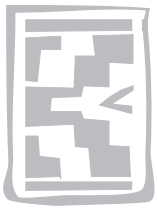

Changes in the renal handling of urea in sheep on a low protein diet exposed to saline drinking water

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ABSTRACT

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Previous trials have demonstrated that sheep on a low protein diet and free access to water, and sheep dosed with boluses of NaCl intraruminally also with free access to water, showed decreases in urea loss via the urine compared to control animals.

We monitored urea excretion in sheep on a relatively poor protein diet when they were exposed to saline drinking water, i.e. they were unable to vary their intake of NaCl:water.

Sheep on isotonic saline drinking water (phase 3) excreted significantly more urea via the urine (284 mM/day) compared to phase 1 when they were on non-saline drinking water (urea excretion = 230 mM/day) and phase 2 when they were on half isotonic saline drinking water (urea excretion = 244 mM/day).This finding was explained by the high glomerular filtration rate (GFR) 91.9 ℓ/day, compared to 82.4 ℓ/day (phase 1) and 77.9 ℓ/day (phase 2), together with a significantly raised fractional excretion of urea (FE_{urea}) (51.1 %) during this phase, and was in spite of the significantly lower plasma concentrations of urea in phase 3 compared to phase 1. The FE_{urea} probably results from the osmotic diuresis caused by the salt. There were indications of a raised plasma antidiuretic hormone (ADH) concentration and this would have opposed urea loss, as ADH promotes urea reabsorption. However, this ADH effect was probably counteracted to some extent by a low plasma angiotensin II concentration, for which again there were indications, inhibiting urea reabsorption during the phases of salt loading.

As atrial natriuretic peptide both increases GFR and decrease sodium reabsorption from the tubule, it was probably instrumental in causing the increase in GFR and the increase in the fractional excretion of sodium (FE_{Na}).

Keywords: Kidney, low protein diet, saline drinking water, sheep, urea

INTRODUCTION

The return of urea via the blood and saliva to the gastrointestinal tract of ruminants provides a valuable source of nitrogen to rumen microbes for protein synthesis (Godwin & Williams 1986). Therefore, the role of the kidney in conserving urea particularly during periods of low nitrogen intake, e.g. during drought conditions, is of immense importance in the ruminant.

Renal function during times of nitrogen shortage changes in such a way as to significantly decrease urea loss via the urine. This was found to be achieved both through a decrease in glomerular fil-

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tration rate (GFR) and a decrease in the fractional excretion of urea (FE_{urea}) (Leng, Szanyiova & Boda 1985). Van der Walt, Boomker, Meintjes & Shultheiss (1999), although unable to demonstrate any change in GFR in sheep on a low protein diet, showed an almost 50 % reduction in the FE_{urea}.

During times of water depletion there is also a reduction in the amount of urea lost via the kidneys. This has been ascribed mainly to a reduction in GFR and therefore less urea being made available for excretion rather than to a decrease in the FE_{urea} (Leng, Szanyiova, Varady & Boda 1987). In this respect it was later established that restriction of water intake for 3 days results in an upgrading of urea transporter mRNA in the inner stripe of the outer medulla (Shayakul, Smith, Mackenzie, Lee, Brown & Hediger 2000).

The intraruminal infusion of NaCl at doses in excess of 1 250 mM/day in sheep with free access to drinking water, resulted in a decline in renal urea loss (Godwin & Williams 1986). Although the saline water had the effect of increasing GFR, effective renal plasma flow (ERPF) and even the FE_{urea}, less urea was excreted in the urine. This finding was ascribed solely to a lower concentration of urea in the plasma during the phase of NaCl supplementation than during the control phase.

