The Potassium and Sodium Contents of Sheep Tissues in Relation to the Potassium Content of the Erythrocytes and the Age of the Animal

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MATERIAL AND METHODS

General

Abderhalden (1898) and Kerr (1937) showed that the concentrations of electrolytes in the erythrocytes of different mammalian species varied considerably. Kerr (1937) observed that a wide range occurred in the sodium and potassium concentrations of the erythrocytes of sheep and Widdas (1954) and Evans (1954) showed that the concentrations varied around either of two means. These two types of sheep have been named HK (high potassium: approximately 83 m-equiv. of potassium/l. of erythrocytes) and LK (low potassium: approximately 23 m-equiv. of potassium/l. of erythrocytes). It is also possible to recognize intermediate types (Evans, 1957*a*) but these are rare in British breeds. The HK and LK characters are heritable (Evans & King, 1955; Evans, King, Cohen, Harris & Warren, 1956), and it is possible that the two types may differ in other physiological characters as their frequency varies from breed to breed (Evans & Mounib, 1957; Evans, Harris & Warren, 1958*a*) and their geographic distribution is not a random one (Evans, Harris & Warren, 1958*b*).

The object of the work reported here was to see if comparable differences could be found in other tissues of the sheep. The erythrocytes of the rat and the cat contain 100 and 6 m-equiv. of potassium/l. of erythrocytes respectively, and these can therefore be considered as HK and LK animals respectively. The data of Harrison & Darrow (1938) and Yannet & Darrow (1938) suggest that a significant difference also exists between the potassium and sodium concentrations of tissues such as the skeletal muscle and liver of these animals.

Sheep of different ages had to be used in the experiments and as the age of the animal, irrespective of the blood type, influenced the results, further attention was given to this factor.

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The tissues of sheep from three age groups were used, namely lambs approx. 1 year old (88 animals); adult sheep approx. 4 years old (33 animals) and aged sheep approx. 6–8 years old (30 animals). Most specimens were obtained from the local abattoir but the 10 aged ewes which were used to obtain a number of different tissues were maintained on a constant ration for at least 2 weeks before slaughter. The sheep used were Scottish Blackface or crosses with that breed, or Border Leicester ewes (six animals only).

The animals were shot with a captive-bolt humane killer and then were bled. A blood sample was taken at this time. Tissues were collected within 3 min. after slaughter. The amount of blood which remains in tissues is relatively small if the animal is allowed to bleed freely before tissue samples are taken (Fenn, 1939).

The investigation was carried out in two stages: (a) The potassium and sodium concentrations in kidney, heart and liver tissue were determined in three age groups of sheep. All age groups included HK and LK animals. (b) Ten aged Scottish Blackface sheep (five HK and five LK) were used to provide a wider range of tissues and a more detailed study of them in relation to the erythrocyte type of the animals. Skeletal muscle, liver, spleen, kidney, heart, brain, duodenum, rumen and rumen epithelium were examined for their potassium and sodium concentrations as well as for their water and fat contents. The blood content was determined for all tissues except the brain, duodenum, rumen and rumen epithelium, as Laramore & Grollman (1950) found it to be of negligible importance in these tissues after exsanguination.

Tissues were taken from the same part of each organ each time. Skeletal muscle: biceps femoris. Liver: peripheral tissue devoid of large ducts or vessels. Spleen: the capsule was removed and tissue which did not contain large vessels was selected. Kidney: the cortex of the left kidney; Eichelberger & Bibler (1940) found identical potassium and sodium values for the right and left kidneys. Heart: Davies, Davies, Francis & Whittam (1952) reported that potassium and sodium concentrations vary from one part of the heart to another; heart muscle was taken from a point approx. 5 mm. to the right of the apex, where the ventricular septum and the wall of the left ventricle meet (endo- and epi-cardial tissue was removed). Brain: cortical and medullary tissue in approximately equal quantities were taken from the anterior part of the dorsal aspect of

the cerebrum adjacent to the longitudinal fissure. Small intestine: a section through the whole wall of the organ was taken approx. 45 cm. caudal to the opening of the common bile duct. Rumen and rumen epithelium: samples were taken from the anterior part of the dorsal sac of the rumen about 12 cm. from the point of entry of the oesophagus; the epithelium is easily stripped.

