

CHAPTER 5

RENAL FUNCTION AND PLASMA ELECTROLYTE RESPONSES OF
LAMBS ACCUSTOMED TO LOW OR HIGH INTAKE OF SODIUM CHLORIDE
AFTER ROUTINE ORAL DOSING WITH HYPERTONIC SALINE

5.1 INTRODUCTION

Increases in urine output and Na and Cl excretion have been reported in sheep consuming either food, water or both that contained a high content of NaCl (Peirce 1957; Potter 1961; Wilson 1966b; Wilson and Dudzinski 1973 and Tomas *et al.* 1973). Urine osmolarity and Na^+ concentration remained relatively constant over a range of intakes of NaCl (Wilson 1966b; Wilson and Dudzinski 1973). Plasma Na^+ and Cl^- concentrations have been observed to increase after NaCl consumption (Potter 1968; Tomas *et al.* 1973) but the long term plasma electrolyte changes reported in the literature are difficult to analyse (see Discussion, Chapter 4). Sampling times and sample collection techniques may be associated with variations in results reported.

The differences in renal function and plasma electrolytes between lambs given either low or high intakes of NaCl have been reported in Chapter 4. In this study, renal function and plasma electrolytes were measured for the same lambs over a 24 hour period encompassing their normal daily routine. The experimentation was undertaken to: (a) obtain further information to aid in understanding the mechanisms lambs utilize to maintain fluid and electrolyte homeostasis when exposed to high intake of NaCl and (b) complement data obtained in experiments reported in Chapters 3 and 4.

5.2 MATERIALS AND METHODS

5.2.1 Animals, Treatments and Measurements

The lambs used in the experiments reported here and their routine maintenance has been described previously (see Chapter 3, section 3.2.1). Experimentation was undertaken at the Armidale site when the lambs were approximately seven (7) months of age and at the Fowler's Gap site when the lambs were approximately seven and one half (7.5) months of age. On

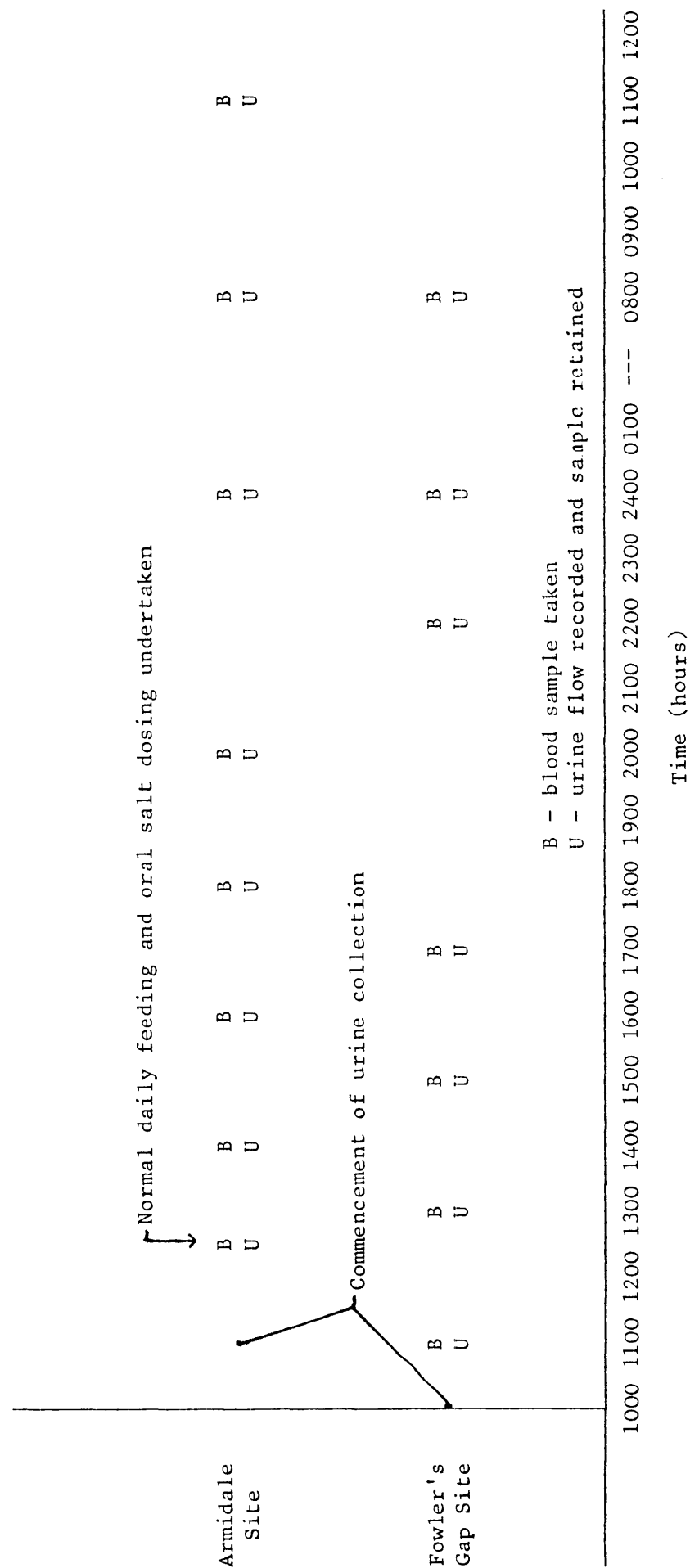
the day of the experiment at each site, one jugular vein of each lamb was cannulated using 1.00 mm ID medical grade polyethylene tube (Dural Plastics and Engineering, Dural, N.S.W., Australia) to allow blood sampling. Also the bladder was catheterized (10 ch x 3 ml Paediatric Balloon Catheter, Euro Medical Ind., Sussex, England) and urine collection bags (Tuta, Lane Cove, N.S.W., Australia) were fitted to allow continuous urine collection. At 1100 hours at the Armidale site and 1000 hours at the Fowler's Gap site urine collection was commenced and production rate recorded. A sample was retained at regular intervals for 24 hours at the Armidale site and 22 hours at the Fowler's Gap site as shown in Table 5.1. Blood samples were obtained at regular intervals over the respective periods of measurement at both sites as shown in Table 5.1. At approximately 1215 - 1230 hours the normal routine of feed and water intake measurement, feed and water provision and oral salt dosing of HS lambs was undertaken. Urine samples obtained were frozen until analysis. Blood samples were centrifuged immediately after collection and the plasma retained and frozen until analysis.

Plasma and urine osmolarity and Na^+ , Cl^- and K^+ concentrations were estimated as described in section 4.2.1 of Chapter 4. Plasma osmolar, Na^+ , Cl^- and K^+ clearances and urinary osmolar, Na^+ , Cl^- and K^+ excretion rates were calculated. Free water clearance was also calculated. Plasma and urine creatinine concentrations were estimated spectrophotometrically using a Cobas-Bio Automated Centrifugal Analyser (F Hoffmann-La Roche Co. Ltd. Co. Diagnostica, Basle, Switzerland) and used to calculate glomerular filtration rate.

5.2.2 Analysis of Data

Split plot analysis of variance was performed on data. Missing data points were accounted for in the analysis. Further analysis of variance was undertaken when significant interactions between treatment groups and measurement period were observed. An example of the analysis of variance conducted on data is shown in Appendix 2, Table 2.

Table 5.1 Plan of experimental regime for lambs during oral saline dosing at Armidale and Fowler's Gap sites



5.3 RESULTS

The estimations (means $\pm$ SD) of renal function and plasma electrolytes at the Armidale and Fowler's Gap sites during the period of measurement are shown in Table 5.2. Also any statistical differences between the LS and HS lambs at each interval of measurement are shown as superscripts in Table 5.2. The levels of significance attained in the split plot analysis of variance of data are shown in Table 5.3. The period of measurement was representative of the routine treatments that the lambs had received each day for several months.

Plasma osmolarity and Na^+ , Cl^- and K^+ concentrations of the LS lambs at the Armidale site remained relatively stable throughout the measurement period. In contrast after the HS lambs received the oral dose of NaCl the plasma Na^+ and Cl^- concentrations increased and plasma K^+ concentrations decreased. All three electrolytes then gradually returned to approximately pre-dosing levels by the end of the measurement period. The plasma Cl^- responses at the Fowler's Gap site were similar to those observed at the Armidale site but plasma Na^+ for both LS and HS lambs increased and then returned to a level higher than the initial observations. Plasma K^+ concentration changes were similar for both LS and HS lambs at the Fowler's Gap site but the HS lambs generally had a lower concentration of plasma K^+ than the LS lambs throughout most of the measurement period.

Plasma Na^+ concentrations of the LS lambs were higher than the HS lambs at the initial pre-feeding estimations and at the end of the measurement period but this difference was statistically significant only at the Armidale site. For a number of estimations after the HS lambs were given the oral dose of NaCl their plasma Na^+ concentration was higher than the LS lambs but a statistically significant difference was observed only at the Armidale site. Plasma osmolarity differences between LS and HS lambs at both sites were similar to the plasma Na^+ responses between groups at the Armidale site. Plasma Cl^- concentration differences between LS and HS lambs were similar to those observed for plasma Na^+ concentrations although the differences were not as pronounced at the initial and end of measurement period estimations.

The urine osmolarity of LS lambs was always greater than HS lambs. The LS lambs had a gradual increase in urine osmolarity during the

measurement period at the Armidale site but a gradual decrease was observed at the Fowler's Gap site. The HS lambs responded to the oral dose of NaCl with a reduction of urine osmolarity followed by a gradual increase. The increase reached approximately pre-dosing values by the end of the measurement period at the Armidale site but not at the Fowler's Gap site.

Urine flow rate, urine osmolar, Na^+ and Cl^- concentrations and excretion, plasma osmolar, Na^+ and Cl^- clearances and free water clearances of the LS lambs displayed an increased after feeding that peaked towards the middle of the measurement period and then gradually returned to approximately pre-feeding values by the completion of the experiment. After feeding and oral dosing with NaCl the HS lambs demonstrated rapid increases in the parameters just reported for the LS lambs. The increases were substantially greater in the HS lambs than in the LS lambs. After the large increases the HS lambs demonstrated a gradual return to pre-dosing values by the end of the measurement period. The pre-feeding and final measurement levels for urine Na^+ and Cl^- concentration and excretion and plasma Na^+ and Cl^- clearance of the HS lambs were higher than for the LS lambs but urine flow rate, urinary osmolar excretion, plasma osmolar clearance and free water clearance were similar for both groups of lambs.

Relative to pre-feeding levels LS lambs had increased urine K^+ concentration throughout the measurement period and urinary K^+ excretion and plasma K^+ clearance increased until approximately the middle of the experimental period thereafter falling to pre-feeding levels by the end of the measurement period. The pre-feeding and dosing and final measurements of urine K^+ concentration of HS lambs was similar to LS lambs. After the HS lambs were given the oral dose of NaCl urine K^+ concentration quickly fell to a value much lower than that recorded by LS lambs for most of the experiment and plasma K^+ clearance increased quickly then decreased quickly to be lower than LS lambs for most of the experiment.

The glomerular filtration rate (GFR) of LS and HS lambs was similar and very little change occurred over the duration of the experiments. GFR of both groups of lambs at the Fowler's Gap site appeared to be lower than at the Armidale site.

Diarrhoea was evident in the HS lambs approximately 6 hours after the oral dose of NaCl but ceased by approximately 12 hours after the oral dose of NaCl.