In the trial referred to above, the sheep were able to regulate the ratio of water:NaCl taken in, as they were given free access to non-saline drinking water. The question, however, arises as to how the kidney would handle urea in a situation where animals on a high salt intake are unable to vary the ratio of water:salt. Under such conditions, the need to excrete excess NaCl while at the same time retaining sufficient water to prevent dehydration, requires definite changes in kidney function. These changes may or may not favour the retention of urea by the kidney. In many parts of the world, e.g. in the vicinity of large salt pans, animals have access to only salt-laden water, i.e. they are faced with a large intake of salt and are unable to vary the ratio of salt: water taken in. If, in addition, as is often the case, their nitrogen (protein) intake is also low, the renal handling of urea becomes critical. This issue, i.e. the effect of saline drinking water together with a low protein diet on the renal handling of urea was investigated in the trial reported here.

MATERIAL AND METHODS

Six German Merino wethers (body mass 30–35 kg) were individually housed in metabolic crates in a room where the ambient temperature was kept at 22 °C. The crates were equipped for the collection of urine into refrigerated containers. The sheep were fitted with faecal bags for the collection of faeces so as to minimize contamination of the urine. Throughout the trial the animals were fed a diet containing 8 % protein in the form of *Eragrostis* teff hay at the rate of 2 % of their body mass per day (Van der Walt *et al*. 1999).

The trial consisted of three phases, each lasting 7 days:

Phase 1 Fresh water was made available *ad libitum*.

Phase 2 Half isotonic saline (4.5 g NaCl/ℓ water) was offered *ad libitum* as the sole source of drinking water.

Phase 3 Isotonic saline (9 g NaCl/ℓ water) was offered *ad libitum* as the sole source of drinking water.

During each phase the animals were allowed 4 days to adapt to the drinking water before samples were taken on a daily basis for 3 consecutive days for analyses. Everyday at 08:30 the feed and water intakes and urine output of each sheep were recorded and a sample of urine was retained for later analysis. Blood samples were collected by venipuncture into heparin and EDTA for later analysis.

The following analyses were carried out on both urine and blood samples:

1. Urea concentration using a method described by Fawcett & Scott (1960) for plasma and urine
2. Creatinine concentration using the Jaffé method (plasma was first deproteinated).
3. Na+ and K+ concentrations by the ion-specific electrode method (Eschweiler Ecolyte electrolyte analyser).

Furthermore, haematocrits of the blood samples were determined by centrifugation (using microhaematocrit tubes) and total plasma protein was estimated using a refractometer (Bellingham & Stanley Ltd., Tunbridge Wells, England).

From the analytical data the following derived values were calculated:

1. Glomerular filtration rate. This was taken to be equal to the plasma clearance of endogenous creatinine, on the assumption that creatinine is neither absorbed nor secreted by the renal tubule in the sheep (Nawaz & Shah 1984; Bastl, Rudnick & Nairns 1985).

2. Fractional excretion of urea (FE_{urea}) (Bastl *et al*. 1985).
3. Fractional excretion of sodium (FE_{Na}) (Bastl *et al*. 1985).
4. Plasma clearance of urea (C_{urea}) (Bastl *et al*. 1985).
5. Urea filtered per day ($Urea_{filtd}$)
6. Electrolyte-free water reabsorption/excretion (T^c_{H2O}) (Rose 1986).

$$T^c_{H2O} = U_{vol}\,(U_{[Na]} + U_{[K]} - 1)\ (\ell/\text{day})$$

$$\overline{(Pl_{[Na]} + Pl_{[K]})}$$

where

$U_{[Na]}$ and $U_{[K]}$ = the urine concentrations of Na^+ and K^+ respectively, and $Pl_{[Na]}$ and $Pl_{[K]}$ = the plasma concentrations of Na^+ and K^+ respectively.

Statistical methods: As, within a phase, there was no valid reason for the readings obtained for the variables to differ from day to day, a one way repeated measure analysis of variance (ANOVA) test was applied to the values. If having established that between the phases there was a significant difference (at the $P < 0.05$ confidence level) in a variable, Duncan's Multiple Comparison Test was used to indicate which phases in fact differed from each other. The NCSS – Pass 2000 programme was used.

