STUDIES ON THE SODIUM–POTASSIUM BALANCE IN ERYTHROCYTES OF AUSTRALIAN MERINO SHEEP

I. CHANGES IN CONCENTRATIONS IN THE ERYTHROCYTES OF LAMBS FROM BIRTH TO 98 DAYS

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Summary

Estimates are given of the sodium–potassium balance in the erythrocytes of 16 Australian Merino and Merino cross lambs at ages ranging from 0 to 98 days. The [Na^+] values rose and the [K^+] values fell from birth till an age of approximately 60 days, after which each variable fluctuated about a value comparable with those obtained for the dams of the same lambs, all of which were adult Merinos. Curves were fitted up to 57 days, their equations being $y[Na^+]=21\cdot47+2\cdot63x-0\cdot0203x^2$ and $y[K^+]=99\cdot60-2\cdot78x+0\cdot0221x^2$ (x=age in days).

I. INTRODUCTION

Evans (1954) reported differences in the sodium and potassium concentrations of the red blood cells of British breeds of sheep, which led to the recognition of two types of animal—*HK* (high potassium) and *LK* (low potassium). Evans and King (1955) concluded that the sodium–potassium balance was under genetic control and could be altered significantly by the presence of one or another of a pair of alleles at one locus, the gene for *HK* being recessive to that for *LK*. Evans and Mounib (1957) presented the results of a survey of a number of sheep breeds in Britain, which showed considerable variation in the proportion of *HK* animals in each sample of sheep. This proportion was higher for animals living in less favourable environments in the north of Britain, and the suggestion was made that the sodium–potassium balance might have some bearing on survival in mountain and upland regions in the temperate zone. Evans (1957) adapted Kerr's (1937) classification, which extended the number of recognizable types of animals from two to four, the original *LK* and *HK* now being designated $Ke\alpha$ and $Ke\gamma$ respectively. Some observations on Merinos, sampled in Britain, were included in this paper, with the observation that most of them were of type $Ke\alpha$, but with an even lower value of potassium (in m-equiv/l of red blood cells) than had been recorded for other breeds. Attention was drawn to the fact that Denton *et al.* (1951) and Harris, McDonald, and Williams (1951) had also obtained very low potassium values when working with Australian Merinos, but Bernstein's (1954) values for the South African Merino were not exceptionally low.

Lamb-marking percentages are notoriously poor in the Australian Merino, frequently because of considerable losses between birth and marking, which takes

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place shortly after the end of the lambing period. In a sheep-breeding trial, one of us (H.N.T.) had observed a greater mortality in young lambs among the progeny from half-sib than from unrelated matings, and there seemed a possibility that some detrimental recessive gene might be contributing to the losses. The work of Evans and his co-authors suggested that the recessive gene for high levels of potassium might be present in Australian Merino flocks, and it was thought that, for some reason homozygous recessives might be less fitted to survive in hot, dry areas than animals with a low potassium level.

To test this hypothesis, it was originally planned to sample lambs at birth, and to compare the potassium level of those which died as lambs and those which survived. At the time, no data were available on changes in the sodium–potassium balance in lamb erythrocytes. Evans and his associates have worked with sheep 6 months of age or older, and have found (Evans 1957) that repeated sampling of the same animal at various time intervals has given values which do not differ significantly from each other. Hallman and Karvonen (1949) and Widdas (1954) showed that concentrations of potassium in foetal erythrocytes were higher than those of adults in Finnish and Welsh sheep respectively, the sodium concentrations being correspondingly lower. Widdas also showed that there was a fall in the potassium concentration (y) with foetal age (d in days), as expressed by the equation $y = 115-0\cdot196d$. Green and Macaskill (1928), Groenewald (1935), and Wise *et al.* (1947) all found a downward postnatal trend in the potassium level in cattle erythrocytes. In the data presented by Green and Macaskill and by Groenewald, there was a corresponding rise in the sodium level, but Wise *et al.* found a fall in sodium level to 14 days of age, followed by a rise.

The present set of observations was undertaken to give information on changes in the sodium–potassium balance of the erythrocytes of Merino and Merino cross lambs immediately after birth. Subsequently, Wright *et al.* (1958) published observations on four lambs, at ages ranging from 14 to 63 days, no details of breed being given. The pattern was similar to that for cattle, with a rise in [Na^+] and a corresponding fall in [K^+].

