

A STUDY OF THE PLASMA SODIUM AND POTASSIUM
LEVELS IN NORMAL MERINO SHEEP

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INTRODUCTION

During work on metabolic diseases in sheep such as "Geeldikkop" and "Enzootic Icterus" it became apparent that disturbances of electrolyte balance and adrenal cortical function played a very big role in the pathogenesis of such diseases. It was therefore decided to investigate this aspect further but very little was known of the normal sodium and potassium levels in the plasma of sheep under South African conditions. In fact no figures based on a large number of samples could be found in the literature. Hence it was decided to carry out a sufficient number of determinations on normal sheep to establish the normal range and the effects, if any, of salt supplementation, sex, season and adrenocortical stimulation by A.C.T.H. as simulating stress conditions such as sudden cold etc

METHODS

The instrument used was an "EEL" flame photometer Model A and the method was adapted from that described by King & Wootton (1956). Preliminary experiments indicated certain modifications to the standards which were finally adopted as follows:—

Plasma Sodium

A stock solution containing 11.691 gm. pure NaCl (= 200 m. eq. Na) per litre of de-ionized water was prepared. From this a series of working solutions containing up to 2.0 m. eq./litre was made. The photometer was adjusted to read 0 for distilled water and 100 for the 2.0 m. eq./litre solution and readings for the intermediate solutions were taken. From these figures a curve was drawn up from which subsequent readings were converted. Plasma was diluted 1:100 for sodium determinations.

Plasma Potassium

The stock solution contained 0.7456 gm. KCl per litre (= 10 m. eq. K per litre). In order to compensate for the interference effect of the high sodium content of plasma all potassium working solutions contained 1.4 m. eq. Na (i.e. 7 ml. Na stock solution per litre). Solutions containing up to 0.25 m. eq. K per litre were used to draw up the standard curve. The plasma was diluted 1:50 for potassium determinations.

Urine

Owing to the high preponderance of potassium over sodium in most sheep urine, stock solutions were made up as follows:—

Na—11·691 gm. NaCl (= 200 m. eq.) per litre.

K—7·456 gm. KCl (= 100 m. eq.) per litre.

From these the following working solutions were prepared (m. eq. per litre):—

<i>Na</i>	<i>K</i>
0·25 plus	1·25
0·50 plus	2·50
0·75 plus	5·00
1·00 plus	7·50
1·50 plus	10·00

Using the same solutions but the appropriate filter, curves were drawn up for both sodium and potassium. This range worked well for sheep not receiving salt when the urine was diluted 1:100 but for sheep on a high salt intake a dilution of 1:200 had to be used.

Where the daily output of sodium and potassium was to be measured, the sheep were placed in metabolism cages and fitted with faeces bags. The volume of urine and weight of wet faeces were recorded. A 10 gm. sample of wet faeces was then ashed at low temperature, digested with HCl and the volume made up to 50 c.c. This was then read against the urine standards and the total daily output calculated.

Blood Samples

In the majority of cases the blood was also required for other determinations requiring samples collected with heparin and under oil. In order to test whether serum could also be used, a series of 20 sheep was bled into heparin — oil bottles and into dry sterile bottles. The sodium and potassium in the heparinised plasma and serum were then compared. In the case of sodium the comparative figures were within the normal experimental error and the averages were — Plasma 144·2, serum 144·7. The potassium, however, was consistently higher in the serum, the average figures being:— Plasma 5·07 and Serum 5·5. This is undoubtedly due to slight haemolysis which was noted in all the sera. Sheep's blood is peculiarly prone to undergo haemolysis while clotting, resulting in the escape of intracellular potassium into the serum. Serum is, therefore, satisfactory for sodium determination but not for potassium. All figures used in this report are from heparinised plasma collected under oil in which haemolysis very rarely occurs.

Dilution and Reading

As the method was designed to deal with large numbers of samples extreme accuracy had to be sacrificed for simplicity. One ml. plasma was diluted to 50 ml. with water and the potassium concentration determined. The total volume was then brought up to 100 ml. and the sodium determined.

When using the flame photometer the zero and high standard readings were checked after approximately every 10 samples. Provided an interval of about 10 minutes was given for the apparatus to "warm up" before readings were started very little drift was found to occur.

