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Effects of sodium intake during two parities on Na status in Blackface sheep

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1. A low-sodium diet was given to Blackface ewes over two reproductive seasons. This diet provided a total of 3–7 mmol/d except during lactation when the intake was about 11 mmol/d. Control ewes were given the same diet supplemented with sodium chloride to provide recommended levels which were about tenfold that of the experimental diet.

2. The output of Na in urine and faeces from ewes given the low-Na diet was very low, about 3 mmol/d, from early in the experiment and continued at about this level throughout.

3. Lambs born to low-Na ewes and given a low-Na diet similar to that of their dams during lactation, grew, after weaning, more slowly than corresponding lambs from control ewes, but at 6 months of age when six from each group were killed, tissue Na contents were only slightly lower in experimental than control lambs.

4. Fluids and tissues obtained at the end of the second lactation from four ewes of each group that had suckled twin lambs, were analysed for Na and potassium. The Na concentration in saliva and rumen fluid of low-Na ewes was about half that of control ewes and there were corresponding increases in K: the differences were significant. Although Na concentrations for experimental ewes were generally lower than those for control ewes in the tissues analysed (liver, kidney, heart, brain and bone), the difference was significant only for the heart ($P < 0.05$). Haemoglobin and packed cell volume were significantly elevated in low-Na ewes ($P < 0.01$), indicating decreased body fluid volume. Enlargement of the adrenal glands in low-Na ewes ($P < 0.01$) and specifically in the zona glomerulosa ($P < 0.001$), showed the marked hormonal response to Na status of experimental ewes given a very-low-Na diet over two reproductive cycles.

An experiment was carried out over two reproductive cycles to determine the effects of a low sodium intake on productive performance and health in Blackface ewes and their lambs. The efficiency of reproduction of these experimental ewes and of corresponding control ewes has been described recently (Vincent *et al.* 1986*b*): no significant difference was observed in any aspect of reproduction between these two groups of ewes. Furthermore, there were no differences in milk yield and growth of the lambs (Vincent *et al.* 1986*a*). The experiment provided an opportunity to determine Na and potassium levels in excreta from the ewes, and in tissues and fluids from small groups of first-year lambs, and of ewes killed at the end of the second lactation.

These three sets of values, the Na and K contents of excreta of ewes, tissues of lambs and tissues of ewes are described in the present paper. They provide information on the Na status of these animals, particularly the ewes.

MATERIALS AND METHODS

Animals and diets

Forty-two mature Blackface ewes were divided into two groups of similar mean live weight, one group was given a control diet (C) providing adequate Na, the other group a low-Na diet (T). They were housed on wooden slats throughout the two seasons, except when the lambs were small. From about 3 weeks of age, their lambs were offered a creep diet similar in composition to the lactation diet of the dams. They were weaned at about 7 weeks of age.

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Both diets consisted of oats, bran and wheat straw, supplemented with maize gluten in late pregnancy and lactation to provide additional protein. They also contained a supplement of calcium, phosphorus, trace elements and vitamins. This mix, diet T, contained 10 mmol Na/kg air dry feed and provided less than 10 mmol Na/d during maintenance feeding (Vincent *et al.* 1986*b*). Diet C contained a further 55 mmol Na as sodium chloride/d during maintenance feeding (65 mmol/kg air dry feed) to bring the level to that recommended by the Agricultural Research Council (1980). Diet T-fed ewes were given rain-water to drink, containing 0·07 mmol Na/l, since tap water levels were high, about 3 mmol Na/l. K in the diet was more than adequate: the level during maintenance feeding was about 200 mmol/kg air dry feed. Feed allowances were based on values from Ministry of Agriculture Fisheries and Food (1976) and have been described in detail by Vincent *et al.* (1986*b*).

Collection of samples for analysis

Urine and faeces were collected from four ewes in each group kept in metabolism cages, during the first and second pregnancies of the experiment. Samples were obtained at mid-term of the first pregnancy and during the last 4 weeks of the second pregnancy.

Urine samples were collected from lambs after brief respiratory restriction. Samples of mixed saliva were obtained using a sponge held with forceps and wiped round the sublingual and submaxillary gland areas.

Lambs. Tissues were obtained from representative subgroups of lambs, six from each treatment group, when they were killed at 6 months of age. From 3 weeks of age they were given access to the lactation diet of the dams. Diets C and T contained 40–60 and 3–4 mmol Na/kg air-dry feed respectively.