II. Material and Methods

(a) *Sheep*

Data in this first series of observations came from Merino ewes and Merino or Merino × Border Leicester crossbred lambs born to them at three centres in or near Sydney during September–October 1955—the McMaster Animal Health Laboratory, Glebe, N.S.W., Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., and the F. D. McMaster Field Station, Badgery's Creek, N.S.W. Sheep at the two laboratories were maintained under pen conditions, while those at the field station were run at pasture. No differences in potassium or sodium concentrations were detected between sheep from the three locations, nor were the data sufficiently numerous to demonstrate any possible differences between sheep of different sexes, or between pure Merinos and crossbreds. All observations have therefore been considered together.

A total of 16 ewes and 21 lambs were sampled, including five sets of twins. One ewe sample was lost before measurement.

The animals were sampled as soon as possible after the birth of the lamb, and at intervals from then on. Each surviving lamb was bled from four to seven times. Samplings were fairly regular up to seven weeks of age, but thereafter only a few readings were made, the oldest lamb sampled being aged 98 days.

(b) *Sampling and Measurement*

The method used was based on that of Love and Burch (1953). About 5 ml of blood was collected from the jugular vein of the sheep, using an 18-gauge needle, and was run directly into a plastic centrifuge tube (see below) containing 0·5 mg of crystalline heparin (Boots) dissolved in 0·1 ml of a 0·154M NaCl solution. The tubes containing the blood were immediately placed in a centrifuge and spun at 3000 r.c.f. for 30 min. After spinning, a clamp was fastened just below the top of the red cell layer. The top part of the tube above the clamp, which contained plasma, white cell layer, and portion of the packed red cells was cut off and discarded. The clamp was then released and the remaining lower portion of the tube sealed, care being taken that the cork stopper did not touch the surface of the packed red cells. The sample was then immersed in a mixture of dry ice and alcohol, the frozen samples being later placed in the freezing chamber of a refrigerator. Immediately before analysis, the samples were thawed by placing the tubes in tap water. The resulting haemolysed solutions were diluted with double-distilled water, appropriately for the estimation of Na and K on the "EEL" flame photometer. Measurements are expressed in milli-equivalents per litre of packed red blood cells (m-equiv/l) and denoted by $[Na^+]$ and $[K^+]$ respectively.

The plastic centrifuge tubes were prepared from polythene tubing of 15 mm bore and 1 mm wall thickness. The hose was cut into pieces 10 cm long, one end of each piece then being rounded off and heat-sealed above a bunsen flame. These tubes could be used over a period of 2–3 months, after which the sealed end seemed to become brittle and reopened.

III. Results and Discussion

Full data for lambs which survived till the end of the period of observations are given in Table 1, and for those which died in Table 2.

To obtain homogeneous sets of data, with as many observations as possible at each day of age, the readings shown in brackets in Table 1 were interpolated on trends fitted by eye for each lamb; in most cases linear interpolation between adjacent readings was appropriate. Using these interpolated values and grouping consecutive days, three sets of homogeneous data were obtained, with means for lambs 1–4, 5–10, and 12–16. The group of lambs 1–4, for example, has mean values for days 1–2, 5–6, 12–13, 19–20, 26–27, 40–41, and 61–62. By further interpolations of the same kind, the two Merino sets (lambs 1–4 and 5–10) were combined into one, with readings at ages ranging from day 1 to day 56/57. Two minor extrapolations were used for lambs 8 and 9, the readings for days 47 and 50 respectively being repeated at day 57. Extensive extrapolation was not considered warranted, and

TABLE 1

VALUES OF SODIUM AND POTASSIUM (IN M-EQUIV/L PACKED RED BLOOD CELLS) FOR LAMBS WHICH WERE STILL SURVIVING AT THE END OF THE PERIOD OF OBSERVATIONS