Experimental Error

In order to determine the experimental error of the method, duplicate dilutions were made from over 100 different plasma samples. The sodium and potassium concentrations were then determined in each dilution on two separate occasions. Analysis of the results showed that the error was: Reading ± 3 for sodium and Reading ± 1.25 for potassium.

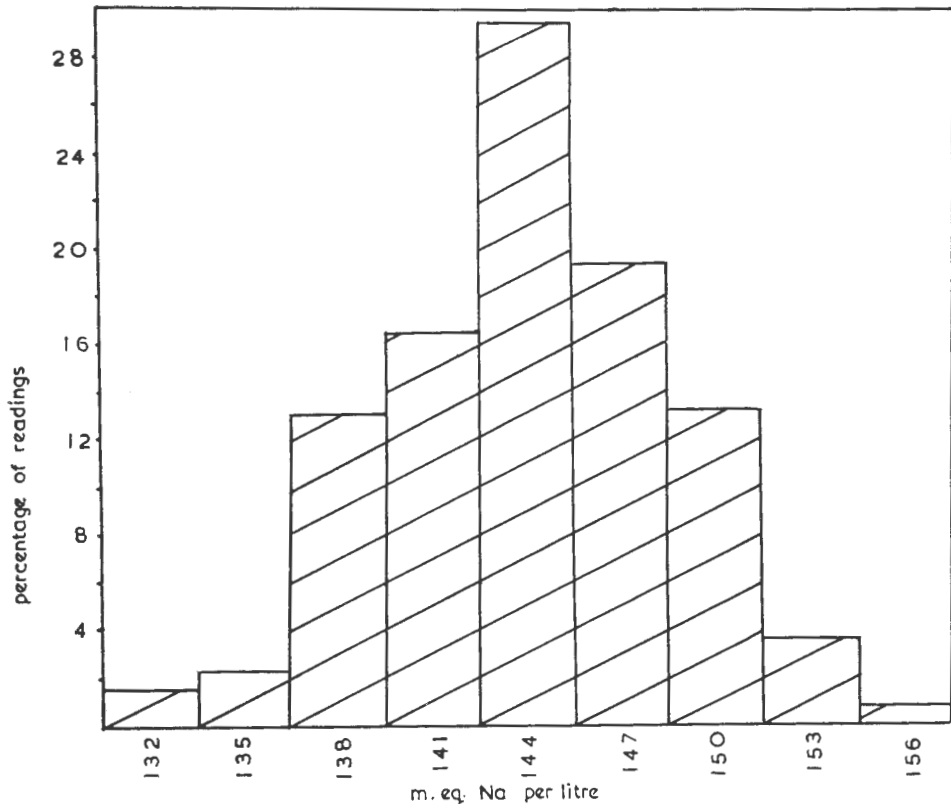
Possible Factors Affecting the Plasma Sodium and Potassium Levels

As the object was to lay down a range which could be looked upon as normal for all Merino sheep kept under a variety of conditions, the effects of the following factors were investigated.

(1) Salt Dosing

Twenty four Merino wethers were placed on a diet of lucerne hay *ad lib.* Twelve of them were dosed daily with 5 gm. NaCl. After 50 days there was found to be no difference between the plasma sodium or potassium levels of the two groups. It can therefore be concluded that the lucerne hay supplied sufficient sodium and an extra intake had no effect on the blood levels.

GRAPH I.
PLASMA Na. NORMAL DISTRIBUTION



(ii) *Season*

Again no difference could be found in figures from a group of sheep in June and July and those from the same sheep in November.

(iii) *Sex*

The figures from a flock of mixed dry and pregnant ewes were the same as those from wethers running with them.

(iv) *Starvation*

The figures from a group of eight sheep were not altered after 96 hours complete starvation with water *ad lib*.

The Normal Levels of Plasma Sodium and Potassium

The following figures are based on 500 readings for both sodium and potassium taken from 70 sheep (40 wethers and 30 ewes) over a period extending from May to November 1958. All readings used are from apparently normal sheep not under experiment.

Sodium

The distribution is shown in Graph I.

It must be noted that, in the part of the curve involved, one degree deflection on the galvanometer equals three milli-equivalents per litre. The readings, therefore, progress in steps of three units and e.g. the figure 144 can be taken as 143 to 145. The extreme range was 132 to 156 with a mode of 144 and an arithmetic average for all figures of 144.11; 91.6 per cent of the readings fell between 138 and 150 and 97.6 per cent between 135 and 153. The figure for 90 per cent of apparently "normal" sheep can therefore be taken as 144 ± 6 .