RESULTS

There was a significant increase in water intake as the water source changed from fresh water to half isotonic saline water and then again with the change to isotonic saline (see Table 1). The average daily water intakes for the respective phases resulted in average daily NaCl intakes (via the drinking water) of 0 mM/day (phase 1), 304 mM/day (phase 2) and 801 mM/day (phase 3).

The volume of urine voided per day corresponded to the increase in water intake with each phase. So, too, did the amount of sodium excreted via the urine and the fractional excretion of sodium (Table 1).

Both plasma sodium and urea concentrations decreased significantly with the change to saline drinking water, although no further change was recorded with the further increase in salt loading (phase 3).

Plasma potassium concentration increased from when fresh to half isotonic drinking water was offered, and increased even further with the change from the latter to isotonic drinking water.

The haematocrit and total plasma protein content remained unchanged throughout all three phases, indicating that there was neither haemoconcentration nor haemodilution with the various treatments.

The amount of urea filtered per day was significantly lower in phase 2 compared to phases 1 and 3. This was due to the relatively low GFR together with a low plasma urea concentration.

The amount of urea excreted via the urine increased significantly when the sheep were offered isotonic saline (phase 3). Similarly, GFR rose significantly during the same phase. The fractional excretion of urea increased significantly with the change to saline drinking water, there being no difference between phases 2 and 3. Both the plasma clearance of urea and the electrolyte-free water reabsorbed increased significantly from phase 1 to phase 2 and then again from phase 2 to phase 3. In fact, T^c_{H2O} changed from excretion (negative value) during the control

TABLE 1 Daily drinking water intake (H_2O in) and urine variables, *viz*. volume (U_{vol}), sodium concentration ($U_{[Na]}$), potassium concentration ($U_{[K]}$), sodium excreted via the urine (Na ex ur) and fractional excretion of sodium (FE_{Na}) for sheep on fresh water (phase 1), half isotonic saline (phase 2) and isotonic saline (phase 3). Mean values (SD); $n = 6$ are given

Variable	Phase 1	Phase 2	Phase 3
H_20 in (ℓ/day)	3.192^a (0.284)	3.660^b (0.447)	5.008^c (0.537)
U_{vol} (ℓ/day)	0.526^a (0.140)	0.907^b (0.402)	2.258^c (0.529)
$U_{[Na]}$ (mM/ℓ)	16^a (13)	220^b (48)	273^c (43)
$U_{[K]}$ (mM/ℓ)	106^a (7)	109^a (22)	79^b (22)
Na ex ur (mM/day)	10^a (11)	193^b (62)	597^c (96)
FE_{Na} (%)	0.061^a (0.094)	1.866^b (0.709)	4.539^c (0.867)

^{abc} Values in rows with different superscripts differ significantly from each other at the $P < 0.05$ level

TABLE 2 Plasma concentrations of sodium ($Pl_{[Na]}$), potassium ($Pl_{[K]}$), urea ($Pl_{[urea]}$) and total proteins (TPP) as well as the haematocrit (Hct) for sheep on fresh water (phase 1), half isotonic saline (phase 2) and isotonic saline (phase 3). Mean values (SD); n = 6 are given

Variable	Phase 1	Phase 2	Phase 3
$Pl_{[Na]}$ (mM/ℓ)	146[a] (7)	137[b] (1)	137[b] (1)
$Pl_{[K]}$ (mM/ℓ)	2.34[a] (0.27)	3.04[b] (0.49)	6.08[c] (0.56)
$Pl_{[urea]}$ (mM/ℓ)	6.8[a] (0.7)	6.0[b] (1.0)	6.1[b] (1.0)
TPP (g/ℓ)	55[a] (7)	53[a] (7)	54[a] (4)
Hct (%)	22[a] (4)	21[a] (3)	22[a] (3)

