quantities of urea. The UFR and U_{urea} increased up until 15.6 g/d of urea-N was infused. Further input of urea resulted in little change in UFR and U_{urea}, but P_{urea} increased to very high levels (24.4 mmol/L). When 500 mmol/d of NaCl or KCl were added to the infusate at this level of urea input, the P_{urea} decreased substantially, due to an increased UFR and U_{urea}.

It was concluded that urea alone has a limited ability to promote a diuresis that will increase its excretion to maintain P_{urea} levels below about 11 mmol/L. Urea input above this level causes little change in renal urea output, and consequently P_{urea} rises dramatically. However, the addition of sufficient of the diuretic agents NaCl or KCl reduced the P_{urea} to about 11 mmol/L by increasing the UFR.

The changes in U_{urea} noted with the increase in food intake in the first experiment and in McIntyre's (1970) study, are thus due to both the diuretic effect of urea, whose production is elevated with increased N intake, and the diuretic effect of the increased intake of dietary electrolytes.

Because endogenous urea has substantial nutritional value to animals fed low protein diets, and in view of the effects of NaCl on urea excretion of animals loaded with urea in the earlier experiments it was decided to investigate the effects of increasing levels of NaCl intake in animals fed a low protein roughage diet.

Sheep were fed a basal low protein roughage ration and were infused intraruminally with increasing quantities of NaCl. The animals increased their water intake and urine flow rates virtually linearly with salt input. The excretion of urea was not affected until 1000 mmol/d of NaCl was infused. The excretion of urea then decreased with increasing salt intake, despite an increase in FE_{urea}. The P_{urea} level was dramatically reduced by the higher salt infusions. This was primarily due to a reduction in the apparent digestibility of N.

The reduction in the apparent digestibility of N was caused by an increased flow rate of digesta through the rumen and hence a decrease in the fermentative release of N. A decrease in U_{URAL} also suggested a reduction in rumen microbial protein synthesis.

Despite the increased flow rate of digesta from the rumen there was no stimulus to appetite when the animals were allowed food ad libitum. Thus it appears that high levels of salt with this type of ration would probably be deleterious to the production of animal protein.

Animals freely grazing high quality green pastures frequently have N intakes in excess of 50g of digestible N per day, ie., substantially higher than the N intakes of both McIntyre's (1970) and the infused intakes reported in chapter 1. Preliminary data obtained from sheep grazing a legume based pasture (appendix 6) revealed that P_{urea} levels were seldom much greater than about 11 mmol/L. It appears from the data obtained in chapter 5 that the urinary excretion of urea to maintain P_{urea} below 11 mmol/L is achieved by a water diuresis caused by the inherent high intake of dietary water.

Changes in acid-base status of ruminants is a common occurrence, particularly when the diet of the animals is changed from a roughage to a predominantly grain ration. Recent reports have suggested that metabolic urea production may

play an important role in the maintenance of acid-base balance. The data presented in chapter 3 tends to refute an important role for urea production in controlling acid-base balance, atleast during an acute change of acid-base status. There was no change in P_{urea} or U_{urea} in response to the changes in acid-base status.

Head-out water immersion is a procedure which causes a shift in the distribution of fluid within the extracellular water compartments in man. In chapters 1 and 2 an increased electrolyte intake resulted in an expanded ECV. The experiment reported in chapter 5 shows that firstly, the sheep is a suitable experimental animal for immersion studies and that secondly, plasma volume can be altered in sheep non-invasively and without concurrent changes in electrolyte input. The ECV remained constant, but changes in the $P_{protein}$ level suggested a substantial expansion of plasma volume. Under these conditions a massive diuresis and urea excretion occurred, indicating again that one of the major factors governing U_{urea} is the UFR.

During the experimental manoeuvres outlined in chapters 1-5 there were substantial changes noted in renal haemodynamics. Generally an increase in electrolyte intake caused an increase in GFR, whilst an increase in urea intake had little effect.

The well known positive relationship between protein intake and GFR is thus probably due to the inherent increase in electrolyte intake associated with the high protein diets.

The excretion of electrolytes was generally increased by any degree of diuresis. Some points of note were that the excretion of Ca parallelled that of Na, and that the digestibility of Ca was also increased by NaCl infusion. High sodium inputs also caused an increase in U_{Mg} which then resulted in a decrease in P_{Mg}. Changes in acid-base balance led to substantial derangements in Ca and Pi metabolism which appeared to be related to the packed cell volume of the blood.

The overall data indicate that the major controlling factors determining the

excretion of urea by sheep are the P_{urea}, UFR and GFR. Combining of all data presented in this thesis yielded the following multiple regression equation, which accounted for 82 % of the variance associated with U_{urea}.

$$U_{urea} = 41.6P_{urea} + 44.2UFR + 0.314GFR - 189.$$

$$(r^2 = 0.822, P < 0.001)$$

This equation highlights the practical importance of reducing the levels of material in N limiting diets which are likely to cause increases in UFR or GFR, as any urea which is excreted on such diets could be considered as potential body protein loss.