Potassium

The distribution is shown in Graph 2.

As in humans (King & Wootton, 1956) the distribution was found to be lognormal. The extreme range was 3.8 to 6.0 with a mode 4.8 and an arithmetic average of 4.804 with 92.6 per cent of readings between 4.1 and 5.5.

The normal range of plasma potassium for Merino sheep can therefore be said to be 4.8 ± 0.7 m. eq. per litre.

The Excretion of Excess Sodium

Two Merino wethers were placed in metabolism cages and the urine and faeces collected and measured daily. The sodium and potassium contents were determined and the total daily output calculated. The animals were given 1 Kg. lucerne hay per day and water *ad lib*. As the daily ration was consumed completely, the intake of sodium and potassium remained more or less constant. The animals were given no salt for a period of 10 days and then 10 gm. NaCl each daily for a further 10 days. The results are summarised in Table 1.

It will be noted that the entire sodium content of the daily dose of 10 gm. NaCl (173 m. eq. Na) was accounted for in the increased urinary output. When salt dosing was discontinued the urinary output of sodium fell to the "no salt" level within 24 hours.

GRAPH 2.
PLASMA K. LOG NORMAL DISTRIBUTION

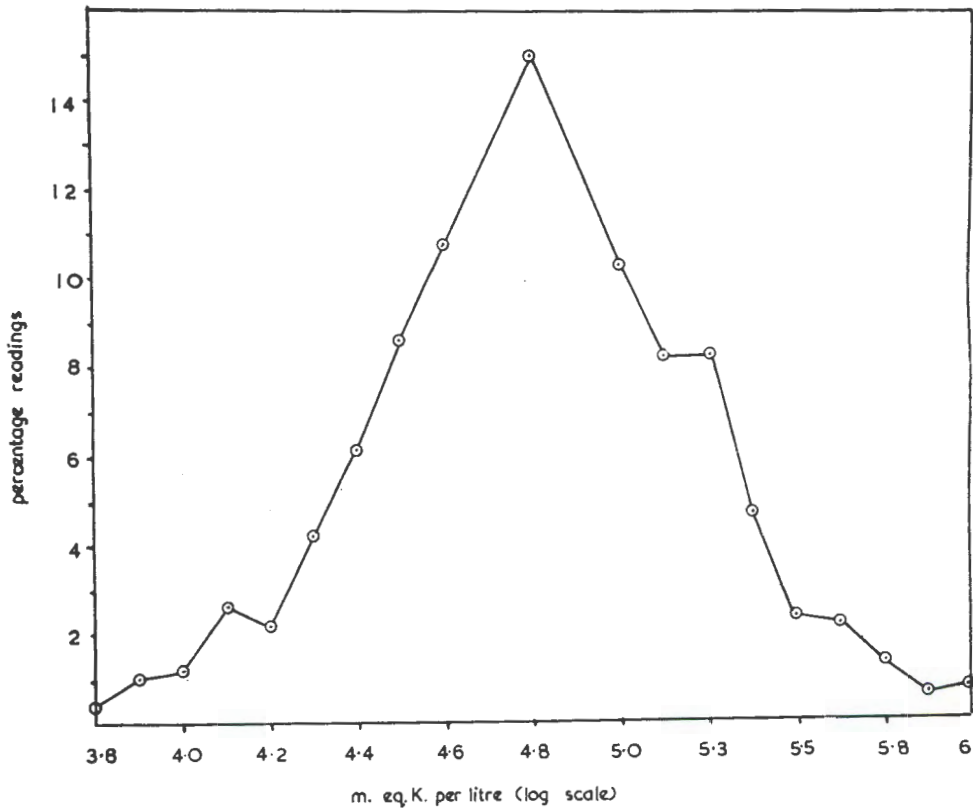


TABLE 1.