Ewes. At about 60 d of lactation when the experiment terminated, four ewes from each treatment group that were suckling twins were killed and tissues and fluids were taken for analysis.

In general, tissues and fluids considered probable sources of Na (Aitken, 1976) were analysed. The range was larger for ewes than for lambs and included liver, kidney, adrenal gland, heart, brain, parts of the skeleton, blood, rumen fluid, saliva and urine. These were kept in sealed polystyrene containers or plastic bags: fluids were frozen and solids dried if not analysed immediately. Bones were selected to provide samples representative of the skeleton, e.g. rib, vertebra, long bone and mandible. These were cut to give samples that were largely compact (long bone shaft), cancellous (vertebra) or a mixture (proximal rib): marrow was removed from the medullary cavity of the radius shaft and mandible but not from other more cancellous bones.

Analyses

Polystyrene or Pyrex glass containers only were used, previously rinsed with distilled–deionized water. Na and K were determined by atomic absorption spectroscopy in rumen fluid and other fluids after diluting as necessary with distilled–deionized water.

Weighed samples of dried tissues and faeces were digested in a nitric–perchloric acid mixture. The digest was made up to volume and portions taken for dilution to determine Na and K. Bone was treated differently: samples were extracted with acetone, and dried and weighed. The dry fat-free bone was digested in a nitric–perchloric acid mixture and the solution was adjusted to suitable volume for determination of Na and K (McDougall *et al.* 1974).

Determinations of blood glucose were made using glucose oxidase (*EC* 1.1.3.10), total protein by biuret, aspartate aminotransferase (glutamic-oxalacetate transaminase) (*EC* 2.6.1.1) from the rate of formation of oxalacetic acid, haemoglobin colorimetrically with cyanmethaemoglobin as standard and packed cell volume using microhaematocrit tubes.

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Table 1. *Sodium output in urine and faeces (mmol/d) at mid-term of the first pregnancy and throughout the second pregnancy in ewes given an adequate (C)- or low (T)-Na diet*

(Mean values with their standard errors for four ewes)

Treatment	Mid-term of first pregnancy			End of second pregnancy		
	Urine	Faeces	Total	Urine	Faeces	Total
C						
Mean	7·2	31·2	38·4	41·4	30·0	71·4
SE	2·0	3·5		4·7	2·9	
T						
Mean	0·7	2·1	2·8	1·0	2·2	3·2
SE	0·1	0·4		0·7	0·3	

Histology

Adrenal glands were weighed and then prepared for histological examination by embedding in paraffin wax. Sections, cut at 5 μm, were stained with haematoxylin and eosin.

Statistical analyses

Means with their standard errors were calculated and statistical significance was assessed by the two-tailed Student's t test. Where the population variances were very different the t test was preceded by log transformation of the data. Na and K contents of different bones within an animal were compared using the paired t test.

RESULTS

Excreta of ewes

Na concentration of urine and faeces and daily output from ewes at mid-pregnancy of the first year and late pregnancy of the second year are given in Table 1. By mid-term of the first pregnancy the concentrations of Na in urine and faeces from group T ewes were much lower than those from group C ewes, giving a total daily output of 2·8 mmol for group T ewes, compared with 38·4 mmol for group C ewes. Corresponding values from group T ewes towards the end of the second pregnancy were similar to those obtained in the early stages of the experiment, about 14 months earlier.

Lambs

Mean body-weights of the lambs at slaughter were 33·1 kg for group T and 27·2 kg for group C, and the difference was not significant ($P < 0·1$).

The Na and K contents of urine, plasma, liver, kidney and bone from a cervical vertebra and the left mandible are given in Table 2. The Na concentration of urine from group T lambs was extremely low, but that of plasma was normal by comparison with corresponding values for group C lambs. A similar pattern of differences was seen for K. Mean Na contents of liver, kidney, cervical vertebra and mandible were lower for group T than for group C lambs, but the difference was significant only for the cervical vertebra. There were no statistically significant treatment effects on the K content of the tissues.