LEGEND - Table 5.2

T.G. Treatment Group

Time (hrs) Time (in hours) elapsed after measurements
 commenced

a $P < 0.001$

b $P < 0.01$

c $P < 0.05$

Table 5.2 Renal function and plasma electrolytes (means $\pm$ SD) of lambs during oral saline dosing experiments at Armidale and Fowler's Gap sites. Superscripts denote the level of significance between treatment groups at each point of sampling

ARMIDALE SITE							
T.G. Time (hrs)	Plasma Osmolarity (mosm/l)		Urine Osmolarity (mosm/l)		Urine Flow Rate (ml/min)		
	LS	HS	LS	HS	LS	HS	
1.5	297 $\pm$ 3.1	286 $\pm$ 4.8 ^b	1113 $\pm$ 366	1275 $\pm$ 396	0.87 $\pm$ 0.37	0.72 $\pm$ 0.33	
3	297 $\pm$ 5.2	309 $\pm$ 4.4 ^a	1388 $\pm$ 184	946 $\pm$ 475	0.70 $\pm$ 0.15	3.20 $\pm$ 2.48	
5	297 $\pm$ 3.4	317 $\pm$ 7.8 ^b	1993 $\pm$ 429	923 $\pm$ 282	0.89 $\pm$ 0.24	3.47 $\pm$ 1.98	
7	298 $\pm$ 1.9	305 $\pm$ 3.6 ^b	1645 $\pm$ 691	925 $\pm$ 132	1.14 $\pm$ 0.44	2.90 $\pm$ 0.79	
9	301 $\pm$ 4.1	298 $\pm$ 2.1	1698 $\pm$ 888	1035 $\pm$ 193	1.10 $\pm$ 0.69	2.89 $\pm$ 0.63	
13	299 $\pm$ 1.3	299 $\pm$ 5.4 ^b	1495 $\pm$ 297	875 $\pm$ 635	1.18 $\pm$ 0.26	3.20 $\pm$ 2.27	
21	299 $\pm$ 1.7	289 $\pm$ 4.0 ^b	1838 $\pm$ 385	1057 $\pm$ 164	0.52 $\pm$ 0.34	1.91 $\pm$ 0.67	
24	298 $\pm$ 2.9	291 $\pm$ 3.1 ^a	1905 $\pm$ 683	1150 $\pm$ 572	0.56 $\pm$ 0.43	0.87 $\pm$ 0.39	

T.G. Time (hrs)	Urinary Osmolar Exc. (mosm/min)		Plasma Osmolar Clear. (ml/min)		Free Water Clear. (ml/min)		
	LS	HS	LS	HS	LS	HS	
1.5	0.90 $\pm$ 0.28	0.86 $\pm$ 0.36	3.03 $\pm$ 0.97	3.02 $\pm$ 1.25	-2.16 $\pm$ 0.74	-2.30 $\pm$ 0.98	
3	0.99 $\pm$ 0.30	2.19 $\pm$ 1.09	3.31 $\pm$ 0.98	7.07 $\pm$ 3.48	-2.61 $\pm$ 0.85	-3.87 $\pm$ 1.08	
5	1.70 $\pm$ 0.20	2.92 $\pm$ 1.15	5.72 $\pm$ 0.63	9.20 $\pm$ 3.54	-4.83 $\pm$ 0.42	-5.73 $\pm$ 2.26	
7	1.69 $\pm$ 0.22	2.65 $\pm$ 0.63	5.68 $\pm$ 0.69	8.67 $\pm$ 2.01	-4.54 $\pm$ 0.67	-5.77 $\pm$ 1.36	
9	1.46 $\pm$ 0.27	2.96 $\pm$ 0.66	4.84 $\pm$ 0.94	9.94 $\pm$ 2.26	-3.74 $\pm$ 1.48	-7.05 $\pm$ 1.88	
13	1.71 $\pm$ 0.22	1.87 $\pm$ 0.48	5.73 $\pm$ 0.73	6.30 $\pm$ 1.53	-4.55 $\pm$ 0.58	-3.10 $\pm$ 1.23	
21	0.90 $\pm$ 0.60	1.95 $\pm$ 0.41	3.00 $\pm$ 2.01	6.73 $\pm$ 1.35	-2.48 $\pm$ 1.70	-4.82 $\pm$ 0.72	
24	0.87 $\pm$ 0.42	0.89 $\pm$ 0.31	2.91 $\pm$ 1.38	3.10 $\pm$ 1.11	-2.35 $\pm$ 1.14	-2.19 $\pm$ 1.01	

T.G. Time (hrs)	Plasma Na ⁺ Conc. (mmol/l)		Urine Na ⁺ Conc. (mmol/l)		Urinary Na ⁺ Exc. (mmol/min)		
	LS	HS	LS	HS	LS	HS	
1.5	140 $\pm$ 3.5	131 $\pm$ 2.5 ^b	35 $\pm$ 44	75 $\pm$ 41	0.02 $\pm$ 0.02	0.05 $\pm$ 0.04	
3	135 $\pm$ 2.5	140 $\pm$ 3.5	81 $\pm$ 56	158 $\pm$ 35	0.06 $\pm$ 0.03	0.45 $\pm$ 0.30 ^a	
5	139 $\pm$ 0.6	148 $\pm$ 2.6 ^c	165 $\pm$ 21	215 $\pm$ 69	0.15 $\pm$ 0.05	0.67 $\pm$ 0.26 ^b	
7	136 $\pm$ 1.3	140 $\pm$ 1.7 ^a	113 $\pm$ 42	220 $\pm$ 42	0.12 $\pm$ 0.04	0.63 $\pm$ 0.18 ^b	
9	136 $\pm$ 2.2	136 $\pm$ 2.2	95 $\pm$ 58	239 $\pm$ 61	0.08 $\pm$ 0.04	0.67 $\pm$ 0.10 ^b	
13	137 $\pm$ 1.0	136 $\pm$ 1.5	96 $\pm$ 53	195 $\pm$ 156	0.11 $\pm$ 0.08	0.40 $\pm$ 0.10 ^b	
21	134 $\pm$ 1.3	129 $\pm$ 2.2 ^a	28 $\pm$ 31	156 $\pm$ 35	0.02 $\pm$ 0.02	0.30 $\pm$ 0.09 ^b	
24	130 $\pm$ 3.6	130 $\pm$ 2.5	18 $\pm$ 29	70 $\pm$ 56	0.02 $\pm$ 0.03	0.07 $\pm$ 0.07	

Table 5.2 (continued)

ARMIDALE SITE

T.G. Time (hrs)	Plasma Na Clear. (ml/min)		Plasma Cl Conc. (mmol/l)		Urine Cl Conc. (mmol/l)	
	LS	HS	LS	HS	LS	HS
1.5	0.17 ± 0.15	0.41 ± 0.30	107 ± 3.1	104 ± 1.9 ^b	118 ± 28	236 ± 68
3	0.40 ± 0.23	3.20 ± 2.16 ^a	106 ± 1.6	116 ± 3.4 ^b	163 ± 35	267 ± 84
5	1.07 ± 0.34	4.59 ± 1.76 ^b	108 ± 1.7	125 ± 5.6 ^c	159 ± 47	284 ± 75
7	0.89 ± 0.31	4.52 ± 1.23 ^b	108 ± 3.2	119 ± 4.2 ^b	146 ± 53	291 ± 40
9	0.62 ± 0.29	4.92 ± 0.82 ^c	108 ± 1.7	114 ± 1.9 ^c	128 ± 68	319 ± 68
13	0.83 ± 0.54	2.95 ± 0.74 ^b	110 ± 2.2	112 ± 2.6	177 ± 46	243 ± 183
21	0.14 ± 0.16	2.23 ± 0.65 ^c	109 ± 2.7	105 ± 1.7	194 ± 61	245 ± 66
24	0.13 ± 0.26	0.52 ± 0.57	107 ± 1.6	104 ± 3.9	143 ± 58	198 ± 90

T.G. Time (hrs)	Urinary Chloride Exc. (mmol/min)		Plasma Chloride Clear. (ml/min)		Plasma K Conc. (mmol/l)	
	LS	HS	LS	HS	LS	HS
1.5	0.09 ± 0.04	0.16 ± 0.05	0.91 ± 0.33	1.50 ± 0.47	4.1 ± 0.5	4.1 ± 0.4
3	0.12 ± 0.03	0.72 ± 0.44 ^a	1.08 ± 0.29	6.05 ± 3.62 ^a	3.9 ± 0.3	3.2 ± 0.3
5	0.14 ± 0.05	0.91 ± 0.35 ^b	1.29 ± 0.48	7.29 ± 2.91 ^b	4.0 ± 0.6	3.2 ± 0.4
7	0.15 ± 0	0.84 ± 0.21 ^c	1.39 ± 0.08	7.01 ± 1.74 ^c	4.0 ± 0.3	3.0 ± 0.7
9	0.11 ± 0.04	0.91 ± 0.23 ^c	1.02 ± 0.36	8.02 ± 1.94 ^c	4.0 ± 0.1	3.2 ± 0.4
13	0.21 ± 0.08	0.50 ± 0.10 ^b	1.89 ± 0.67	4.48 ± 0.84 ^b	4.0 ± 0.2	3.7 ± 0.6
21	0.11 ± 0.08	0.44 ± 0.07 ^c	1.04 ± 0.75	4.19 ± 0.72 ^c	3.9 ± 0.3	3.5 ± 0.6
24	0.06 ± 0.03	0.15 ± 0.03 ^b	0.59 ± 0.31	1.46 ± 0.30 ^b	3.6 ± 0.1	3.6 ± 0.4

T.G. Time (hrs)	Urine K Conc. (mmol/l)		Urinary K Exc. (mmol/min)		Plasma K Clear. (ml/min)	
	LS	HS	LS	HS	LS	HS
1.5	163 ± 23	268 ± 102	0.14 ± 0.05	0.17 ± 0.09	32.3 ± 7.0	43.8 ± 15.3
3	220 ± 14	105 ± 69 ^a	0.16 ± 0.04	0.22 ± 0.09	39.8 ± 7.5	69.6 ± 31.2
5	210 ± 42	39 ± 5 ^c	0.18 ± 0.03	0.14 ± 0.08	45.0 ± 6.3	46.2 ± 35.2
7	243 ± 127	36 ± 9 ^a	0.25 ± 0.08	0.11 ± 0.05 ^a	62.8 ± 22.4	40.7 ± 28.4
9	280 ± 175	41 ± 20 ^a	0.23 ± 0.09	0.12 ± 0.05	56.8 ± 21.2	37.3 ± 17.3
13	251 ± 86	35 ± 34 ^b	0.28 ± 0.05	0.06 ± 0.02 ^c	70.4 ± 15.5	17.7 ± 5.7 ^c
21	426 ± 103	74 ± 49 ^c	0.20 ± 0.14	0.13 ± 0.07	53.2 ± 38.8	39.9 ± 16.0
24	354 ± 132	133 ± 152	0.16 ± 0.08	0.08 ± 0.06	45.7 ± 22.3	21.9 ± 15.5

Table 5.2 (continued)

FOWLER'S GAP SITE

T.G. Time (hrs)	Plasma Osmolarity (mosm/l)		Urine Osmolarity (mosm/l)		Urine Flow Rate (ml/min)	
	LS	HS	LS	HS	LS	HS
1			2421 ± 384	1564 ± 156	0.53 ± 0.14	0.88 ± 0.15 ^a
3			2243 ± 310	1480 ± 511	0.52 ± 0.10	1.34 ± 0.43 ^a
5			2053 ± 488	888 ± 204	0.89 ± 0.37	3.94 ± 0.79 ^c
7		N/A	1638 ± 97	846 ± 225	1.04 ± 0.33	3.01 ± 1.11 ^a
12			1595 ± 166	978 ± 400	1.07 ± 0.33	1.81 ± 0.33 ^a
14			1583 ± 185	863 ± 311	1.08 ± 0.52	1.89 ± 1.53
22			1638 ± 154	930 ± 181	0.39 ± 0.16	0.66 ± 0.45

T.G. Time (hrs)	Urinary Osmolar Exc. (mosm/min)		Plasma Osmolar Clear. (ml/min)		Free Water Clear. (ml/min)	
	LS	HS	LS	HS	LS	HS
1	1.29 ± 0.44	1.38 ± 0.35				
3	1.17 ± 0.28	1.85 ± 0.35 ^a				
5	1.75 ± 0.54	3.41 ± 0.63 ^b				
7	1.68 ± 0.45	2.41 ± 0.60		N/A		N/A
12	1.68 ± 0.43	1.74 ± 0.79				
14	1.63 ± 0.66	1.40 ± 0.71				
22	0.64 ± 0.27	0.63 ± 0.45				