	No salt		10 gm. NaCl	
	Sh. 1	Sh. 2	Sh. 1	Sh. 2
Plasma Na (m. eq./l.).....	139	141	141	140
Plasma K (m. eq./l.).....	4.2	4.3	4.2	4.0
Water Intake (l.).....	2.7	2.8	2.9	3.5
Urine Volume (l.).....	0.87	1.04	1.22	1.41
Urine Conc. Na (m. eq./l.).....	41	41	186	145
Urine Conc. K (m. eq./l.).....	563	511	426	407
Urine Na Output (m. eq./24 hr.).....	36	43	227	207
Urine K Output (m. eq./24 hr.).....	490	532	520	574
Faeces Na loss (m. eq./24 hr.).....	11	18	10	15
Faeces K loss (m. eq./24 hr.).....	12	20	14	15

All figures = averages for 10 consecutive days.

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The Sodium Retaining Capacity of the Kidneys

During the last 24 hours of a 96 hour fast (water *ad lib.*) the urinary output of 2 sheep was found to be:—

Sheep	Concentration (m. eq./l.)		Total 24 hour output	
	Na	K	Na	K
1.....	6	140	1·8	42
2.....	4	116	1·2	35

The Effect of A.C.T.H.

Four sheep were injected with 25 units A.C.T.H. intramuscularly and blood samples were taken at 0, 6 and 24 hours after injection. The results are summarised in Table 2.

TABLE 2.

Time after injection	Plasma Na	Plasma K	Blood sugar	Eosino- phile count
Prior to.....	142	4·2	49	180
6 hours.....	146	4·0	67	12
24 hours.....	145	4·3	48	206

Figures = averages for 4 sheep.

Although there would appear to be a slight rise in the plasma sodium, the change was not significant. That the A.C.T.H. was active is shown by the rise in blood sugar and drop in eosinophiles. It will also be noted that the effect lasted less than 24 hours although the preparation used was of the long acting type.*

The failure to demonstrate a rise in plasma sodium after injection with A.C.T.H. did not exclude sodium retention as a proportional amount of water might well be retained concomitantly. Four sheep were, therefore, placed in metabolism cages and given 700 gm. lucerne hay and 5 gm. NaCl each daily with water *ad lib.*

The total urinary output of sodium and potassium was measured over 8 consecutive days. The sheep were then injected with 25 units A.C.T.H. at 8 a.m. and 8 p.m. for two days, followed by a second collection period of 8 days. Again the plasma sodium and potassium figures showed no significant variations, but the total output of both sodium and potassium was reduced as shown by the figures in Table 3. (Average daily output for all figures.)

* "Acton Prolongation" brand of A.C.T.H. Fredericksberg Chemical Laboratories. Copenhagen Denmark.

TABLE 3.

	8 days prior	2 days Injected	Following Day	8 days subsequent
Total Urinary Na.....	117	70	215	106
Total Urinary K.....	398	320	502	422
Water Intake minus Urine volume.....	1.5	2.0	1.1	1.9

It will be noted that there was a retention of sodium and apparently also of potassium over the two days of treatment followed by an increased excretion on the following day. The figures for "water intake minus urine volume" indicate a greater retention of water under the influence of A.C.T.H. but, owing to the marked day to day variation always encountered in sheep in regard to these two factors, a very large number of figures would be required before any definite conclusions could be drawn.

DISCUSSION

The figures found for Merino sheep are almost identical to those given by Harrison (1957) for humans, viz.

	<i>Humans</i>	<i>Sheep</i>
Plasma Sodium.....	138-148 m.eq./litre	138-150 m. eq./litre
Plasma Potassium.....	4.0-5.5 m. eq./litre	4.1-5.5 m. eq./litre

SUMMARY

(1) The following figures were deduced from determinations made on 500 samples of plasma from apparently normal Merino sheep.

Plasma Sodium

Extreme range 132-156 m. eq./litre, mode 144, arithmetic average 144.11, 90 per cent of samples between 138 and 150. Distribution normal.

Plasma Potassium

Extreme range 3.8-6.0 m. eq./litre, mode 4.8, arithmetic average 4.804, 90 per cent of samples between 4.1 and 5.5. Distribution lognormal.

(2) No differences could be found due to sex, season, salt dosing (on a basic diet of lucerne hay) or starvation for 96 hours.

(3) The administration of A.C.T.H. caused retention of sodium and potassium but had no effect on the plasma levels due to concomitant water retention.

REFERENCES

- HARRISON, G. A. (1957). *Chemical Methods in Clinical Medicine*. London: J. and A. Churchill.
- KING, E. J. & WOOTTON, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*. London: J. and A. Churchill.