Values in brackets have been estimated from the trend line for each lamb

Age (days)	Merino Lambs: Lamb No.																						Crossbred Lambs: Lamb No.									
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16	
	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$	$[K^+]$
0													29	88					38	91												
1	26	97	29	95					29	93	26	98			19	84	25	115														
2					24	91	27	76																								
4																									48	88	39	83	22	96		
5	32	98	30	98																												
6					36	88	(39)	(73)																								
7																					29	56										
8									40	89	30	100	35	74	23	89	38	72	(50)	(75)												
11																							51	74	49	79	45	70	31	95	58	72
12	27	41	42	84																												
13					49	76	59	69											58	65												
15									65	50	62	83			46	86	56	69	(59)	(63)												
16													81	55																		
19	68	55	72	50																												
20					90	43	65	34																								
22									77	43	72	74	(82)	(45)	68	55	(64)	(57)	(62)	(55)												
26	80	34	82	31																			76	43	74	40	80	48	59	30	93	51
27					94	20	89	24											65	50	77	54										
29													82	33			72	44														
36									80	17	80	38	(102)	(32)	62	28	(82)	(32)	(83)	(32)												
39																							107	14	102	21	98	21	92	26	111	9
40	84	21	126	26																												
41					87	15	98	14																								
43													122	31																		
46																			102	13												
47									(91)	(14)	(91)	(23)	(118)	(26)	102	12	(98)	(14)														
50																	102	9			112	14										
56													108	16																		
57									102	12	102	9			(102)	(12)	(102)	(9)	(110)	(11)												
61	111	9	100	12																												
62					98	8	137	12															112	13	84	10	109	14	74	10	106	9
69																			118	8												
79																					111	9										
91																							115	9	98	15	95	12	105	16	106	9
98																			120	10												

the means for crossbred lambs (12–16) were kept separate, with readings at ages from day 11 to day 91.

TABLE 2

VALUES OF SODIUM AND POTASSIUM (IN M-EQUIV/L PACKED RED BLOOD CELLS) FOR LAMBS WHICH DIED DURING THE PERIOD OF OBSERVATIONS

Age (days)	Merino Lambs				Crossbred Lambs					
	No. 17		No. 18		No. 19		No. 20		No. 21	
	[Na+]	[K+]	[Na+]	[K+]	[Na+]	[K+]	[Na+]	[K+]	[Na+]	[K+]
0	22	88	26	110	23	96	23	103		
4									20	100
7	52	84								
27	81	51								
50	120	15								

The means of [Na+] and [K+] for the 10 Merino and 5 crossbred lambs are plotted on Figure 1. Individual values lying outside the range of days used for

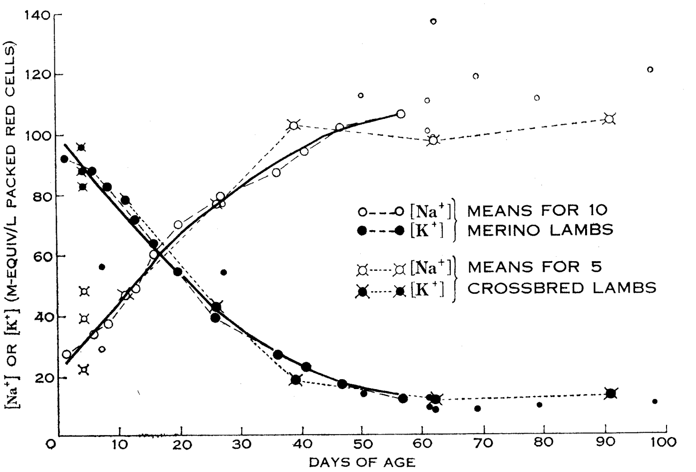

Fig. 1.—Changes with age of [Na+] and [K+]. Individual readings not included in the derivation of the means (see text) are shown in the same code with smaller symbols. Full lines indicate the curves fitted to the means of the 10 Merino lambs up to 56/57 days, the equations being

$$y[\mathrm{Na}^+] = 21{\cdot}47 + 2{\cdot}63x - 0{\cdot}0203x^2,$$
$$y[\mathrm{K}^+] = 99{\cdot}60 - 2{\cdot}78x + 0{\cdot}0221x^2.$$

the means are shown separately, together with the readings for Merino lamb 11, which had no readings before day 7.