T.G. Time (hrs)	Plasma Na Conc. (mmol/l)		Urine Na Conc. (mmol/l)		Urinary Na Exc. (mmol/min)	
	LS	HS	LS	HS	LS	HS
1	132 ± 5.2	127 ± 2.4	9 ± 3	114 ± 106	0.01 ± 0.00	0.11 ± 0.11 ^b
3	133 ± 1.2	139 ± 5.5	20 ± 20	231 ± 22	0.01 ± 0.01	0.31 ± 0.11 ^b
5	144 ± 16.5	140 ± 10.0	108 ± 45	244 ± 52	0.10 ± 0.08	0.94 ± 0.18 ^c
7	143 ± 5.9	134 ± 5.6	123 ± 35	250 ± 66	0.12 ± 0.05	0.71 ± 0.16 ^c
12	138 ± 2.2	134 ± 2.1	123 ± 33	200 ± 141	0.14 ± 0.07	0.38 ± 0.31
14	145 ± 7.6	136 ± 3.0	63 ± 31	191 ± 126	0.08 ± 0.06	0.32 ± 0.23
22	141 ± 8.9	135 ± 5.5	26 ± 18	173 ± 134	0.01 ± 0.00	0.09 ± 0.06 ^a

Table 5.2 (continued)

FOWLER'S GAP SITE							
T.G. Time (hrs)	Plasma Na Clear. (ml/min)		Plasma Cl Conc. (mmol/l)		Urine Cl Conc. (mmol/l)		
	LS	HS	LS	HS	LS	HS	
1	0.04 ± 0.01	0.85 ± 0.82 ^b	91 ± 0.6	90 ± 1.3	230 ± 52	313 ± 143	
3	0.07 ± 0.06	2.24 ± 0.80 ^b	94 ± 0.6	106 ± 1.7 ^c	286 ± 61	367 ± 142	
5	0.72 ± 0.58	6.73 ± 1.43 ^c	95 ± 0.8	111 ± 13.4 ^a	220 ± 48	338 ± 62	
7	0.86 ± 0.33	5.33 ± 1.36 ^c	96 ± 1.3	100 ± 4.8	241 ± 28	312 ± 82	
12	0.98 ± 0.46	2.83 ± 2.31	95 ± 2.2	95 ± 3.4	220 ± 46	263 ± 77	
14	0.54 ± 0.44	2.38 ± 1.75	95 ± 2.2	94 ± 3.0	239 ± 10	284 ± 111	
22	0.86 ± 0.02	0.68 ± 0.45 ^a	91 ± 2.7	90 ± 4.0	224 ± 41	291 ± 142	
T.G. Time (hrs)	Urinary Chloride Exc. (mmol/min)		Plasma Chloride Clear. (ml/min)		Plasma K Conc. (mmol/l)		
	LS	HS	LS	HS	LS	HS	
1	0.12 ± 0.04	0.30 ± 0.17 ^b	1.33 ± 0.39	3.22 ± 1.95 ^b	4.5 ± 0.4	4.6 ± 0.5	
3	0.15 ± 0.02	0.46 ± 0.12 ^c	1.55 ± 0.17	4.38 ± 1.20 ^c	3.9 ± 0.3	3.9 ± 0.4	
5	0.18 ± 0.05	1.31 ± 0.26 ^c	1.93 ± 0.49	11.73 ± 1.26 ^b	4.7 ± 0.7	4.8 ± 0.7	
7	0.24 ± 0.06	0.88 ± 0.20 ^c	2.54 ± 0.62	8.92 ± 2.29 ^a	5.7 ± 0.8	4.7 ± 0.6	
12	0.23 ± 0.07	0.48 ± 0.20	2.40 ± 0.67	5.09 ± 2.01 ^a	5.1 ± 0.3	5.1 ± 0.5	
14	0.26 ± 0.14	0.46 ± 0.23	2.71 ± 1.38	4.88 ± 2.41	5.6 ± 0.4	5.1 ± 0.6	
22	0.09 ± 0.04	0.19 ± 0.12	0.97 ± 0.47	2.10 ± 1.33	5.8 ± 0.5	4.7 ± 0.3	
T.G. Time (hrs)	Urine K Conc. (mmol/l)		Urinary K Exc. (mmol/min)		Plasma K Clear. (ml/min)		
	LS	HS	LS	HS	LS	HS	
1	254 ± 102	195 ± 39	0.12 ± 0.03	0.17 ± 0.06	28.0 ± 8.1	37.4 ± 10.3	
3	310 ± 114	215 ± 50 ^b	0.16 ± 0.07	0.30 ± 0.18	42.6 ± 21.9	80.2 ± 51.2	
5	258 ± 98	66 ± 28 ^c	0.24 ± 0.15	0.25 ± 0.07	52.2 ± 35.8	50.7 ± 8.9 ^a	
7	330 ± 89	46 ± 25 ^c	0.35 ± 0.18	0.13 ± 0.06 ^b	60.2 ± 24.1	27.4 ± 11.9 ^b	
12	368 ± 81	43 ± 27 ^c	0.38 ± 0.12	0.08 ± 0.06 ^c	75.6 ± 26.8	15.9 ± 12.5 ^a	
14	396 ± 29	62 ± 21 ^b	0.42 ± 0.19	0.10 ± 0.04 ^c	75.9 ± 34.7	19.0 ± 7.2 ^a	
22	393 ± 15	144 ± 89 ^b	0.15 ± 0.07	0.12 ± 0.11	27.0 ± 13.8	25.6 ± 23.6	
T.G. Time (hrs)	Glomerular Filtration Rate (ml/min)				Fowler's Gap Site		
	Armidale Site				LS	HS	
3	44.8 ± 8.4	59.9 ± 3.3			35.3 ± 8.3	32.2 ± 10.9	
5	48.4 ± 10.1	47.8 ± 24.0			29.9 ± 3.8	27.8 ± 6.2	
7	45.3 ± 16.0	53.8 ± 9.0			27.8 ± 6.2	34.0 ± 5.2	
9	51.4 ± 10.4	63.8 ± 20.2			30.9 ± 1.7	19.7 ± 8.5	
13	55.2 ± 3.0	50.5 ± 17.7			29.6 ± 2.6	19.3 ± 4.7	
21	46.5 ± 14.1	33.4 ± 14.4			30.9 ± 10.1	22.0 ± 3.6	

Table 5.3 Levels of significance attained in the AOV of data shown in Table 5.2

	ARMIDALE SITE			FOWLER'S GAP SITE		
	Between treat- ments	Over measure- ment period	MPxT	Between treat- ments	Over measure- ment period	MPxT
Plasma Osmolarity	NS	***	***	-	-	-
Urine Osmolarity	*	NS	NS	***	***	*
UFR	**	*	NS	**	**	*
Osmolar Exc.	**	***	NS	*	***	*
Osmolar Clear.	**	***	NS	-	-	-
Free Water Clear.	*	**	NS	-	-	-
Plasma Na ⁺	NS	***	***	*	NS	NS
Urine Na ⁺	*	**	NS	*	**	NS
Na ⁺ Exc.	***	***	**	***	***	**
Na ⁺ Clear.	***	***	**	***	***	**
Plasma Cl ⁻	**	***	***	*	**	*
Urine Cl ⁻	*	NS	NS	NS	NS	NS
Cl ⁻ Exc.	***	**	*	***	***	**
Cl ⁻ Clear.	***	**	*	***	***	**
Plasma K ⁺	*	NS	*	NS	**	NS
Urine K ⁺	**	NS	*	***	*	**
K ⁺ Exc.	*	NS	*	NS	NS	**
K ⁺ Clear.	NS	NS	*	NS	NS	**
GFR	NS	NS	NS	NS	NS	NS

5.4 DISCUSSION

5.4.1 Plasma Osmolarity and Plasma Na^+ , Cl^- and K^+ Concentrations

The LS lambs showed a trend of having higher plasma Na^+ and Cl^- values than HS lambs at the beginning and end of both experiments. The trend was similar to results reported in Chapter 4 (see section 4.4.1) but other factors may have modified the results reported here. Plasma clearance and urinary excretion of Na^+ and Cl^- of the HS lambs were greater than LS lambs both before feeding and salt dosing and at the end of each experiment. This indicates that the HS lambs may have had a residual NaCl load during the initial and final measurements and therefore plasma Na^+ and Cl^- of the HS lambs may have been elevated in comparison to observations made during other experiments (see Ch. 4). When this factor is taken into account the results of the present experiments agree with previous observations when the lambs were given intravenous infusions of saline (Chapter 4, section 4.4.1). The lower plasma osmolarity of the HS lambs before salt dosing at the Armidale site is in agreement with observations reported previously (Chapter 4, section 4.4.1) and gives further evidence that a difference exists in plasma electrolytes between LS and HS lambs.

Although substantial differences in climate exist between the Armidale and Fowler's Gap sites it was evident that after the HS lambs were given their dose of NaCl a substantial elevation of plasma Na^+ and Cl^- occurred at both sites and plasma osmolarity increased at the Armidale site. Tomas *et al.* (1973) reported elevations in plasma Na^+ and Cl^- after consumption of NaCl. The elevation in plasma Na^+ and Cl^- was within the range that sheep can tolerate before viability is impaired as they have been observed to maintain homeostasis with plasma electrolytes much higher (Macfarlane 1967; Tomas *et al.* 1973) than observed in experiments reported here. The plasma concentration and clearance and urinary concentration and excretion of Na^+ and Cl^- increases occurred more slowly during oral saline loading than when lambs (see Chapter 4) or sheep (Potter 1968) were given intravenous NaCl loads. This demonstrates the effect of the NaCl load initially becoming part of the reticulo-rumen water and electrolyte pool.

5.4.2 Renal Function

Renal function of lambs given no supplementation of NaCl and lambs given a high intake of NaCl, at the Armidale site followed a pattern in accord with the relative load of NaCl presented to each group. The HS lambs were able to excrete most of the NaCl load by the end of the experiment. Differences in urine osmolarity, flow rate and Na^+ , Cl^- and K^+ concentration of HS lambs between the Armidale and Fowler's Gap sites may have been due to the Fowler's Gap lambs having a greater upper respiratory tract water loss and hence a greater need to concentrate urine. The GFR of lambs at the Fowler's Gap site was smaller than at the Armidale site which further demonstrates a greater water stress on the Fowler's Gap lambs. Higher plasma clearances and urinary excretion of Na^+ and Cl^- by the HS lambs at the Fowler's Gap site in comparison to the Armidale site provides further evidence that the lambs at Fowler's Gap had a greater water requirement due to the hotter and drier climate.

Large negative free water clearances but a reduction in urine osmolarity after NaCl dosing of HS lambs indicates that although water was being conserved efficiently by the lambs, water was excreted when excreting the NaCl load. The response was similar to that observed after the lambs were given intravenous infusion of hypertonic saline.

5.5 CONCLUSION

The lower plasma Na^+ and Cl^- concentrations and plasma osmolarity of lambs exposed to a high intake of NaCl were similar to observations made previously. This provides further evidence to suggest that a high intake of NaCl causes permanent changes in plasma electrolyte concentrations.

When lambs were exposed to a high intake of NaCl, renal function variables demonstrate that efficient elimination of excessive NaCl occurred. The responses observed indicated that when the lambs were given NaCl by the oral route, renal function was similar to when the NaCl was given intravenously.

Changes in plasma electrolytes, efficient kidney function and transient diarrhoea were in agreement with observations recorded when lambs were given an NaCl load intravenously.

CHAPTER 6

RUMEN WATER, ELECTROLYTES AND EXTRACELLULAR FLUID VOLUME
RESPONSES OF YOUNG SHEEP ACCUSTOMED TO LOW OR HIGH
INTAKE OF SODIUM CHLORIDE

6.1 INTRODUCTION

Large variations in the ingestion of water, electrolytes or both may cause large changes in rumen electrolyte concentrations and water content. This could cause osmoregulatory changes due to the rates at which an electrolyte load disturbs the internal environment of a sheep. Changes in renal excretion of electrolytes (Dobson *et al.* 1966; Warner and Stacy 1972) and rumen electrolyte concentrations (Warner and Stacy 1972), have been demonstrated after alterations in the potassium concentration of diets. Rumen electrolyte concentrations (Potter *et al.* 1972; Tomas and Potter 1975) volumes and fluid outflows (Warner and Stacy 1972; Hemsley 1975) all increased when sheep were given a high intake of NaCl. Salivary flows decreased when sheep were given a high intake of NaCl (Tomas and Potter 1975) or rumen water fill was increased (Wilson 1963). Extracellular fluid volumes of sheep, given a high intake of NaCl by ruminal infusion, have been reported to be increased (Godwin and Williams 1984).