All tissues were quickly blotted on ashless filter paper and visible fat was removed. Tissues from the digestive tract received a quick wash in water to remove adherent digesta. Duplicate or triplicate samples of each tissue were analysed.

Chemical methods

Potassium and sodium. The method described by Mounib & Evans (1957) was used in which tissues were extracted with 2% (w/v) trichloroacetic acid for 24 hr. in the cold.

Water. Tissues were weighed, frozen at -20° and then dried over P_2O_5 under vacuum to constant weight.

Fat. This was determined on dry tissues by the method of Hastings & Eichelberger (1937).

Residual blood in tissues. The benzidine method of Bing & Baker (1931) as modified by Laramore & Grollman (1950) was used.

Statistical analysis. When the numbers of animals in the subclasses were not equal, the analysis of variance was made by the method for disproportionate subclass numbers as suggested by Snedecor (1955).

RESULTS

Effect of blood type and age on the concentrations of potassium and sodium in the kidney, heart and liver

The concentrations of potassium and sodium in the kidney, heart and liver of lambs, adult and aged

sheep of both blood types are given in Table 1. The great majority of these samples were obtained from the abattoir and it was not possible therefore to obtain equal numbers of HK and LK animals. Cross-bred sheep were most commonly available and therefore approximately three times as many LK as HK sheep were sampled for kidney and heart tissue and approximately twice as many for liver tissue. As the total number of animals sampled was large the unequal distribution of the two types does not seriously detract from the results.

The LK sheep had a significantly higher concentration of sodium in the kidney than did HK sheep ($P < 1.0\%$). No other significant difference with respect to blood type and tissue concentrations was found. Age and blood type were significantly related for the potassium concentrations in the kidney ($P < 5\%$). The effect of age was highly significant with respect to the potassium ($P < 1.0\%$) and sodium ($P < 0.1\%$) concentrations of the kidney. The older sheep had lower concentrations of potassium and higher concentrations of sodium. The potassium concentration of the heart also decreased significantly with age ($P < 0.1\%$). The HK animals had a higher potassium to sodium ratio in all three tissues but the difference was significant only for the kidney ($P < 5\%$). There was a tendency for the ratio to decrease with advancing age (Table 2). The effect of age on the ratio was highly significant in kidney ($P < 0.1\%$) and heart tissue ($P < 1.0\%$).

Table 1. Concentrations of potassium and sodium in kidney, heart and liver tissue of LK and HK sheep of different ages

Differences between LK and HK sheep (irrespective of age) are not significant except for the sodium concentrations in the kidney ($P < 1.0\%$). Effects of age (irrespective of the potassium type of the animal) are significant for the potassium ($P < 1.0\%$) and sodium ($P < 0.1\%$) concentrations of kidney and for the potassium concentrations of the heart muscle ($P < 0.1\%$). Interaction between type and age is significant only for the potassium concentration in the kidney. The numbers of animals examined in each class are shown in Table 2.