From Figure 1 it is clear that the [Na+] values rise with age, while the [K+] values fall, until each is fluctuating about a relatively constant level. The exact point at which this level is reached cannot, of course, be determined, and is likely to vary from sheep to sheep. In the present data there is clearly no further trend in either variable after 60 days, and very little trend after 50 days. Beyond 60 days the [Na$^+$] values appear to fluctuate more than those for [K$^+$], but as only a few figures are available no definite conclusion can be drawn on this point.

Quadratic curves were fitted to the means for the 10 Merino lambs, the equations being:

$$y[\mathrm{Na}^+] = 21{\cdot}47 + 2{\cdot}63x - 0{\cdot}0203x^2,$$
$$y[\mathrm{K}^+] \;= 99{\cdot}60 - 2{\cdot}78x + 0{\cdot}0221x^2,$$

where x = age in days. The curves have been plotted on Figure 1 up to $x = 60$. The fit is good; there is a suggestion of a sigmoidal shape in each case, but observations at early ages are not sufficiently close for the more refined curve to be fitted accurately. Variances about the fitted lines, calculated from the deviations of observed values, were similar, the standard errors of the regression coefficients being 0·75 for the linear and 0·013 for the quadratic terms.

The two curves are thus reversed, the two pairs of coefficients being opposite in sign but not differing significantly in magnitude. The sum of [Na$^+$] and [K$^+$] remains relatively constant, but there is a slight tendency for a downward trend with age. This can be seen by comparing the mean sums at earlier and later ages for the same sheep; for example, the weighted mean difference between the mean sum before and after 20 days of age (chosen as having approximately the same number of observations before and after) was −3·0 for lambs 1–10, the change being from 121 to 118. This trend is reflected in the fact that the negative linear regression coefficient for [K$^+$] is greater than the corresponding positive coefficient for [Na$^+$]. The difference in magnitude was not significant, and neither was the linear coefficient fitted to [Na$^+$] + [K$^+$] whose value was $-0{\cdot}103 \pm 0{\cdot}222$. The tendency towards a downward trend in the sum is, however, worth noting.

The mean values for the five crossbred lambs follow the general pattern for the Merino lambs, as do the additional individual values (Fig. 1), though there are occasional aberrant observations.

It is interesting to compare the rates of change in [K$^+$] with those given by Widdas (1954) for foetal lambs. Widdas found a negative linear trend of [K$^+$] on foetal age, the fall being at the rate of −0·196 m-equiv/l per day of foetal age. If the quadratic term is omitted, the linear trend for [K$^+$] in the fitted line of Figure 1 is −1·605, which is 8 times the foetal value found by Widdas. As mentioned previously, Figure 1 gives an indication that the fall in [K$^+$] is at a lower rate during the first few days of life. The estimated combined means for day 1 and day 5·5 for lambs 1–10 were 92·8 and 88·0 m-equiv/l respectively, giving a daily fall of −0·873, which is still 4·5 times Widdas' figure.

The mean value of [Na$^+$] from 60 days onward is 105·8, including all values, the range being from 74·0 to 137·0 m-equiv/l. The mean value of [K$^+$] from 60 days onwards is 11·0, the range being from 8·3 to 16·0. Both ranges are comparable with the values obtained for the adult ewes in this experiment, which had an average for [Na$^+$] of 97·4 (range 88–114) and for [K$^+$] of 11·5 (range 9–22). It would therefore appear that the adult value for both criteria has been reached on the average by 60 days of age, though in some cases it may be attained earlier.

The original aim of this work was to investigate the possibility of distinguishing $[K^+]$ values at birth or early in life between lambs which died and those which survived The number of deaths (five) in the present group was small. Table 2 shows readings at birth for four of them; the $[K^+]$ values range from 88 to 110, compared with a range of 84 to 115 for readings at birth or 1 day old for eight lambs which survived. (Table 1). The ranges overlap completely, and the numbers are too small for any conclusions to be drawn about differences between dead and surviving animals. All $[K^+]$ values at birth appear to be so high, however, that the chance of demonstrating any difference is very slight.

A new series of observations was therefore planned, estimates being made on adult ewes and rams at mating, with a view to investigating the possibility of an association between the $[K^+]$ values of the parents and the outcome of the mating.

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