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Table 2. *The sodium and potassium contents of urine, plasma (mmol/l), and liver, kidney, and fat-free bone (mmol/kg dry matter) of lambs given an adequate (C)- or low (T)-Na diet*

(Mean values with their standard errors for six lambs)

Element	Treatment	Urine	Log_{10} urine	Plasma	Liver	Log_{10} liver	Kidney	Log_{10} kidney	Vertebra	Mandible
Na	C									
	Mean	26·0	1·1748	144	123	—	376	2·5551	238	258 (*n* 3)
	SE	9·68	0·2340	0·8	22·1	—	55·9	0·0547	7·1	4·6
	T									
	Mean	1·0	0·0502***	143	103	—	348	2·5350	204**	245 (*n* 4)
	SE	0·26	0·1229	1·1	23·6	—	27·1	0·0856	7·0	4·2
K	C									
	Mean	138·0	2·0798	4·5	224	2·3463	208	—	96·9	29·8 (*n* 3)
	SE	24·49	0·1186	0·16	1·5	0·0281	15·3	—	5·0	7·1
	T									
	Mean	38·3	1·4075*	4·2	243	2·3821	231	—	82·6	34·3 (*n* 4)
	SE	11·76	0·4926	0·23	13·5	0·0233	15·1	—	6·1	6·4

Mean values were significantly different from those for controls: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

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Table 3. *The sodium and potassium concentrations (mmol/l) in rumen fluid taken during year 2, from ewes given an adequate (C)- or low (T)-Na diet*

(Mean values with their standard errors)

Element	Treatment	November (non-pregnant) Rumen fluid (*n* 3)	November (non-pregnant) Log_{10} rumen fluid	June (lactating) Rumen fluid (*n* 4)	June (lactating) Log_{10} rumen fluid
Na	C				
	Mean	111	2·0435	110	—
	SE	0·8	0·0334	3·7	—
	T				
	Mean	115	2·0575	44***	—
	SE	6·6	0·0251	5·8	—
K	C				
	Mean	14·5	—	22·7	1·3502
	SE	1·77	—	1·94	0·0838
	T				
	Mean	29·8**	—	69·2	1·8315***
	SE	1·52	—	7·50	0·0487

Mean values were significantly different from those for controls: ** $P < 0·01$, *** $P < 0·001$.

Ewes

Throughout the experiment the Na intake of group T ewes was at least ten times lower than that of group C ewes; it rose to 10–12 mmol Na/d during lactation only. Further details are given elsewhere (Vincent *et al.* 1986*a*,*b*). The group T ewes remained clinically healthy and showed no evidence of feed refusal.

Na and K concentrations of plasma, saliva and urine for the subgroups killed in the present study were similar to those reported earlier (Vincent *et al.* 1986*a*) for ewes in their second lactation. Plasma values for group T ewes remained similar to those for group C ewes for both Na and K, saliva values for group T ewes were significantly lower for Na and higher for K than those of group C ewes and urine values of group T ewes were significantly lower for Na and unchanged for K by comparison with those of group C ewes.

The Na concentration of rumen fluid, shown in Table 3, was much lower and the K concentration was much greater during lactation in group T than group C ewes: both differences were highly significant. In samples taken the previous autumn from non-pregnant ewes (Table 3) Na values were similar for both groups, but K concentration was significantly elevated in group T ewes.

Blood glucose was not significantly affected by treatment (Table 4), but haemoglobin content and packed cell volume were significantly greater in group T than group C ewes. Plasma total protein was greater in group T than group C ewes: there was no difference in aspartate aminotransferase activity between treatment groups.

Na and K contents of liver, kidney, heart and brain are shown in Table 5. The concentration of Na was lower in all tissues of group T than group C ewes, but the difference was significant only in the heart. Except for liver, the K values showed a trend towards a concomitant increase in group T ewes.

Na and K contents of bone (Table 6) showed no significant effects of treatment. The Na levels in vertebra and mandible of group T ewes were lower than those for group C ewes,

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Table 4. *Blood constitutents in late lactation of year 2, of ewes given an adequate (C)- or low (T)-sodium diet*

(Mean values with their standard errors for four ewes)

	Blood				Plasma	
Treatment	Glucose (mmol/l)	Log_{10} glucose	Hb (g/l)	PCV	Total protein (g/l)	Aspartate aminotransferase (*EC* 2.6.1.1) (iu/l)
C						
Mean	2·18	0·3361	104	0·300	74·5	45·0
SE	0·946	0·0197	3·2	0·0071	2·18	7·50
T						
Mean	2·13	0·3268	120**	0·360**	81·8*	42·5
SE	0·629	0·0127	2·7	0·0092	1·44	5·95

PCV, packed cell volume; Hb, haemoglobin.
Mean values were significantly different from those of controls: * $P < 0·05$, ** $P < 0·01$.