Potter (1972) postulated that the rumen was involved in the tolerances shown by sheep to high intakes of NaCl. The range of modifications reported in extracellular fluid volumes and observations of plasma electrolytes in both sheep and other species may be due to a complete range of functional modifications occurring that allow sheep to achieve osmoregulation when given a high intake of NaCl. This enables sheep to be functionally efficient under extreme conditions that could not be tolerated by many other domesticated animals.

In view of these findings an experiment was carried out to observe rumen and extracellular fluid volume responses of young sheep accustomed to either a low or high intake of NaCl. The young sheep had been used previously for experiments reported in Chapters 3, 4 and 5 and plasma electrolytes and urine electrolytes were measured again to allow comparison with earlier observations (see Chapters 4 and 5).

6.2 MATERIALS AND METHODS

6.2.1 Animals, Treatments and Measurements

Rumen cannulae were inserted surgically in five young sheep (three accustomed to a low intake of NaCl (LS sheep) and two accustomed to a high intake of NaCl (HS sheep)) that had been used in experiments reported in Chapter 3. After a recovery period these sheep were returned to the routine maintenance schedule described in section 3.2.1 of Chapter 3. A number of measurements, as detailed below, were made over a 24 hour period when the lambs were approximately 14 months of age.

On the day of experimentation both jugular veins were cannulated and the bladder catheterized for each animal as described in section 4.2.1 of Chapter 4. Urine collection bags (Tuta, Lane Cove, N.S.W. Australia) were fitted to allow continuous urine collection which commenced at 1100 hours. The rate of urine production was recorded at selected intervals as shown in Table 6.1 and a sample retained for each consecutive period of collection. Five observations were made, at intervals over the next 24 hours, of extracellular fluid volume as shown in Table 6.1. Rumen volume and turnover rate were observed on two occasions as shown in Table 6.1. Extracellular fluid volume was estimated by injecting 1 ml of $\text{Cr}^{51}\text{EDTA}$ (Australian Atomic Energy Commission, Lucas Heights, Australia) into one jugular vein and obtaining blood samples from the opposite vein at 5, 10, 15, 30, 45, 60, 75 and 100 minutes after injection. Cr^{51} was counted in each sample using a gamma counter ('Kontron Gammamatic 1-Automatic Gamma Counting System', W & W Electronics Scientific Instruments, Basle, Switzerland). A regression equation was calculated from the estimations and the Cr^{51} counts extrapolated to zero time by solving the regression equation. Extracellular fluid volume was then calculated by dilution factors.

Rumen volume was estimated by giving a bolus dose of 200 ml of CrEDTA complex, prepared using the methods of Binnerts *et al.* (1968), into the rumen and obtaining rumen samples at 2, 4, 6, 7, 8, 10 and 12 hours after dosing. Samples were centrifuged and filtered and chromium concentration determined by atomic absorption spectrophotometry (S8900 Atomic Absorption Spectrophotometer, GBC Scientific Equipment Pty. Ltd., 7/63 Park Drive, Dandenong, Victoria, Australia). A regression equation was calculated from the estimations of Cr concentration and extrapolated to

zero by solving the regression equation. Rumen volumes were then calculated from dilution factors. Rumen outflow rates were calculated by the methods of Grovum (1971).

Blood, urine and rumen samples were analysed for osmolarity and Na^+ , Cl^- and K^+ concentration at selected intervals as shown in Table 6.1. Plasma and urine estimations were made using the techniques described in section 4.2.1 of Chapter 4. Rumen estimations of osmolarity and Na^+ , Cl^- and K^+ concentration were obtained using similar techniques.

6.2.2 Analysis of Data

Split plot analysis of variance was performed on data obtained for plasma, urine and rumen osmolarity and Na^+ , Cl^- and K^+ concentrations. Other data were compared using a Student's 't' test. Each treatment group contained only a small number of sheep and therefore the possibility of reduced accuracy in the statistical analysis must be considered. Statistical analysis of the data is therefore of limited value in interpreting results. An example of the analysis of variance conducted on data is shown in Appendix 2, Table 2.

6.3 RESULTS

Plasma and urine osmolarity and Na^+ , Cl^- and K^+ concentrations, urine production rate and the levels of significance attained in an analysis of variance of the data are shown in Appendix 4. The above measurements follow similar trends to those reported in Chapters 4 and 5. The levels of significance attained in the statistical analysis of this data did not have a high correlation with similar results reported in Chapters 4 and 5.

Rumen volumes and outflow rates, osmolarity and Na^+ , Cl^- and K^+ concentrations are shown in Table 6.2. The levels of significance attained in an analysis of variance of rumen osmolarity, Na^+ , Cl^- and K^+ concentration are also shown in Table 6.2. Rumen osmolarity increased for up to 12 hours after feeding for the LS sheep and thereafter declined to a relatively stable level. The HS sheep demonstrated a substantial reduction immediately after feeding following by an increase in rumen osmolarity to a level somewhat lower than the LS sheep up until about 12 hours after feeding and oral saline dosing, thereafter declining to a relatively stable value that was similar to that observed for LS sheep.

Rumen Na^+ and Cl^- concentration of the LS sheep were stable for about 12 hours after feeding. For the final period of measurement Na^+ concentration increased to a new plateau but Cl^- concentration decreased to a lower plateau. The HS sheep had substantially higher Na^+ and Cl^- concentration immediately after feeding and saline dosing which persisted for a longer period for rumen Cl^- but after about 12 hours values had become very similar to LS sheep.

Rumen K^+ concentration of LS sheep tended to increase slightly during the initial 12 hour measurement period followed by a large dramatic decrease and then remained relatively stable. The HS sheep had substantially lower K^+ concentration immediately after feeding and dosing when compared to LS sheep. Thereafter K^+ concentration increased to a similar level observed for LS sheep.

Rumen volumes and fluid outflow rates of the LS sheep were lower than those of the HS sheep on both occasions. Student's 't' test did not indicate any significant differences between the groups at either occasion when rumen volume was estimated but the differences may not be statistically significant due to the small sample size. When measurements for each group were pooled a significant difference ($P < 0.001$) existed between the groups.

The extracellular fluid volume (ECF) of both LS and HS sheep increased after feeding then decreased followed by a gradual increase until the end of the measurement period (Figure 6.1). Initial measurements showed the HS sheep to have a greater ECF than LS sheep but this difference had disappeared by about 12 hours after the commencement of the experiment (Figure 6.1). Means and S.D. of ECF and ECF/kg BW and the levels of significance attained in an analysis of variance of the results are shown in Table 6.3. ECF/kg BW followed a similar trend to ECF and no significant differences between LS and HS sheep were observed.

The general health of the sheep was not visibly affected by the experiment. The presence of a transient diarrhoea several hours after oral saline dosing was observed in the HS sheep.

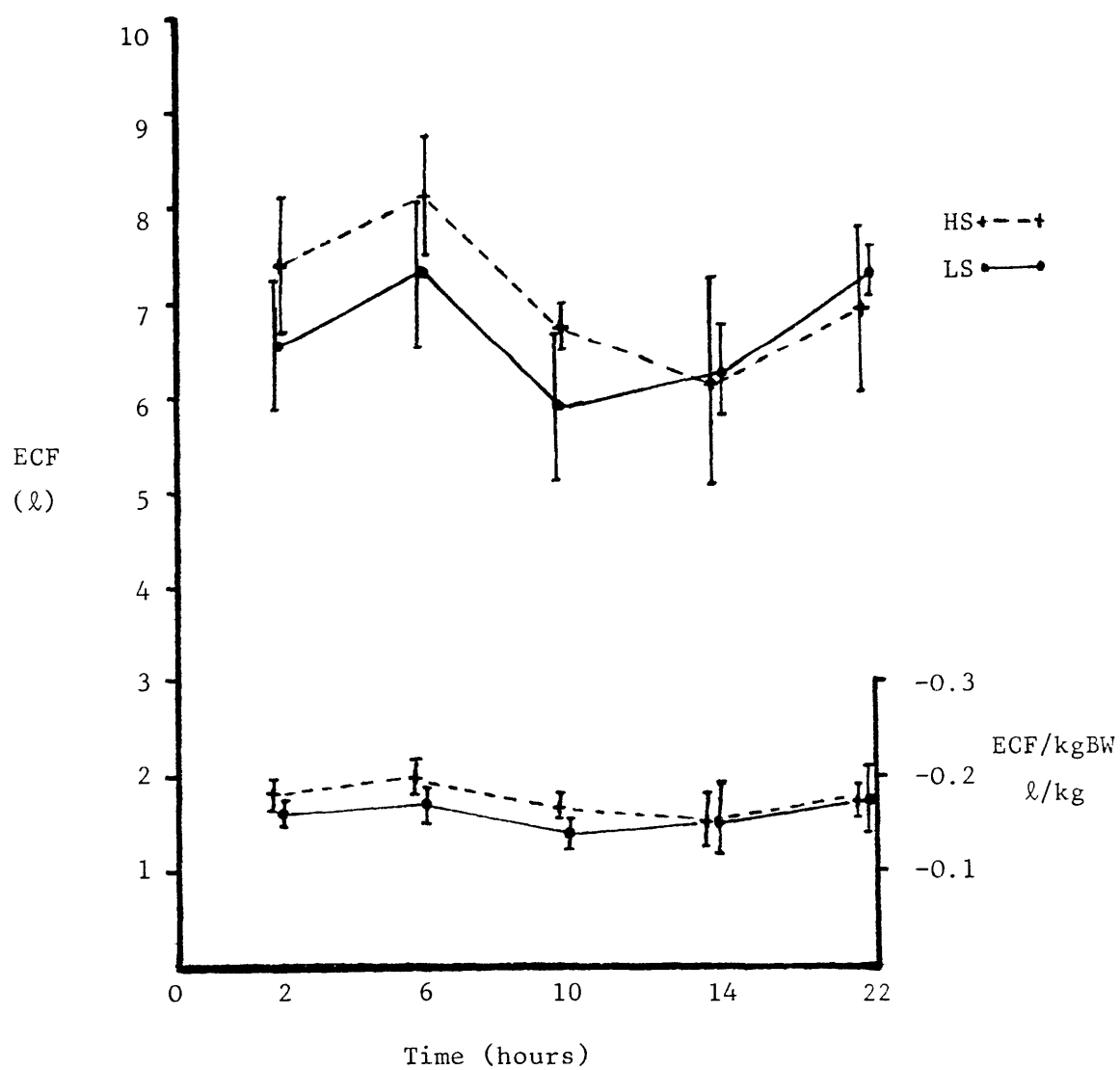


Figure 6.1 Extracellular fluid volume and extracellular fluid volume/kg BW of LS and HS sheep

Table 6.2 Rumen parameters (means $\pm$ SD) measured for LS and HS sheep and the levels of significance attained in AOV of the data except for rumen volume and water turnover rate

T.G. Time (hrs)	Osmolarity (mosm/l)		Na Conc. (mmol/l)		Cl Conc. (mmol/l)	
	LS	HS	LS	HS	LS	HS
2	425 $\pm$ 110	443 $\pm$ 32	99 $\pm$ 26	152 $\pm$ 11	34 $\pm$ 8	74 $\pm$ 21
4	458 $\pm$ 44	308 $\pm$ 110	91 $\pm$ 12	132 $\pm$ 17	29 $\pm$ 6	42 $\pm$ 16
6	453 $\pm$ 25	398 $\pm$ 39	84 $\pm$ 11	80 $\pm$ 0	29 $\pm$ 1	44 $\pm$ 1
8	492 $\pm$ 60	418 $\pm$ 11	87 $\pm$ 8	84 $\pm$ 6	28 $\pm$ 2	42 $\pm$ 0
10	463 $\pm$ 20	403 $\pm$ 32	88 $\pm$ 0	186 $\pm$ 3	23 $\pm$ 3	34 $\pm$ 10
12	363 $\pm$ 32	340 $\pm$ 42	132 $\pm$ 4	124 $\pm$ 6	15 $\pm$ 4	12 $\pm$ 1
8	363 $\pm$ 32	345 $\pm$ 35	117 $\pm$ 12	116 $\pm$ 6	29 $\pm$ 8	19 $\pm$ 0
12	367 $\pm$ 38	350 $\pm$ 29	132 $\pm$ 7	130 $\pm$ 3	18 $\pm$ 5	12 $\pm$ 2