Age group	Blood type	Potassium (m-equiv./kg. wet wt.; mean $\pm$ s.e.)			Sodium (m-equiv./kg. wet wt.; mean $\pm$ s.e.)		
		Kidney	Heart	Liver	Kidney	Heart	Liver
Lambs	LK	71.54 $\pm$ 0.43	83.98 $\pm$ 0.47	81.45 $\pm$ 0.80	66.20 $\pm$ 0.58	38.84 $\pm$ 0.30	33.30 $\pm$ 1.92
	HK	73.66 $\pm$ 1.32	85.92 $\pm$ 1.01	85.71 $\pm$ 3.24	63.76 $\pm$ 1.06	37.96 $\pm$ 0.78	32.95 $\pm$ 2.58
	Total	71.91 $\pm$ 0.43	84.23 $\pm$ 0.43***	83.15 $\pm$ 1.53	65.77 $\pm$ 0.52**	38.73 $\pm$ 0.28	33.16 $\pm$ 1.33
Adults	LK	73.08 $\pm$ 1.81	80.25 $\pm$ 0.79	79.11 $\pm$ 0.63	71.59 $\pm$ 2.42	38.63 $\pm$ 0.68	34.58 $\pm$ 0.90
	HK	68.84 $\pm$ 0.90	78.22 $\pm$ 1.25	78.02 $\pm$ 1.28	66.07 $\pm$ 1.43	37.33 $\pm$ 0.61	34.59 $\pm$ 1.16
	Total	71.54 $\pm$ 1.32	79.76 $\pm$ 0.68	78.85 $\pm$ 0.56	69.59 $\pm$ 1.90	38.32 $\pm$ 0.55	34.58 $\pm$ 1.05
Aged	LK	67.08 $\pm$ 1.93	77.98 $\pm$ 0.98	79.47 $\pm$ 0.90	72.57 $\pm$ 1.69	38.89 $\pm$ 0.69	36.51 $\pm$ 0.91
	HK	68.73 $\pm$ 1.14	79.39 $\pm$ 0.74	81.03 $\pm$ 1.34	69.04 $\pm$ 2.15	38.91 $\pm$ 1.07	34.20 $\pm$ 0.99
	Total	67.91 $\pm$ 1.09*	78.59 $\pm$ 0.64	80.14 $\pm$ 0.77	70.82 $\pm$ 1.42	38.90 $\pm$ 0.59	35.51 $\pm$ 0.69

* Differences between the potassium contents of the kidneys of aged sheep and those of adult sheep are on the borderline of significance at the 5% level; differences between aged sheep and lambs are highly significant ($P < 0.1\%$).

** Differences between the sodium concentrations of the kidney tissue of lambs and those of aged sheep are highly significant ($P < 1\%$).

*** Differences between the potassium concentrations of heart muscle of lambs and those of both adult and aged sheep are highly significant ($P < 0.1\%$).

Table 2. *Ratio of potassium to sodium in kidney, heart and liver tissue in HK and LK sheep of different ages*

Effect of blood type (irrespective of age) is significant only in the kidney ($P < 5.0\%$). Effect of age (irrespective of blood type) is highly significant in the kidney ($P < 0.1\%$) and heart ($P < 1.0\%$).

Age group	LK sheep		HK sheep		Total	
	No. of animals	Mean ratio $\pm$ s.e.	No. of animals	Mean ratio $\pm$ s.e.	No. of animals	Mean ratio $\pm$ s.e.
Kidney						
Lambs	47	1.09 $\pm$ 0.01*	10	1.16 $\pm$ 0.03	57	1.10 $\pm$ 0.01
Adult	7	1.03 $\pm$ 0.05	4	1.04 $\pm$ 0.04	11	1.04 $\pm$ 0.04
Aged	5	0.93 $\pm$ 0.05	5	1.00 $\pm$ 0.03	10	0.97 $\pm$ 0.03**
Heart						
Lambs	48	2.17 $\pm$ 0.01	7	2.27 $\pm$ 0.06	55	2.18 $\pm$ 0.02***
Adult	16	2.09 $\pm$ 0.05	5	2.10 $\pm$ 0.06	21	2.09 $\pm$ 0.04
Aged	17	2.02 $\pm$ 0.06	13	2.06 $\pm$ 0.07	30	2.04 $\pm$ 0.05
Liver						
Lambs	3	2.47 $\pm$ 0.16	2	2.63 $\pm$ 0.30	5	2.53 $\pm$ 0.13
Adult	16	2.31 $\pm$ 0.07	5	2.34 $\pm$ 0.21	21	2.32 $\pm$ 0.02
Aged	17	2.20 $\pm$ 0.07	13	2.40 $\pm$ 0.10	30	2.29 $\pm$ 0.06

* Differences between LK and HK lambs are significant ($P < 5.0\%$).

** Differences between aged sheep and lambs are highly significant ($P < 0.1\%$).

*** Differences between lambs and adult sheep are significant ($P < 5.0\%$) and those between lambs and aged sheep are highly significant ($P < 1.0\%$).