Table 5. *The tissue sodium and potassium contents (mmol/kg dry matter) in late lactation of year 2, of ewes given an adequate (C)- or low (T)-Na diet*

(Mean values with their standard errors for four ewes)

Element	Treatment	Liver	Kidney	Heart	Brain
Na	C				
	Mean	151	344	192	375
	SE	21·7	29·4	9·1	19·6
	T				
	Mean	133	289	148*	320
	SE	19·9	25·0	10·8	16·5
K	C				
	Mean	275	261	298	384
	SE	25·1	15·2	35·2	23·1
	T				
	Mean	255	290	322	403
	SE	25·1	22·0	21·0	21·2

Mean values were significantly different from those of controls: * $P < 0·05$.

but the differences were small, and values for radius and rib were very similar for both groups of ewes. There were significant differences among bones in Na and K content: for example, Na between vertebra and mandible ($P < 0·01$) and K between proximal rib and proximal radius ($P < 0·001$).

The adrenal gland showed marked evidence of the effect of the low-Na diet (Table 7): T ewes had heavier adrenals ($P < 0·05$) and the zona glomerulosa was much wider ($P < 0·001$) than in group C ewes.

DISCUSSION

There was very little faecal Na loss in group T ewes, an effect of enhanced active reabsorption of Na from the lower gut that has been demonstrated in Na-deficient animals

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Table 6. *The sodium and potassium contents of dry fat-free bone (mmol/kg) in late lactation of year 2, of ewes given an adequate (C)- or low (T)-Na diet*

(Mean values with these standard errors for four ewes)

Element	Treatment	Vertebra	Mandible	Rib		Radius		
				Proximal	Distal	Proximal	Mid-shaft	Distal
Na	C							
	Mean	305*	345	313	303	314	317	306
	SE	8·1	8·6	11·0	6·2	7·9	6·4	5·8
	T							
	Mean	283	330	317	305	320	314	295
	SE	7·1	6·4	7·2	6·3	5·5	3·6	4·0
K	C							
	Mean	49·5*	20·0	57·0	51·0	13·6	8·8	12·8
	SE	5·70	1·67	2·96	4·11	1·47	0·33	1·01
	T							
	Mean	43·8	21·3	55·4	44·4	13·2	9·6	13·2
	SE	4·47	2·15	4·43	2·38	1·39	0·75	1·19

* Mean of only two ewes. Bone biopsy samples had already been taken from lumbar vertebrae of the other two ewes.

Table 7. *Adrenal weight and width of the zone glomerulosa and capsule in late lactation of year 2, of ewes given an adequate (C)- or low (T)-sodium diet*

(Mean values with their standard errors for four ewes)

Treatment	Adrenal wt (g)	Adrenal wt (g) body-wt (kg)	Zone glomerulosa width		Capsule width	
			μm	Log_{10}	μm	Log_{10}
C						
Mean	5·88	0·10	244	2·3864	194	2·2865
SE	0·915	0·020	6·3	0·0115	6·3	0·0145
T						
Mean	9·60*	0·17**	838	2·9175 ***	213	2·3235
SE	0·720	0·030	74·5	0·0401	16·2	0·0334

Mean values were significantly different from those for controls: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

by several workers (Goodall & Kay, 1965; Blair-West *et al.* 1968). Renal conservation of Na was also very efficient, similar to that reported by numerous workers (e.g. Denton, 1957; Scott & Dobson, 1965). In the present long-term study a maximum degree of conservation occurred during the first 2 or 3 months of the experiment and during the major part of the 20-month period only about 3·0 mmol Na were lost daily in faeces and urine from group T ewes compared with about 40 mmol or more from group C ewes. Lambs on a low-Na diet in the present study conserved K in the urine as well as Na, possibly in response to partial replacement of Na ions by K, for example, in gut fluids (Blair-West *et al.* 1970; Beal *et al.* 1974). No economy of Na was evidently possible by reducing the concentration in plasma or tissues: the major response to very low intake was reduced rate of weight gain.