[abc] Values in rows with different superscripts differ significantly from each other at the $P < 0.05$ level

TABLE 3 Urine urea concentrations ($U_{[urea]}$), urea excreted daily via the urine (Ur exd), glomerular filtration rate (GFR), urea filtered (urea filtd), fractional excretion of urea (FE_{urea}), plasma clearance of urea (C_{urea}) and electrolyte-free water reabsorbed (T^c_{H2O}) for sheep on fresh water (phase 1), half isotonic saline (phase 2) and isotonic saline (phase 3). Mean values (SD); n = 6 are given

Variable	Phase 1	Phase 2	Phase 3
$U_{[urea]}$ (mM/ℓ)	484[a] (96)	304[b] (98)	139[c] (44)
Ur exd (mM/day)	230[a] (27)	244[a] (31)	284[b] (47)
GFR (ℓ/day)	82.4[a] (16.3)	77.9[a] (10.8)	91.9[b] (19.8)
Urea filtd (mM/day)	559[a] (61)	451[b] (64)	569[a] (143)
FE_{urea} (%)	41.6[a] (6)	53.9[b] (12.5)	51.1[b] (11.6)
C_{urea} (ℓ/day)	34[a] (6.1)	41.5[b] (4.9)	47.6[c] (5.0)
T^c_{H2O} (ℓ/day)	–0.07[a] (0.06)	1.01[b] (0.24)	2.19[c] (0.55)

[abc] Values in rows with different superscripts differ significantly from each other at the $P < 0.05$ level

phase to reabsorption (positive value) during the saline loading phases.

DISCUSSION

In sheep 24–50 % of filtered urea is normally excreted by the kidneys in the urine (Bickhardt & Dungelhoef 1994). The remainder is reabsorbed by a process of simple diffusion by the renal tubular epithelium. The diffusion gradient favouring urea reabsorption does not exist to begin with, i.e. on the arrival of the filtrate in a particular section of the nephron tubule, the concentration of urea in the filtrate only rises above that in the plasma following water reabsorption (Costanzo 1998). It follows therefore that urea can only be reabsorbed in those parts of the nephron where water is first reabsorbed, *viz*. the proximal convoluted tubule, the descending limb of Henlé's loop and the collecting duct (Wang, Butler, Nielsen, Nielsen, Knepper & Masilamani 2002). Urea reabsorbed from the inner medullary collecting duct contributes to the hypertonicity of the inner medulla interstitium. Much of this urea reenters the thin part of the ascending limb of Henlé's loop to recycle in the distal half of the nephron tubule (Imai & Kokko 1974).

Different types of urea transporter proteins have been identified, *viz*. UT1 transporters in the terminal inner medullary collecting duct and UT2 and UT3 transporters in the thin limb of Henlé's loop, and in the descending vasae rectae respectively (Tsukaguchi, Shayakul, Berber & Hediger 1988) .

In the current trial, urea loss via the urine was significantly higher when the NaCl intake of a sheep reached an average of about 800 mM/day (phase 3), compared to phases 1 and 2. This was most likely due to two factors working together. Firstly, the significantly greater GFR during phase 3 (91.9 ℓ/day) compared to phases 1 (82.4 ℓ/day) and 2 (77.9 ℓ/day). This had the effect of making as much urea available to the nephron tubule during phase 3 compared to phase 1, in spite of the lower urea con-

centration in the plasma (6.1 mM in phase 3 compared to 6.8 mM during phase 1). Secondly, the fractional excretion of urea was significantly higher during phase 3 than in the control phase.

During phase 2, although the fractional excretion of urea was also significantly higher than in the control phase (and similar to that in phase 3), the amount of urea excreted was similar to that of the control phase because of a similar GFR to the control phase together with a significantly lower plasma urea concentration than in the control phase, both factors favouring less urea finding its way into Bowman's capsule in phase 2 than in phase 3 (see $Urea_{filtd}$ in Table 3).