A study of the concentrations of potassium and sodium in individual samples of all three tissues showed that they were inversely related in spite of a considerable scatter. Regression equations indicate that a negative correlation existed, varying from -0.36 to -0.56 (P 0.1–2.0%).

The concentrations of potassium in the heart and in the liver from the same adult or aged sheep were positively related (r 0.41 and $P < 1\%$); that is, they moved in the same direction.

Potassium and sodium concentrations of the tissues of aged sheep corrected for fat content or blood content or both

Five HK and five LK aged Scottish Blackface ewes were used. The quantities of fat, water and trapped blood were determined in nine tissues. The fat content of the skeletal muscle was significantly higher in LK than in HK sheep ($P < 5\%$). There were no other significant differences between HK and LK sheep in relation to fat content, water content or trapped blood. Because the full nutritional history of these ewes was not known and because of the importance of skeletal-muscle fat content in animal husbandry a further comparison of the fat content of muscle was made between two groups of HK and LK lambs of the same breed. In this experiment no difference was found.

No significant differences were found between the potassium and sodium concentrations or the potassium to sodium ratios in any tissue other than rumen epithelium (values for HK animals were

higher than those for LK animals: $P < 1.0\%$) although there was a tendency (approaching significance) in four of the tissues examined (skeletal muscle, liver, spleen and kidney) for the LK sheep to have lower potassium concentrations, higher sodium concentrations and lower potassium to sodium ratios than the HK sheep (Table 3). In each of the nine tissues examined there was a tendency for an inverse relationship to exist between the potassium and sodium concentrations, in agreement with the previous results.

DISCUSSION

Suitable techniques for obtaining tissues from experimental animals without any transfer of fluids from one compartment to another are difficult, and although exsanguination is known to cause a movement of interstitial fluid into the vascular system many previous investigations on the potassium and sodium contents of tissues have been made after the animal has been so treated. It is also likely that a movement of fluid from the intracellular to the extracellular phase will take place but this latter movement will probably be confined, as far as electrolytes are concerned, to ions not electrostatically held by non-diffusible material such as proteins. It is impossible to say how far the results presented here have been influenced by such movements but as all animals were treated in the same way any bias introduced will be in the same direction.

Table 3. *Concentrations of potassium and sodium, the ratio of potassium to sodium and the percentage of blood, fat and water in different tissues of the sheep*

(1), Estimations are for fat-free, blood-free, wet tissue; (2), estimations are not corrected for fat or blood in wet tissue; (3), estimations are for fat-free wet tissue.

Tissue	Calculation	Potassium, sodium and ratio (m-equiv./kg. of wet tissue)				
		LK (five animals)		HK (five animals)		
		K	Na	K/Na	K	Na
Skeletal muscle	(1)	108.8 ± 1.73	21.6 ± 0.93	5.00 ± 0.319	110.4 ± 1.67	19.4 ± 0.80
Liver	(1)	86.7 ± 1.66	36.0 ± 1.67	2.43 ± 0.114	88.6 ± 1.66	31.8 ± 1.15
Spleen	(1)	111.0 ± 1.60	34.0 ± 1.00	3.28 ± 0.120	115.1 ± 2.55	31.0 ± 0.90
Kidney	(1)	68.8 ± 2.04	73.0 ± 1.60	0.95 ± 0.049	70.1 ± 1.19	69.6 ± 2.30
Heart	(1)	83.4 ± 1.82	35.6 ± 1.16	2.36 ± 0.121	82.7 ± 1.15	36.4 ± 1.37
Brain	(2)	89.6 ± 1.26	53.8 ± 1.30	1.67 ± 0.035	88.7 ± 1.40	53.6 ± 1.23
Small intestine	(3)	70.9 ± 1.44	51.5 ± 2.42	1.39 ± 0.070	70.3 ± 2.18	52.8 ± 2.66
Rumen	(3)	68.2 ± 1.43	55.3 ± 1.53	1.24 ± 0.076	69.3 ± 2.25	57.1 ± 2.33
Rumen epithelium	(3)	42.6 ± 1.55	60.2 ± 2.71	0.72 ± 0.054	51.8 ± 0.98**	64.5 ± 1.93