T.G. Time (hrs)	K Conc. (mmol/l)		Fluid Volume (l)		H ₂ O Turnover Rate (l/h)	
	LS	HS	LS	HS	LS	HS
2	52 $\pm$ 14	36 $\pm$ 0	5.8 $\pm$ 0.3	8.2 $\pm$ 0.7	0.705 $\pm$ 0.08	1.136 $\pm$ 0
4	63 $\pm$ 6	36 $\pm$ 11	NS*		NS*	
6	75 $\pm$ 6	58 $\pm$ 3				
8	76 $\pm$ 11	70 $\pm$ 3				
10	68 $\pm$ 7	66 $\pm$ 3				
12	32 $\pm$ 0	36 $\pm$ 6				
8	30 $\pm$ 6	42 $\pm$ 3	6.0 $\pm$ 0.3	7.6 $\pm$ 0.4	0.480 $\pm$ 0.04	0.640 $\pm$ 0
12	33 $\pm$ 5	37 $\pm$ 1	NS*		NS*	

* Levels of significance between LS and HS sheep from Student's 't' test on data

Levels of Significance

	Between Treatment	Over M.P.	M.P.x B.T.
Rumen Osmolarity	NS	*	NS
Na	NS	*	NS
Cl	*	**	*
K	NS	**	NS

Legend as for Table 5.2

Table 6.3 Extracellular fluid volume and extracellular fluid volume/kg BW of LS and HS sheep (means $\pm$ SD) and levels of significance between treatment groups

Treatment group Time (hrs)	ECF (ℓ)		Levels of signifi- cance	ECF/kg BW (ℓ/kg)		Levels of signifi- cance
	LS	HS		LS	HS	
2	6.54 $\pm$ 0.64	7.37 $\pm$ 0.84	NS	0.16 $\pm$ 0.02	0.18 $\pm$ 0.02	NS
6	7.35 $\pm$ 0.88	8.07 $\pm$ 0.66	NS	0.17 $\pm$ 0.03	0.20 $\pm$ 0.02	NS
10	5.94 $\pm$ 0.88	6.78 $\pm$ 0.22	NS	0.14 $\pm$ 0.02	0.17 $\pm$ 0.01	NS
14	6.25 $\pm$ 0.51	6.17 $\pm$ 1.10	NS	0.15 $\pm$ 0.04	0.15 $\pm$ 0.03	NS
22	7.22 $\pm$ 0.25	6.98 $\pm$ 0.91	NS	0.17 $\pm$ 0.04	0.17 $\pm$ 0.02	NS

Legend as for Table 5.2

6.4 DISCUSSION

The plasma and urine osmolarity and electrolyte concentration responses of HS sheep after oral saline dosing and the differences between LS and HS sheep during the experimental period were similar to observations reported in Chapters 4 and 5. Transient diarrhoea was also observed in the HS sheep during the experiments reported here. The results indicate that the LS and HS sheep responded in a similar manner to that observed in experiments reported in Chapters 4 and 5.

Rumen osmolarity and electrolyte responses were similar for both LS and HS sheep except for an initial period of a few hours immediately after oral saline dosing of the HS sheep and some minor variations. The high Na^+ and Cl^- concentrations and lower osmolarity and K^+ concentrations soon after saline dosing are a result of the HS sheep drinking a large volume of water immediately after dosing thereby reducing osmolarity and K^+ but still leaving a relatively high concentration of Na^+ and Cl^- . The ability of the HS sheep to adjust rumen osmolarity and electrolytes to levels similar to those observed in LS sheep within about 6-8 hours is remarkable and illustrates an ability to rapidly manipulate the rumen environment. Potter, Walker and Forrest (1972) reported different responses when sheep were continuously fed for most of the day and given drinking water containing 1.3% NaCl. Undoubtedly the different experimental regime utilised during the experiments reported here would give rise to different rumen responses. The changes observed in rumen osmolarity and electrolytes during the 24 hour measurement period indicates that dramatic changes occur when sheep are exposed to once daily feeding and oral saline dosing. The experimental regime of once daily feeding and saline dosing may be similar to the pattern of feeding and drinking that sheep could encounter when grazing salt bush (*Atriplex* sp.) in arid situations. The values of osmolarity and electrolyte concentrations of the rumen reported by Tomas and Potter (1975) do not appear to show much similarity to the results reported in this experiment. When the observations are averaged over the measurement period for this experiment then values obtained are similar to those of Tomas and Potter (1975). Results obtained for rumen environmental conditions, of sheep continuously fed or where estimations are made of bulked samples, do not appear to give a good indication of the daily functional responses that occur in sheep consuming different diets or different levels of NaCl. Rumen volumes of the LS sheep were lower than the HS sheep demonstrating that HS

sheep had a greater water content than LS sheep. The lower rumen osmolarity of HS sheep supports this possibility.

The increases in ECF in both LS and HS sheep after feeding would be expected due to water and electrolyte intakes during food intake which would then give rise to ECF expansion. The higher ECF of the HS sheep is an indication of the higher intake of Na and Cl causing a need for ECF to be expanded to a greater extent to keep plasma Na and Cl concentrations as close as possible to normal. About 12 hours after feeding ECF of both LS and HS groups had merged to similar values maintaining the similarity for the rest of the measurement period. The time of sampling could therefore affect the results obtained when sheep are given high intakes of NaCl. The effect of NaCl in large amounts does cause ECF to increase but in this experiment the sheep were able to quickly return to a level similar to sheep given a low intake of NaCl.

Both LS and HS sheep, for about 10-12 hours after feeding, had a similar pattern of response for plasma, urine and rumen parameters and ECF. The HS sheep demonstrated a dramatic increase in some parameters in response to the higher load of NaCl. About 12 hours after feeding both groups of sheep had a relatively stable period where the parameters measured were at similar levels in both groups of sheep. The responses observed indicate that the rumen, body fluid and electrolyte compartments and renal function are intimately related in maintaining fluid and electrolyte homeostasis.

Evidence presented shows that young sheep given a high intake of NaCl utilised a number of mechanisms to enable efficient elimination of NaCl. ECF and plasma electrolytes were allowed to increase without any observable deleterious effects. Water intake was rapidly increased to help dilute the high concentration of NaCl in the rumen and renal excretion increased quickly. The HS sheep made adjustments to enable the high intake of NaCl to be rapidly partitioned in the body and then excreted within about 12 hours. The effects of other electrolytes, notably K^+ , and other substances ingested exerted an appreciable effect on osmoregulatory responses. When considered in association with reported toxic effects of NaCl in other species (Boyd 1973) it may be suggested that sheep have an upper threshold of NaCl intake which is determined by a combination of mechanisms that allows successful elimination of a NaCl load.

CHAPTER 7

GENERAL DISCUSSION

7.1 RESPONSES OF YOUNG SHEEP TO CHRONIC HIGH INTAKE OF SODIUM CHLORIDE

7.1.1 Growth Rate and Wool Production

Results obtained from animal house studies showed that lambs given a chronic intake of NaCl, close to maximum tolerable limits, attained a lower liveweight after several months than lambs given no NaCl supplementation. The reduction in growth rate was evident even though growth rates of treatment and control lambs were relatively high (Church 1973). Wool growth of NaCl supplemented lambs appeared to be depressed but more definitive evidence from larger numbers of sheep is required to establish this.

The effects of high intakes of NaCl especially in a pastoral situation are likely to depend on the nutritional and climatic conditions and these factors need to be considered when evaluating experimental results. The reported variations in growth rates (Meyer and Weir 1954; Peirce 1968 a,b) and wool production (Meyer and Weir 1954; Peirce 1959, 1968b; Hemsley 1975) may be associated with differences in these conditions.

7.1.2 Water Intake, Food Intake and Urine Output

Sodium chloride supplementation of lambs was accompanied by increases in water intake and urine output but food intake was reduced. These changes were similar to those reported by other investigators (see Chapter 2). The water intake per gram of NaCl intake in the experiment reported here was similar to results reported for other long term experiments (Meyer and Weir 1954; Peirce 1968a) but was substantially lower than results from short term experiments (Wilson 1966b,c). The cause of these differences needs clarification. Water intake to food intake relationship was altered and shifted to a new plane when compared with control lambs. When allowances were made for changes in maximum daily air temperatures effects were observed only for urine output which indicates that body function was partitioned to allow maximal rates of food intake.

The changed water and food intake and urine output as well as a more rapid consumption of water by the NaCl supplemented lambs indicates that

the water and electrolyte pools and fluxes were altered. The changes observed when considered in association with behavioural patterns recorded in arid zones (Lynch 1974), indicates that sheep would have to make major adjustments to maintain homeostasis when exposed to high intakes of NaCl in those situations.

7.1.3

(a) Renal Function

When NaCl supplemented lambs were given intravenous infusions of hypertonic saline, measurements of renal function parameters indicated rapid and efficient kidney responses to the NaCl load which were maintained with increasing age. Some absolute increases in renal functions were observed as lambs increased in age. Renal function responses when the NaCl supplemented lambs were given an oral dose of NaCl were similar to responses after intravenous infusion of hypertonic saline except for a time lag and some other variations associated with NaCl initially entering the rumen. On the occasions when control group lambs were subjected to intravenous infusions of hypertonic saline they demonstrated renal responses similar to the NaCl supplemented lambs. There was no evidence to indicate that any differences in renal capabilities or function existed or developed between the NaCl supplemented and control lambs from an early age until the experiments concluded at eight months of age.

Renal responses of lambs after being subjected to NaCl challenges were characterised by increased glomerular filtration rate, renal plasma flow, urine production rate, urine concentrations of Na^+ and Cl^- and osmolar, Na^+ and Cl^- urinary excretion and plasma clearances. Although urinary K^+ concentrations were reduced the urinary excretion and plasma clearance remained relatively stable. The excretion of electrolytes appeared to have a diurnal pattern associated with the feeding and saline dosing of the lambs. These responses reflect the accuracy of response to changes in electrolyte loads. The extent of diuresis, reductions in urine osmolarity and increased negative free water clearances were associated with NaCl loading but modifications in the level of response occurred due to other factors that could not be accurately defined in these experiments. Variation in temperature, solar radiation and consumption of bore

water were probably important factors. The effect of the rumen on water and electrolyte movements may also have been important.

The amount of water, electrolytes or both needing to be excreted from the body determines the concentration of solutes in urine (i.e. urine osmolarity). Urine osmolarity during this series of experiments was about 800-1200 mosm/l when the lambs were not stressed which is similar to other reported observations for sheep in 'normal' situations (Potter 1963; Macfarlane 1967; Wilson and Dudzinski 1973). When the stresses of high air temperature and radiant loads were imposed urine concentrations of over 2000 mosm/l were observed. The largest negative free water clearances tended to be associated with the highest urine osmolarities but increased negative free water clearances were observed after NaCl challenges even though urine osmolarity had decreased. During the series of experiments reported here a high NaCl load was associated with extra water in the body and consequently the kidneys had to excrete water as well as Na^+ and Cl^- . Therefore even though water was being used efficiently as shown by the increased negative free water clearance substantial amounts of water were being excreted (in association with Na^+ and Cl^-) which was demonstrated by decreased urine osmolarity. During more stressful situations where temperature and radiation loads were relatively high and water requirements were greater, water was conserved still more efficiently and consequently urine osmolarity remained constant or even increased during saline challenges. The patterns of response observed in urine osmolarity and free water clearance reflect the level of water or electrolyte excretion required in particular situations.

(b) Plasma Electrolyte Concentrations

Plasma Na^+ , Cl^- and osmolarity increased substantially on all occasions when lambs received NaCl challenges but plasma K^+ concentration decreased. These changes reflect the relative electrolyte and water changes that occurred (see later discussion on extracellular fluid). Plasma Na^+ , Cl^- and osmolarity of NaCl supplemented lambs were lower than control group lambs suggesting modification of water-electrolyte relationships in the extracellular pool and possibly changes in intracellular water content. Sheep exposed to high intake of NaCl have been shown to have increased total body water (Jones *et al.* 1970) and the lower values of plasma electrolytes may be a simple dilution effect. Unless total body water is expressed on a fat free bodyweight basis confusion can arise when

attempting to interpret the plasma electrolyte changes observed in these experiments.

Some observations during the present experiments are in contrast to some previously reported results (see Chapters 2 and 4) and further clarification of the effects of high intake of NaCl on plasma electrolyte concentrations is required.