In the experiment by Godwin & Williams (1986), salt loading also led to a significant decrease in plasma urea concentration (from about 6 mM at zero salt loading to 0.8 mM at a NaCl load of 2 000 mM/day). This was in spite of a significant reduction in urinary urea loss with salt loading. No explanation was offered for this finding, but it is also reflected in phase 2 of the current trial, *viz*. a significant reduction in plasma urea concentration compared to the control phase, while urinary excretion of urea was similar during these two phases.

It is possible that the delivery of urea to the rumen is enhanced by the intake of saline drinking water, and that this may have an effect on plasma urea concentrations or possibly under conditions of excess salt intake, the kidney adjusts the ratio of urea to sodium in the medullary interstitium in favour of the former.

Godwin & Williams (1986) found that GFR only rose significantly once a level of 1 500 mM NaCl/day was infused intraruminally. In the current trial, a daily intake of 800 mM NaCl was sufficient to raise the GFR. The differences obtained between the two trials are probably accounted for by the different methods of salt loading, *viz*. different salt:water intakes.

During the phases of salt loading, natriuresis was obligatory for homeostasis. There was also a need to excrete the excess water taken in when saline water was the only source of drinking water available. Accordingly, the fractional excretion of sodium rose significantly from phase 1 to phase 2 and again from phase 2 to phase 3. As atrial natriuretic peptide (ANP) has a potent effect on increasing the FE_{Na}, it is highly likely that plasma concentrations of this hormone were also raised and accounted for the increases in FE_{Na} seen in this trial (Sonnenburg 1990).

Although urine volume also increased in parallel with the excretion of salt, the electrolyte-free water reabsorption also increased. As electrolyte-free water reabsorption is largely governed by antidiuretic hormone (ADH) (Rose 1986), the increase in this variable observed with salt loading in the trial, suggests a concurrent increase in antidiuretic hormone concentration in the plasma. Urea uptake via the UT1 inner medullary collecting duct is stimulated by ADH (Tsukaguchi *et al.* 1988). It is reasonable that increased urea reabsorption brought about by the higher levels of ADH in fact neutralized to some degree the increase in fractional excretion of urea observed with salt loading in this trial. However, saline loading also brought about significant increases in natriuresis. This, taken together with the significant decreases in plasma sodium concentration and increases in plasma potassium concentration, points to inhibition of the renin-angiotensin-aldosterone axis (RAA) (Reid, Morris & Ganong 1978). As the effect of ADH on urea transport (UT1 transporter) is augmented by angiotensin II (Kato, Klein, Zhang & Sands 2000), the amount of urea reabsorption stimulated by ADH was probably minimized in this case, because of simultaneously low levels of circulating angiotensin II.

To conclude, in this trial we showed an increase in the urinary excretion of urea in sheep on a low protein diet with isotonic saline as the sole source of drinking water. This finding is in contrast to the decreased loss of urinary urea in sheep on low protein diets on *ad lib.* non-saline water (Van der Walt *et al*. 1999), and in salt-loaded sheep which were able to adjust their intake of water:salt (Godwin & Williams 1986).

In this trial, where the animals were on a fixed intake of water:salt, the effect of the increased loss of urea probably contributed to the lower urea concentration in the plasma of the animals during phase 3 (although some other mechanism must also have been operating here if one considers the low plasma urea concentration in the absence of increased urea loss in phase 2). Taking the greatly increased flow rate of urine into account with saline loading, the increase in urinary loss of urea, although significant, was not that much greater than that in control animals. This was probably due to the effect of a raised ADH plasma concentration (evidenced by the increase in electrolyte-free water reabsorption), which would increase urea reabsorption in the inner medulla. This effect was presumably neutralized to some extent by low concentrations of angiotensin II (evidenced by the changing patterns in Na^+ and K^+ concentrations in the plasma and the natriuresis with

salt loading). The increases in FE_{Na} are explained by a probable rise in plasma atrial natriuretic peptide (ANP) (Sonnenburg 1990).