Tissue	Percentage of blood in wet tissue (ten animals)	Fat and water (g./kg. wet wt.)				
		LK (five animals)		HK (five animals)		
		Fat	Water	Fat	Water	Water
Skeletal muscle	1.12	44.0 ± 5.8*	779.9 ± 5.8 (1)	26.9 ± 4.0*	779.4 ± 4.7 (1)	779.4 ± 4.7 (1)
Liver	1.46	33.0 ± 4.4	721.8 ± 4.6 (1)	33.6 ± 2.4	717.6 ± 4.9 (1)	717.6 ± 4.9 (1)
Spleen	5.34	15.4 ± 1.2	788.8 ± 3.0 (1)	17.4 ± 0.1	787.7 ± 2.5 (1)	787.7 ± 2.5 (1)
Kidney	1.29	14.9 ± 2.3	806.0 ± 4.0 (1)	12.5 ± 1.6	805.3 ± 2.6 (1)	805.3 ± 2.6 (1)
Heart	1.97	14.4 ± 2.9	793.0 ± 2.2 (1)	16.5 ± 1.6	788.5 ± 1.8 (1)	788.5 ± 1.8 (1)
Brain	—	89.4 ± 10.0	808.3 ± 16.0 (2)	110.2 ± 16.5	810.8 ± 9.3 (2)	810.8 ± 9.3 (2)
Small intestine	—	12.2 ± 1.7	828.3 ± 3.8 (3)	10.4 ± 2.3	835.5 ± 3.5 (3)	835.5 ± 3.5 (3)
Rumen	—	13.8 ± 2.9	812.7 ± 3.4 (3)	10.4 ± 1.9	814.8 ± 3.3 (3)	814.8 ± 3.3 (3)
Rumen epithelium	—	5.8 ± 0.4	808.9 ± 4.8 (3)	7.5 ± 1.5	808.3 ± 0.9 (3)	808.3 ± 0.9 (3)

* Differences in fat content of skeletal muscle between LK and HK sheep are significant ($P < 5\%$).

** Differences in potassium concentration of rumen epithelium between LK and HK animals are highly significant ($P < 1.0\%$).

The concentrations of sodium and potassium in the plasma are the same for HK and LK sheep and show little variation, so that a similar constancy can be expected in the interstitial fluid. For this reason it is unlikely that the residual interstitial fluid present in the tissues examined will have introduced a serious error in the comparisons made unless there was great variation in the quantities present.

The results which were obtained show that with the exception of the kidney and possibly the rumen epithelium (two organs in which active transport of sodium is known to occur) there are no marked differences between HK and LK sheep in the potassium and sodium contents of the tissues examined so that the difference in the erythrocytes is a somewhat unique feature. The meaning of the differences in sodium concentration and ratio of potassium to sodium in the kidney between the HK and LK animals requires closer investigation, as they may be correlated with the differences in water metabolism between these two types of sheep, which was observed by Evans (1957b). He noted a tendency for LK sheep to drink less water and excrete less urine than HK sheep. The differences between the two types of sheep in the potassium concentration of the rumen epithelium are interesting, although the effect of washing this tissue must be taken into consideration. The food which was eaten by these sheep was the same and R. N. B. Kay (personal communication) has not found any difference in the potassium or sodium concentrations in the parotid-gland saliva from HK and LK sheep, so it seems unlikely that external factors such as these could influence the results. However, the high concentration of sodium and the low concentration of potassium in the extracellular fluid compared with the intracellular fluid could result in the differences which were observed in the kidney and rumen epithelium if there is a difference in the proportion of intracellular space between HK and LK animals in these tissues.

Harris, McDonald & Williams (1952), working with anaesthetized sheep, found relatively low values for potassium in skeletal muscle: range 75–85 m-equiv./kg. wet wt. (compared with 109.6 m-equiv./kg. in the present investigation). As Harris *et al.* (1952) were working in Australia and give potassium concentrations for erythrocytes of the same order as those found by Denton, Wynn, McDonald & Simon (1951) and Evans (1957a) in merino sheep it can be assumed that their data for skeletal muscle refer to merino sheep. The difference between the data for skeletal muscle in this paper and those of Harris *et al.* (1952) is of interest in that the merino sheep has a mean erythrocyte-potassium concentration for LK sheep

of approximately 10 m-equiv./l., whereas the mean value for LK British Hill breeds such as the Scottish Blackface is approximately 23 m-equiv./l.