(c) Extracellular Fluid Volume

The volume of extracellular fluid of NaCl supplemented young sheep was probably increased by intake of NaCl. However the influence of factors, such as food and water intake appeared to have an effect in the experiment reported here. There did not appear to be any permanent alterations in extracellular fluid volume due to NaCl supplementation of young sheep. A diurnal pattern of variation in extracellular fluid volume was evident.

The prevailing environmental conditions, time after NaCl, food and water ingestion possibly affects the volume of extracellular fluid observed at any one point of time. These factors may be important in understanding plasma electrolyte changes (discussed previously) in relation to extracellular fluid volume.

(d) Rumen Function

The changes in rumen Na^+ , Cl^- and K^+ concentrations osmolarity and the time course of events of NaCl supplemented lambs after oral saline dosing were similar to changes that occurred in the urine produced after similar oral saline dosing. After oral saline dosing rumen osmolarity decreased, Na^+ and Cl^- concentrations increased and K^+ concentration decreased. The rumen parameters returned to values similar to control sheep in approximately 10-12 hours. Rumen water volume and turnover rate appeared to increased but no pattern of response was seen due to insufficient sampling points. False interpretation of rumen conditions can occur from insufficient sampling points as Corbett and Pickering (1983) have demonstrated. No permanent alterations in rumen electrolytes or osmolarity appeared to have occurred in the NaCl supplemented sheep. These results indicate that the rumen exerts a substantial

influence on rates and patterns of water and electrolyte movement after NaCl ingestion. The rates of movement appear to be well regulated and a diurnal pattern of response probably exists.

7.1.4 General Health and Diarrhoea

All animals appeared to be free from discernable diseases. Diarrhoea and depressed food and water intake was repeatedly observed after several weeks of saline dosing. This was reversed by stopping the saline dosing for a few days. These observations are similar to reported toxicities associated with chronic intake of NaCl by sheep (Blood and Henderson 1974) and rats (Boyd 1973). This syndrome may be attributable to a slow increase in Na^+ and Cl^- levels resulting from incomplete excretion of NaCl. High levels may have been reached precipitating a situation where physiological functions of the large intestine change to allow excretion of excess Na^+ , Cl^- and water.

During acute experiments involving intravenous infusion of hypertonic saline or oral saline dosing, transient diarrhoea was observed in the NaCl supplemented lambs. The rapidity of onset of diarrhoea after NaCl challenge indicates that the effect is not due to passage of digesta through the gastrointestinal tract. Diarrhoea was observed to commence at about the time when kidney excretion of Na^+ and Cl^- was high and rumen and plasma concentration of Na^+ and Cl^- were still elevated. Similarly diarrhoea ceased when kidney excretion and rumen and plasma concentration of Na^+ and Cl^- had returned to relatively normal levels.

The diarrhoea may be associated with interactions between body systems that allows the lower intestinal tract to be utilised for excretion of NaCl and probably water as well when very high NaCl loads are given to sheep. The effects of mineralocorticoids especially aldosterone on the large intestine are essentially similar to the effects on the renal system (Forman and Mulrow 1975; Foster, Zimmerman, Hayslett and Binder 1983) and the importance of the hindgut in retaining electrolytes and water under normal conditions is well documented (Ulyatt, Dellow, Reid and Bauchop 1975). The alterations in hormone levels associated with increased kidney excretory functions possibly explains why diarrhoea occurred in the lambs.

Casual observations during routine animal maintenance indicated that diarrhoea did not occur before daily feeding and saline dosing but was present several hours later. More detailed observations of the pattern of occurrence of diarrhoea during regular saline dosing may have shown that a similar pattern existed as occurred during acute experiments.

7.2 GENERAL SYNOPSIS

High intake of NaCl by young sheep for several months caused detrimental effects which were reflected in reduced growth and wool production. The principal cause of this effect was reduced food intake. This effect was in agreement with some reported observations but not others (see Chapter 2). The alterations in water and electrolyte pools and fluxes that must have occurred were obvious when renal function, plasma electrolytes, extracellular fluid volume and rumen function were measured after lambs were given NaCl challenges. No evidence was found to suggest that chronic NaCl intake caused permanent modifications of extracellular fluid volume, renal function and rumen function parameters measured but plasma Na^+ and Cl^- concentrations were permanently lowered. The well defined phasic responses observed for many of the parameters measured appeared to follow a diurnal pattern of well regulated sequences and time courses as shown in Figure 7.1. Differences in the rate of response occurred depending on whether the NaCl was administered intravenously or orally. Integration of body systems to allow dispersal of NaCl and water throughout the different pools and excrete the excesses over a period of several hours appears to have occurred.

When the lambs were challenged with a high NaCl load a complex response involving efficient function of several body systems, behavioural changes and probably alterations in circadian rhythms occurred that allow tolerance of high intake of NaCl. This series of experiments did not identify any permanent adaptations that occurred in young sheep that would increase tolerance to high loads of NaCl. The reduction in plasma Na^+ and Cl^- concentration of NaCl supplemented lambs was the only exception and the cause of this change cannot be readily defined.

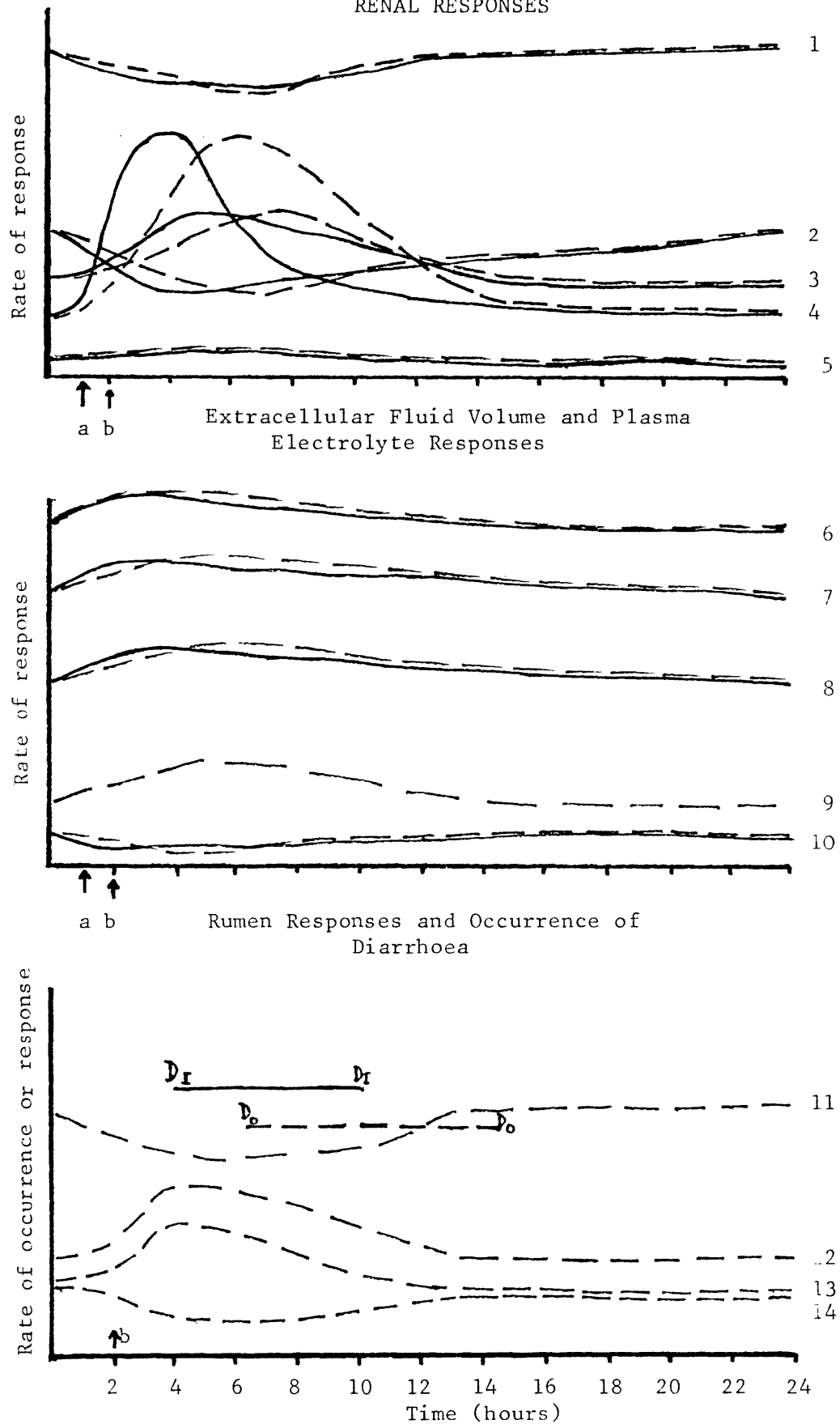


Figure 7.1 Responses of different parameters after NaCl challenge grouped within functional systems, for 24 hours during acute experiments. No absolute values but general magnitude of response is shown

LEGEND FOR FIGURE 7.1

- (1) Urine osmolarity
- (2) Urine K^+ concentration
- (3) Urine Na^+ and Cl^- concentration and excretion rate
Urine osmolar excretion rate
Plasma Na^+ and Cl^- and osmolar clearance
Negative free water clearance
- (4) Urine flow rate
- (5) K^+ urinary excretion rate and plasma clearance
- (6) Plasma osmolarity
- (7) Plasma Na^+ concentration
- (8) Plasma Cl^- concentration
- (9) Extracellular fluid volume
- (10) Plasma K^+ concentration
- (11) Rumen osmolarity
- (12) Rumen Na^+ concentration
- (13) Rumen Cl^- concentration
- (14) Rumen K^+ concentration

D_I Diarrhoea with intravenous saline experiment
 D_O Diarrhoea with oral saline dosing experiment

— — — Oral saline dosing experiment
 ——— Intravenous saline experiment

a Start of 10% NaCl infusion
 b Feeding and oral saline dosing

7.3 MECHANISMS UTILISED BY SHEEP THAT ALLOW TOLERANCE TO NaCl CHALLENGE

Evidence reported in the literature and results obtained in the series of experiments reported here indicates that sheep have an ability to tolerate a high load of NaCl. When sheep are challenged by a NaCl load the whole body is affected and a complex response occurs to enable maintenance of homeostasis.

Food, water or both are the normal sources of NaCl entry into sheep and so the upper gastrointestinal tract is the initial part of the body affected by a high intake of NaCl. After ingestion of NaCl, sheep attempt to dilute rumen contents to bring osmolarity within a range considered satisfactory. This necessitates a substantial intake of water if it is available and saliva may also contribute. Rumen osmolarity is normally maintained at a level isotonic or slightly hypertonic to plasma (Tomas and Potter 1975) but hypertonicity occurs after food intake (see Chapter 6). In the experiments reported here rumen Na^+ and Cl^- concentrations were substantially elevated by NaCl ingestion. The amount and source of NaCl, type of diet and patterns of food intake affect salivary responses and rumen water and electrolyte concentration and fluxes (see Chapter 2). The availability of water also affects the rumen response. The large storage capacity of the rumen is probably used in alleviating the effects of a high intake of NaCl. A rapid flux of Na^+ , Cl^- and water into the extracellular fluid occurs (see Chapter 2) and the rate of passage into the abomasum and small intestine may increase with a high intake of NaCl. Changes in rumen functions on a diurnal basis determined by circadian controls may also be important. The rumen appears to serve an important function in osmoregulation after ingestion of large amounts of NaCl.

After ingestion of excess amounts of NaCl extracellular fluid volume and plasma Na^+ and Cl^- concentration are elevated and so an increased load of Na^+ , Cl^- and water (in many situations) needs to be excreted by the kidney and possibly the large intestine. The renal system responds rapidly and efficiently in excreting the excess Na^+ , Cl^- and water (if necessary) as the experiments reported here indicate. No permanent modifications of kidney function appear to occur due to chronic high intake of NaCl (see Chapter 4). The rate and intensity of renal response varies according to the demands for water for other purposes in the body, the size of NaCl load and in the experiments reported here the size of the water load. The sheep kidney normally produces urine with a

concentration of about 800-1200 mosm/l (see earlier discussion) and only produces a more concentrated urine when a need to conserve water exists. In the experiments reported here, in many instances, although the Na^+ and Cl^- concentration of urine was high urine osmolarity was relatively low. This situation is a result of the need to excrete excess water as well as Na^+ and Cl^- . The concentration of urine produced is a result of the kidney performing an osmoregulatory role and not simply acting as an excretory organ.