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REFERENCES

BASTL, C.P., RUDNICK, M.R. & NAIRNS, R.G. 1985. Assessment of renal function: characteristics of the functional and organic forms of acute renal failure, in *The kidney: physiology and pathophysiology,* edited by D.W. Seldin & G. Giebisch. New York: Raven Press.

BICKHARDT, K. & DUNGELHOEF, R. 1994. Clinical studies of kidney function in sheep. I. Methods and reference values of healthy animals. *Deutsche Tierarzliche Wochenschrifte,* 101: 463–466.

COSTANZO, L.S. 1998. Renal Physiology, in *Physiology*. Philadelphia: W.B. Saunders.

FAWCETT, J.K. & SCOTT, J.G. 1960. A rapid precise method for the determination of urea. *Journal of Clinical Pathology,* 13: 156–159.

GODWIN, I.R. & WILLIAMS, V.J. 1986. Effects of intraruminal sodium chloride infusion on rumen and renal nitrogen and electrolyte dynamics in sheep. *British Journal of Nutrition*, 56:379–394.

IMAI, M. & KOKKO, J.P. 1974. Sodium chloride, urea, and water transport in the thin ascending limb of Henlé. Generation of osmotic gradients by passive diffusion of solutes. *Journal of Clinical Investigation,* 53:393–402.

KATO, A., KLEIN, J.D., ZHANG, C. & SANDS, J.M. 2000. Angiotensin II increases vasopressin – stimulated facilitated urea permeability in rat in inner medullary collecting ducts. *American Journal of Physiology,* 279:F835–F840.

LENG, L., SZANYIOVA, M. & BODA, K. 1985. The renal response of sheep to a low dietary nitrogen intake. *Physiologia Bohemoslovaca (Praha),* 34:147–159.

LENG, L., SZANYIOVA, M., VARADY, J. & BODA, K. 1987. The effect of water depletion on renal excretion of urea and electrolytes in sheep. *Veterinary Medicine (Praha),* 32:371–378.

NAWAZ, M. & SHAH, B.H. 1984. Renal clearance of endogenous creatinine and urea in sheep during summer and winter. *Research in Veterinary Science*, 36:220–224.

REID, I.A., MORRIS, B.J. & GANONG, W.F. 1978. The renin angiotensin system. *Annual Review of Physiology,* 40:377–410.

ROSE, B.D. 1986. New approach to disturbances in the plasma sodium concentration. *The American Journal of Medicine*, 81:1033–1040.

SHAYAKUL, C., SMITH, C.P., MACKENZIE, H.S., LEE, W.S., BROWN, D. & HEDIGER, A. 2000. Long term regulation of urea transporter depression by vasopressin in Brattleboro rats. *American Journal of Physiology,* 278:F620–F627.

SONNENBERG, H. 1990 Mechanisms of release and renal action of atrial natriuretic peptide. *Acta Physiologica Scandenavica*: 138, suppl. 591:80–87.

TSUKAGUCHI, H., SHAYAKUL, C., BERBER, U.V. & HEDIGER, M.A. 1988. Urea transporters in the kidney: molecular analysis and contribution to the urea concentrating process. *American Journal of Physiology,* 275:F319–F324.

VAN DER WALT, J.G., BOOMKER, E.A., MEINTJES, R.A. & SCHULTHEISS, W.A. 1999. Effect of water intake on the nitrogen balance of sheep fed a low or a medium protein diet. *South African Journal of Animal Science*, 29:105–109.

WANG, X., BUTLER, K., NIELSEN, J., NIELSEN, S., KNEPPER, M.A. & MASILAMANI, S. 2002. Decreased abundance of collecting duct urea transporters (UTA-1 and UTA-3) with extracellular fluid volume expansion. *American*