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The more detailed examination of ewes at the end of the second lactation showed that although there was tenacious retention of Na in plasma and tissues, adjustments in metabolism were made to make some body Na available in the face of a deficiency in lactation (Vincent *et al.* 1986*a*) and probably in the last stages of fetal development.

The maintenance of Na concentration in plasma and soft tissues was not surprising but from some studies the release of Na from bone in pregnant and lactating ewes with very low Na intake seemed probable. In rats given low-Na diets decreases in skeletal Na have varied from 3·2% (Ganguli *et al.* 1969) to 50% (Bergstrom & Wallace, 1954). In dogs also, a large decrease was observed (Nichols & Nichols, 1956), but in sheep depleted by draining parotid saliva a 5% decrease occurred (McDougall *et al.* 1974). In the present study the differences in mean values between group T and group C ewes (100) for Na concentration in dry fat-free tissue, varied from −7% for the vertebra to +2% for the proximal radius. Loss from the skeleton can be estimated only approximately from these relatively small samples; however, it is evident that mobilization of Na from the skeleton of adult animals even when there was a severe negative balance during lactation, was very small, probably of the order of 5%. The resorption of bone or the withdrawal of specific elements operates through plasma or an extracellular fluid derived from plasma and it is expected, therefore, that changes would occur more readily in cancellous bone than in compact bone. This seems to be supported by the greater loss from the vertebra, 7%, than the shaft of the radius, 1%, but there were inconsistencies in this respect. Thus, the decrease in the relatively compact mandible was 4%, while in the distal rib, which is largely cancellous, there was an increase of 1%. In studies that showed very large losses of skeletal Na (Bergstrom & Wallace, 1954) the animals were young and Na is more mobile in young than mature bone (Forbes, 1960). Furthermore, it has been suggested that in these cases depletion occurred in association with severe acidosis (Winters *et al.* 1958). Thus present evidence suggests that in mature animals the skeleton does not represent an available source of Na by decrease in concentration *per se*. However, loss of bone occurs during lactation even when the diet provides large quantities of calcium and phosphorus and would release Na for milk production when the diet has a low Na content. In experiments with ewes suckling twins the decrease in dry fat-free bone to 60 d after production was about 0·15 of the amount present in the non-lactating ewe (Benzie *et al.* 1956, 1959). The weight of dry fat-free bone was about 0·03 of body-weight. From these values it is estimated that, assuming no decrease in the Na content of skeletal ash, about 60 mmol Na would be available from the skeleton during lactation.

The large differences in K contents among bones were probably caused by an artifact, namely erythrocytes contained in cancellous tissues. This may be illustrated using values from the rib and vertebra that have a high proportion of cancellous bone and K content of about 50 mmol/kg, compared with the radius that is largely compact bone and had a K content of about 10 mmol/kg.

There were modifications in Na and K concentrations in saliva and rumen fluid to achieve economy in the use of Na in ewes in late lactation. Na concentration in the saliva of group T ewes was almost half that of group C ewes, and K in T ewes was more than double that in group C ewes (Vincent *et al.* 1986*a*). These differences were reflected fairly closely in the rumen fluid, described here (Table 3). A similar partial replacement of Na by K was observed by Kay (1960) and Bott *et al.* (1964). Na absorption from the rumen is enhanced by K (Warner & Stacy, 1972), thus the elevation of K in rumen fluid was an advantageous adaptation in ewes given the low-Na diet.

Blood analyses showed a further modification of metabolism to meet the negative Na balance during lactation. Elevations of haemoglobin and packed cell volume indicated a decrease in extracellular fluid and plasma volume, making Na available while maintaining

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the normal concentration. Further evidence for release of Na via decrease in extracellular fluid volume comes from the difference seen in live weight between the two groups: group T ewes being about 10 kg lighter than group C ewes by the end of the second lactation (Vincent *et al.* 1986*b*).

Close control of Na and K concentrations in body fluids in response to a low Na intake is achieved by increased aldosterone secretion by the adrenal gland (Morris & Gartner, 1971; Hagsten & Perry, 1975). The increase in weight of the adrenal glands of group T ewes and in the width of the zona glomerulosa indicated the marked effect of the low Na intake on aldosterone production in the present study.

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