Changes in hormone levels that permit close to maximal excretion of Na^+ and Cl^- and, in the experiments reported here, substantial excretion of water probably affects large intestine function in sheep. The occurrence of diarrhoea is probably due to a net flux of water and electrolytes into the large intestine. The large intestine would therefore have an enhanced excretory role during periods of high NaCl load.

Integration of body systems and functions to allow controlled and efficient dispersal and excretion of excess Na^+ , Cl^- and water occurs in sheep subjected to a NaCl challenge. A diagrammatic model shown in Figure 7.2 indicates the interactions and interrelationships that occur. The time course of events and diurnal patterns of response further indicate that complex integration and possibly circadian rhythms are important when sheep experience a NaCl challenge. The role of intracellular spaces in tolerance to NaCl loads is not well understood and requires further study. Reductions in plasma electrolytes of lambs receiving chronic high intake of NaCl is difficult to evaluate and clarification of this phenomenon is required.

The experiments reported here indicate sheep maximise tolerance to a high intake of NaCl by optimising physiological function of body systems, and possibly by circadian rhythm and behavioural changes. Genotype determines the maximum capacity an animal has when it is stressed and the genotype-phenotype interactions then determine the functional maximum of an animal. These interactions determined the tolerance of sheep to a high intake of NaCl in a particular set of circumstances.

Information obtained from the experiments demonstrates the importance of integration of body systems and functions for maintenance of homeostasis under stresses such as NaCl challenge. Alterations in Na^+ , Cl^- and water pools and fluxes associated with a high intake of NaCl cause changes in body function that are detrimental to the maintenance of growth and wool

production in sheep. The rate of function of renal and gastrointestinal systems, body Na^+ , Cl^- and water pools and fluxes change to allow tolerance of NaCl loads by sheep. No permanent changes in renal or gastrointestinal system function appear to occur. The need to measure patterns of response to indicate the mechanisms utilised to allow tolerance to NaCl challenge has been demonstrated. Understanding the mechanisms sheep utilise to allow tolerance to a high intake of NaCl when combined with knowledge of grazing behaviour and arid zone ecology may allow better strategies to be devised for sheep management in arid zones.

The effects of circadian rhythms on animal function and behavioural responses under stress such as high intake of NaCl requires further elucidation. Relationships between body composition and electrolyte-water pools needs to be clarified to fully understand the effects of a high intake of NaCl. The function and hormonal control of the large intestine during the stress of a NaCl load requires further study. Further research in these areas should provide useful information to aid in understanding osmoregulation especially during stress.

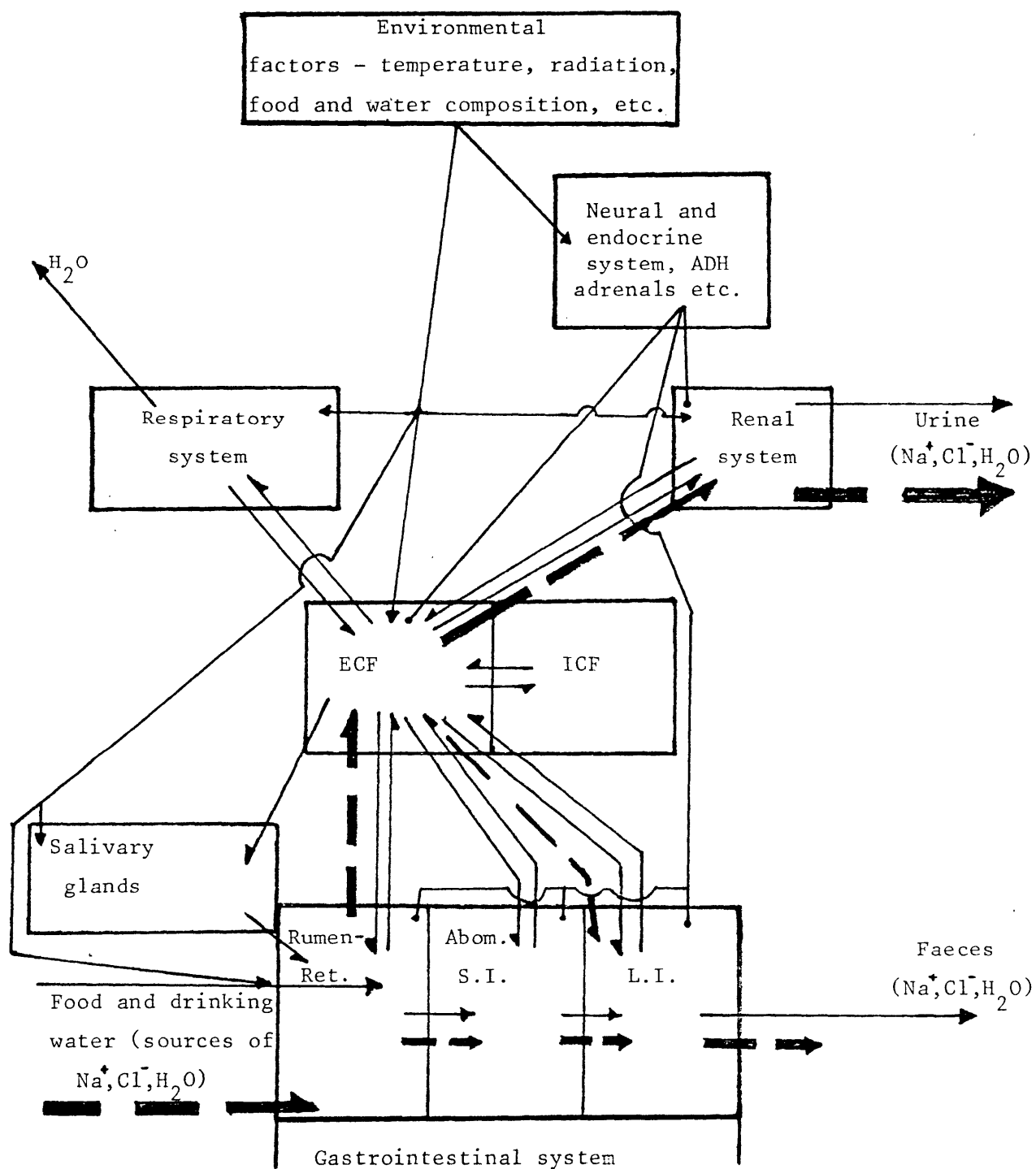


Figure 7.2 Diagrammatic model of Na, Cl and water pools and fluxes and system function of sheep indicating the major interactions and interrelationships that affect responses to NaCl challenge. Solid lines represent normal conditions and broken lines effects of NaCl challenge.

APPENDIX 1

Means $\pm$ Standard deviations for Armidale site lambs for BW from 0-35 weeks of age
 FI from 3-35 weeks of age and for W1 and UO from 6-35 weeks of age

Age	Body Weight (kg)		Av. Daily Food Intake (kg)		Av. Daily Water Intake (L)		Av. Daily Urine Output (L)	
	LS	HS	LS	HS	LS	HS	LS	HS
0	4.44 $\pm$ 0.31	3.75 $\pm$ 0.61						
1	5.25 $\pm$ 0.84	4.44 $\pm$ 0.94						
2	6.94 $\pm$ 1.09	5.38 $\pm$ 1.11						
3	7.55 $\pm$ 1.22	6.13 $\pm$ 1.31	0.39 $\pm$ 0.08	0.32 $\pm$ 0.07				
4	8.08 $\pm$ 1.29	7.13 $\pm$ 1.49	0.28 $\pm$ 0.05	0.24 $\pm$ 0.10				
5	7.55 $\pm$ 1.84	6.38 $\pm$ 0.93	0.17 $\pm$ 0.10	0.16 $\pm$ 0.05				
6	9.58 $\pm$ 1.97	8.00 $\pm$ 0.88	0.35 $\pm$ 0.10	0.35 $\pm$ 0.05				
7	10.55 $\pm$ 1.68	8.70 $\pm$ 1.01	0.47 $\pm$ 0.04	0.40 $\pm$ 0.09				
8	11.30 $\pm$ 1.51	10.10 $\pm$ 0.84	0.50 $\pm$ 0.02	0.48 $\pm$ 0.04				
9	12.30 $\pm$ 1.91	11.50 $\pm$ 1.04	0.63 $\pm$ 0.06	0.59 $\pm$ 0.04				
10	13.80 $\pm$ 2.05	13.50 $\pm$ 1.04	0.69 $\pm$ 0.12	0.69 $\pm$ 0.02	0.76 $\pm$ 0.25	1.05 $\pm$ 0.09	0.22 $\pm$ 0.05	0.50 $\pm$ 0.07
11	16.08 $\pm$ 2.26	15.35 $\pm$ 0.72	0.76 $\pm$ 0.06	0.71 $\pm$ 0.04	1.01 $\pm$ 0.23	1.47 $\pm$ 0.22	0.32 $\pm$ 0.09	0.71 $\pm$ 0.09
12	16.85 $\pm$ 1.18	16.45 $\pm$ 1.06	0.79 $\pm$ 0.03	0.75 $\pm$ 0.04	1.15 $\pm$ 0.09	1.55 $\pm$ 0.14	0.40 $\pm$ 0.08	0.70 $\pm$ 0.16
13	19.45 $\pm$ 1.68	18.30 $\pm$ 0.62	0.92 $\pm$ 0.06	0.77 $\pm$ 0.05	1.34 $\pm$ 0.20	2.03 $\pm$ 0.20	0.45 $\pm$ 0.06	0.94 $\pm$ 0.16
14	20.65 $\pm$ 1.94	19.30 $\pm$ 0.38	0.95 $\pm$ 0.14	0.86 $\pm$ 0.04	1.90 $\pm$ 0.27	2.87 $\pm$ 0.26	0.64 $\pm$ 0.09	1.25 $\pm$ 0.16
15	22.15 $\pm$ 2.38	21.30 $\pm$ 0.77	1.01 $\pm$ 0.19	0.92 $\pm$ 0.04	2.35 $\pm$ 0.32	3.11 $\pm$ 0.14	0.77 $\pm$ 0.14	1.36 $\pm$ 0.14
16	24.00 $\pm$ 2.59	24.15 $\pm$ 2.11	1.15 $\pm$ 0.18	0.98 $\pm$ 0.07	2.46 $\pm$ 0.28	3.27 $\pm$ 0.31	0.90 $\pm$ 0.31	1.81 $\pm$ 0.39
17	25.50 $\pm$ 3.24	24.55 $\pm$ 2.11	1.28 $\pm$ 0.23	1.02 $\pm$ 0.24	2.52 $\pm$ 0.30	3.15 $\pm$ 0.79	1.08 $\pm$ 0.12	1.73 $\pm$ 0.57
18	27.45 $\pm$ 3.59	27.55 $\pm$ 1.11	1.29 $\pm$ 0.20	1.14 $\pm$ 0.08	2.99 $\pm$ 0.36	3.88 $\pm$ 0.44	0.94 $\pm$ 0.06	1.77 $\pm$ 0.33
19	27.25 $\pm$ 3.12	25.18 $\pm$ 1.03	1.18 $\pm$ 0.13	1.15 $\pm$ 0.08	3.01 $\pm$ 0.57	4.26 $\pm$ 1.07	0.87 $\pm$ 0.12	1.64 $\pm$ 0.47
20	28.90 $\pm$ 3.23	28.75 $\pm$ 1.53	1.20 $\pm$ 0.17	1.19 $\pm$ 0.11	3.10 $\pm$ 0.47	5.10 $\pm$ 1.38	0.95 $\pm$ 0.26	2.05 $\pm$ 0.51
21	29.78 $\pm$ 3.33	28.75 $\pm$ 1.53	1.30 $\pm$ 0.07	1.22 $\pm$ 0.08	3.49 $\pm$ 0.88	4.69 $\pm$ 0.88	0.94 $\pm$ 0.24	1.84 $\pm$ 0.45
22	31.05 $\pm$ 3.71	28.30 $\pm$ 1.87	1.31 $\pm$ 0.12	0.93 $\pm$ 0.31	3.78 $\pm$ 0.88	4.83 $\pm$ 0.38	0.94 $\pm$ 0.24	2.22 $\pm$ 0.23
23	33.10 $\pm$ 3.90	31.75 $\pm$ 1.40	1.35 $\pm$ 0.12	1.16 $\pm$ 0.11	3.59 $\pm$ 0.26	4.80 $\pm$ 0.40	0.78 $\pm$ 0.13	2.14 $\pm$ 0.27
24	32.65 $\pm$ 3.96	29.95 $\pm$ 2.43	1.31 $\pm$ 0.14	1.07 $\pm$ 0.12	3.81 $\pm$ 0.39	5.36 $\pm$ 0.64	0.88 $\pm$ 0.17	1.88 $\pm$ 0.54
25	35.20 $\pm$ 3.37	33.10 $\pm$ 1.83	1.37 $\pm$ 0.07	1.22 $\pm$ 0.04	4.04 $\pm$ 0.19	5.47 $\pm$ 0.43	0.72 $\pm$ 0.12	1.73 $\pm$ 0.23
26	36.95 $\pm$ 4.04	35.50 $\pm$ 2.78	1.46 $\pm$ 0.13	1.28 $\pm$ 0.06	4.72 $\pm$ 0.63	4.93 $\pm$ 0.69	0.82 $\pm$ 0.16	1.73 $\pm$ 0.54
27	38.05 $\pm$ 4.00	34.55 $\pm$ 3.65	1.48 $\pm$ 0.16	1.25 $\pm$ 0.10	4.06 $\pm$ 0.57	4.21 $\pm$ 0.74	0.76 $\pm$ 0.19	1.06 $\pm$ 0.63
28	38.30 $\pm$ 3.74	35.25 $\pm$ 4.51	1.48 $\pm$ 0.14	1.19 $\pm$ 0.15	4.20 $\pm$ 0.40	5.62 $\pm$ 0.27	0.74 $\pm$ 0.11	2.43 $\pm$ 0.19
29	39.15 $\pm$ 4.30	36.50 $\pm$ 2.56	1.25 $\pm$ 0.11	1.00 $\pm$ 0.05	4.09 $\pm$ 0.43	5.96 $\pm$ 0.33	0.74 $\pm$ 0.10	2.45 $\pm$ 0.30
30	40.15 $\pm$ 3.89	36.50 $\pm$ 2.56	1.38 $\pm$ 0.12	0.98 $\pm$ 0.22	4.73 $\pm$ 0.61	5.51 $\pm$ 1.41	0.91 $\pm$ 0.07	2.64 $\pm$ 0.48
31	41.18 $\pm$ 3.61	38.07 $\pm$ 2.41	1.32 $\pm$ 0.09	1.20 $\pm$ 0.13	4.55 $\pm$ 0.66	5.68 $\pm$ 0.49	1.24 $\pm$ 0.55	2.29 $\pm$ 0.59
32	41.18 $\pm$ 3.61	38.07 $\pm$ 2.41	1.29 $\pm$ 0.15	1.11 $\pm$ 0.16	4.82 $\pm$ 0.65	6.39 $\pm$ 0.92	1.09 $\pm$ 0.19	3.08 $\pm$ 0.69
33	40.80 $\pm$ 4.22	37.00 $\pm$ 2.85	1.18 $\pm$ 0.17	1.06 $\pm$ 0.14	4.66 $\pm$ 0.76	6.17 $\pm$ 0.82	1.06 $\pm$ 0.29	2.96 $\pm$ 0.68
34	41.05 $\pm$ 3.99	37.00 $\pm$ 2.85	1.20 $\pm$ 0.17	1.14 $\pm$ 0.10	4.96 $\pm$ 0.77	6.44 $\pm$ 1.35	1.27 $\pm$ 0.46	3.02 $\pm$ 0.48
35	43.20 $\pm$ 3.62	39.53 $\pm$ 2.90	1.34 $\pm$ 0.05	1.10 $\pm$ 0.02	5.43 $\pm$ 1.07	8.44 $\pm$ 0.72	1.45 $\pm$ 0.29	3.57 $\pm$ 0.52
					4.26 $\pm$ 0.78	7.25 $\pm$ 0.90	1.03 $\pm$ 0.24	3.00 $\pm$ 0.16
					4.67 $\pm$ 0.65	6.39 $\pm$ 0.48	1.06 $\pm$ 0.25	3.03 $\pm$ 0.46
					4.19 $\pm$ 0.31	6.50 $\pm$ 0.35	1.28 $\pm$ 0.28	3.19 $\pm$ 0.49
					4.36 $\pm$ 0.23	6.42 $\pm$ 0.26	1.15 $\pm$ 0.26	3.35 $\pm$ 0.38