The effects of age on the kidney are similar to those observed by Lowry, Hastings, McCay & Brown (1946) in rats.

The negative correlation between the concentrations of potassium and sodium that was found to exist in the kidney, heart and liver tissue in the first experiment and the confirmatory tendency for such a relationship to exist in the nine tissues subsequently studied in the second experiment may mean that the extra- and intra-cellular phases varied from one animal to another for any one tissue, and that an increase in the extracellular phase (indicated by the increase in the concentration of sodium) is met by a decrease in the intracellular phase (indicated by the decrease in the concentration of potassium). The observation that the concentrations of potassium in the heart and liver are correlated may mean that an increase in the intracellular phase of the liver is associated with an increase in the intracellular phase of the heart in the same animal. On the other hand, this may indicate that individuals have intra- and extra-cellular phases which are specific to themselves and which may be distinctly different from those of other members of the same group or breed. Fowler & Uptill (1955) suggested that the differences in the extracellular phase, estimated as sodium thiosulphate space, which they found between individual rabbits were real differences and specific to individuals. Repeated measurements on one particular tissue (e.g. skeletal muscle) of the same animal will prove or reject this hypothesis. This could be done by the well-established technique of skeletal-muscle biopsy.

SUMMARY

1. Potassium and sodium concentrations in kidney, heart and liver tissue in sheep with either high (HK) or low (LK) concentrations of potassium in their erythrocytes were determined in young, adult and aged individuals.

2. The concentration of sodium in the cortex of the kidneys of LK animals was significantly higher (about 5.6%) than in those of HK animals. Differences of the order of those found in the erythrocytes of sheep were, however, not found in any tissue.

3. The concentration of potassium decreased with age in both the kidney and the heart but not in the liver; the average difference between young and aged sheep was 5.7% in the kidney and 6.9% in the heart. A reciprocal rise in sodium occurred in the kidney and the average difference between young and aged sheep was 7.4%.

4. The ratio of potassium to sodium decreased significantly with advancing age in kidney and heart tissue.

5. Nine tissues were analysed from a further five LK and five HK aged ewes. The skeletal muscle, liver, spleen and kidney (expressed on a fat- and blood-free basis) of the aged HK ewes tended to have higher concentrations of potassium (1.5, 2.2, 3.6 and 1.9% respectively) and lower concentrations of sodium (10.7, 12.3, 9.2 and 4.8% respectively) than those of the aged LK ewes.

6. Rumen epithelium from LK sheep had a significantly lower potassium concentration (19.5%) than that from HK animals.

7. The fat content of the skeletal muscle of aged LK sheep was significantly higher (17 g. of fat/kg. wet wt. or about 48%) than that of aged HK sheep but this difference was not found in a group of lambs of the same breed.

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Comparative Biochemistry of Urea Synthesis

3. ACTIVITIES OF UREA-CYCLE ENZYMES IN VARIOUS HIGHER AND LOWER VERTEBRATES*

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In the first paper of this series we reported on a systematic scheme of analysis for determining quantitatively the activity of urea-cycle enzymes in liver extracts of ureotelic animals (Brown & Cohen, 1959a). Application of this method to livers of *Rana catesbeiana* tadpoles revealed the induction of the cycle at the onset of metamorphosis (Brown & Cohen, 1958, 1959b). It was also

found that the activity of the urea-cycle enzymes could account for the rate of urea synthesis by anuran liver as judged by the rate of urea excretion by intact, metamorphosing tadpoles. This offered final and convincing evidence that the urea cycle as formulated in Fig. 1 (see Brown & Cohen, 1958; Cohen & Brown, 1959; Ratner, 1954, 1955) represents the sequence of reactions for the biosynthesis of urea by a ureotelic organism. Moreover, the favourable free-energy changes associated with the

* Part 2: Brown & Cohen (1959b).