Appendix 1a

Means and Standard deviation for Bodyweight of Fowler's Gap lambs from 0-27 weeks of age

Age	LS	HS
0	4.38 ± 0.83	4.31 ± 0.24
1	5.18 ± 0.94	4.31 ± 0.24
2	-	-
3	-	-
4	6.06 ± 0.76	6.75 ± 1.02
5	8.94 ± 1.73	8.81 ± 0.83
6	10.50 ± 1.29	10.38 ± 1.11
7	11.75 ± 1.71	11.78 ± 0.59
8	13.68 ± 1.81	13.15 ± 0.86
9	15.05 ± 2.30	14.35 ± 0.79
10	15.68 ± 2.37	15.28 ± 1.17
11	16.89 ± 2.68	16.53 ± 0.20
12	18.20 ± 3.09	18.44 ± 0.74
13	19.75 ± 2.77	20.17 ± 0.84
14	21.99 ± 2.82	21.37 ± 1.19
15	23.63 ± 2.81	23.00 ± 1.95
16	24.09 ± 2.90	23.03 ± 1.65
17	23.88 ± 2.65	23.78 ± 1.70
18	24.00 ± 2.10	24.81 ± 1.64
19	26.00 ± 2.00	24.98 ± 0.68
20	28.50 ± 3.53	26.93 ± 2.90
21	29.97 ± 3.39	29.64 ± 1.19
22	30.70 ± 2.51	29.56 ± 1.48
23	30.63 ± 3.94	29.56 ± 1.72
24	29.94 ± 3.05	29.62 ± 0.48
25	33.53 ± 1.72	31.72 ± 0.55
26	34.52 ± 0.92	30.08 ± 0.84
27	35.50 ± 1.29	33.75 ± 1.50

APPENDIX 2

Table 1. An example of regression analysis to give comparison between LS and HS lambs; the comparison is between food intake against age for LS and HS lambs

*** ANALYSIS OF VARIANCE ***

SOURCE	DF	SS	MS	F
OVALL_RE	2	1.73949	0.86974	178.63531
POS_DIFF	1	0.25412	0.25412	52.19361 ***
REG_DIFF	2	0.02588	0.01294	2.65763 *
RESIDUAL	44	0.21423	0.00487	1.00000
TOTAL	49	2.23971	0.04559	9.36285

APPENDIX 2 (continued)

Table 2. An example of the split-plot analysis of variance of data reported in Chapters 4, 5 and 6. Analysis of urine osmolarity from experiments at the Fowler's Gap site during November. The computer printout overlaps result due to printing on A4 size paper.

SOURCE		SUM OF		DEGREES OF		MEAN		F
TAIL	GREENHOUSE	HUYNH	SQUARES	FREEDOM	SQUARE			
PRCE,	GEISSEF	FELDT						
	PRCE,	PRCE,						
MEAN								
0.0000		105451072.34722		1	105451072.34722			210.53
TREAT								
0.4673		204230.01389		1	204230.01389			0.60
ERROR								
		5033827.92778		5	5033827.92778			
INF								
0.6209	0.4917	0.5553	321032.27778	8	40129.03472			0.76
IT								
0.8560	0.6440	0.7541	202027.61111	8	25253.45129			0.49
ERROR								
			2464431.22222	48	51342.31713			

Notes: TREAT = Between treatments (LS vs HS)
 INF = Changes over the measurement period
 IT = Between treatments over measurement period interaction

APPENDIX 3

Typical composition of bore water consumed by
lambs at Fowler's Gap

	Planet Bore	Mandelman Bore
Conductivity ($\mu\text{s}/\text{cm}$)	2820	4300
pH	8.0	7.72
Total Alkalinity as CaCO_3 (mg/ℓ)	208	247
Total dissolved solids (mg/ℓ)	1160	2020
Cl^- (mg/ℓ)	426	780
SO_4^{--} (mg/ℓ)	262	400
NO_3^- (mg/ℓ)	-	-
Na^+ (mg/ℓ)	402	505
K^+ (mg/ℓ)	7	12
Ca^{++} (mg/ℓ)	20	62
Mg^{++} (mg/ℓ)	33	43

Note: This data was obtained from analysis of water conducted by the Department of Chemistry, University of NSW, WS & LB Robinson University College, Broken Hill, and was made available by Mrs L.F. Black of the College.

APPENDIX 4

Plasma and urine osmolarity, and Na, Cl and K concentrations
and urine flow rate and levels of significance attained in
analysis of variance of data for the experiment reported in
Chapter 6

T.G.	Plasma Osm. (mosm/l)		Urine Osm. (mosm/l)		UFR (ml/min)	
	LS	HS	LS	HS	LS	HS
Time (hours)						
12	279 ± 23.7	269 ± 9.9	1195 ± 560	550 ± 389	1.36 ± 0.71	3.01 ± 0.94
4	289 ± 21.5	298 ± 16.3	1075 ± 156	705 ± 205	1.15 ± 0.51	3.02 ± 1.39
8	325 ± 26.0	310 ± 14.1	1037 ± 55	1130 ± 382	1.69 ± 0.65	3.73 ± 1.44
12	300 ± 24.9	274 ± 22.6	1042 ± 210	1263 ± 513	1.24 ± 0.39	2.92 ± 0.35
8	283 ± 4.0	260 ± 0.7	898 ± 423	913 ± 230	0.68 ± 0.34	1.41 ± 0.34
12	293 ± 28.9	298 ± 1.4	995 ± 246	763 ± 265	1.31 ± 0.82	1.41 ± 0.28

T.G.	Plasma Na Conc. (mmol/l)		Urine Na Conc. (mmol/l)		Plasma Cl Conc. (mmol/l)	
	LS	HS	LS	HS	LS	HS
Time (hours)						
12	151 ± 1.7	146 ± 1.4	16 ± 13	10 ± 0	106 ± 5	102 ± 0
4	151 ± 2.7	160 ± 0.7	116 ± 49	148 ± 11	111 ± 2	121 ± 3
8	151 ± 3.2	150 ± 0	131 ± 54	280 ± 42	99 ± 2	112 ± 1
12	148 ± 2.5	146 ± 0	83 ± 47	190 ± 42	109 ± 1	111 ± 4
8	146 ± 4.6	146 ± 2.1	51 ± 72	103 ± 46	107 ± 4	105 ± 1
12	146 ± 2.0	145 ± 0.7	19 ± 19	80 ± 28	110 ± 1	103 ± 4

T.G.	Urine Cl Conc. (mmol/l)		Plasma K Conc. (mmol/l)		Urine K Conc. (mmol/l)	
	LS	HS	LS	HS	LS	HS
Time (hours)						
12	112 ± 28	95 ± 50	4.5 ± 0.1	5.4 ± 0.2	237 ± 131	120 ± 99
4	173 ± 53	233 ± 67	4.9 ± 0.4	4.3 ± 0.1	147 ± 42	60 ± 28
8	182 ± 72	303 ± 60	4.6 ± 0.2	5.3 ± 0.4	223 ± 66	35 ± 7
12	198 ± 23	258 ± 18	4.6 ± 0.4	4.8 ± 0.2	320 ± 35	123 ± 32
8	160 ± 87	265 ± 42	4.3 ± 0.1	4.3 ± 0	228 ± 138	193 ± 39
12	183 ± 47	205 ± 85	4.8 ± 0.5	4.5 ± 0.1	182 ± 52	138 ± 53

Levels of Significance

	Between		
	Treatments	Over M.P.	M.P. x B.T.
Plasma Osmolarity	NS	NS	NS
Urine Osmolarity	NS	NS	NS
UFR	**	NS	NS
Plasma Na	NS	*	*
Urine Na	NS (10%)	**	NS (10%)
Plasma Cl	NS	**	*
Urine Cl	NS	NS	NS
Plasma K	NS	NS	NS
Urine K	**	NS	NS

Legend as for Table 5.2

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