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RENIN RESPONSES TO WATER RESTRICTION AND REHYDRATION

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SUMMARY

1. The effect of restricted water intake and rapid rehydration was studied in three conscious sheep with respect to plasma renin concentration (PRC), blood corticosteroid levels, plasma protein and electrolyte concentrations, and the renal and faecal excretion of sodium and potassium.

2. During water restriction the plasma concentrations of renin, protein and sodium rose while aldosterone levels were low or undetectable. Plasma potassium levels were unchanged. External sodium and potassium balance appeared to be unaffected.

3. During rehydration the sheep drank more than their estimated water deficit in 3–4 min with the following effects: PRC rose three- to fourfold during the ensuing 12 hr. Aldosterone levels too rose, while plasma protein, sodium and potassium concentrations fell. Urinary sodium excretion virtually ceased for 24 hr, and urine flow rate increased only little during this period.

4. If there was a single stimulus to renin release during water restriction and rehydration, it was not an alteration in vascular or extravascular volume, total body sodium, systemic B.P. or plasma sodium concentration.

5. It is concluded that the rise in PRC in these experiments is compatible with the theory that altered sodium transport at the macula densa was the stimulus for renin release.

INTRODUCTION

It is well established that hypotensive haemorrhage and sodium depletion raise plasma renin levels. Internal shifts of body fluids, such as occur in sheep eating their food rapidly, also increase plasma renin concentration (Blair-West & Brook, 1969). In all these situations the concurrent

loss of sodium and plasma volume militates against assessing their individual contribution to the renin response. During water restriction, on the other hand, volume reduction is primary, and extracellular sodium content must be adjusted to strike a balance between the need to maintain plasma osmality and extracellular fluid volume within tolerable limits. Thus, experiments involving water restriction followed by rehydration may permit distinction between sodium loss and reduction in extracellular fluid volume as stimuli for renin release. Previous studies have shown that dehydration increased plasma renin activity in man (Maebashi & Yoshinaga, 1967) and rat (Gross, Brunner & Ziegler, 1965; Rosenthal, Boucher, Rojo-Ortega & Genest, 1969).

The present study was undertaken to examine the effect of restricted water intake on plasma renin and blood corticosteroid levels, and on plasma and urinary electrolytes. The effects of rapidly correcting the water deficit were also investigated.

METHODS

Animals. Three adult merino ewes weighing 31–36 kg were used. They were kept in individual metabolism cages allowing separate collection of urine and faeces, from which daily excretion rates were determined. The animals were given 0·6 kg lucerne chaff daily at 1400 hr. Water was provided *ad libitum* during control periods. The animals were weighed during the control and dehydration periods. The sheep were trained to urinate upon being gently touched on the hindquarters; urine samples were collected by this method during rehydration studies. Pulse rate and systolic B.P. were measured from a carotid artery that had been enclosed in a cervical skin tube. Systolic B.P. was measured by palpation, using a sphygmomanometer with a small pneumatic cuff.

Experimental. Eight experiments were performed, three on each of two sheep and two on one sheep. Each experiment consisted of a control period when the animals had free access to water and drank about 1·5 l./day, followed by a period when water was restricted to 500 ml./day, and, in two experiments, a further 2 days when no water was given. The effects of rehydration were followed once for 6 hr and once for 24 hr in each of the three sheep.

Blood sampling. During periods of water restriction, blood was drawn by venepuncture from a jugular vein. During rehydration periods, blood samples were taken through an indwelling cannula in the jugular vein. About 20 ml. blood was drawn into a heparinized syringe and centrifuged in a refrigerated centrifuge. Part of the plasma was used to determine specific gravity and sodium and potassium concentrations; the remainder was stored at $-20°$ C for plasma renin assay. About 25 ml. of blood was taken for corticosteroid assay.

Analytical procedures. Plasma renin concentration (PRC) was determined by an enzyme kinetic method (Blair-West, Coghlan, Denton, Scoggins, Wintour & Wright, 1967), and the results are expressed as ng angiotensin produced/hr per ml. of plasma. Plasma renin-substrate concentration was measured by incubating 0·2 ml. plasma with 2 u. pig renin (Nutritional Biochemicals Corporation) in 1·8 ml. phosphate buffer, pH 7·5, for 3 hr at 37° C. The incubate was heated at 100° C for 5 min and centrifuged. Angiotensin concentration in the supernate was estimated by the method used in the PRC assay. Renin-substrate concentration is expressed as

ng of angiotensin/ml. plasma. Aldosterone, cortisol and corticosterone concentrations in peripheral blood were estimated by the method of Coghlan & Scoggins (1967). Total protein in plasma was estimated by the copper sulphate specific gravity technique (Varley, 1962). Sodium and potassium concentrations in plasma and urine were determined by flame photometry with a Technicon Autoanalyser. Sodium and potassium in faeces were estimated by homogenizing 50 g samples in 500 ml. distilled water and letting the homogenate stand for 24–48 hr. A 50 ml. aliquot was centrifuged and the sodium and potassium concentration of the liquor determined by flame photometry.

RESULTS

Water restriction. After 5, 7 and 9 days of water restriction PRC had risen above control values (Fig. 1) in seven of the eight experiments. The changes in other parameters are illustrated by one representative experi-

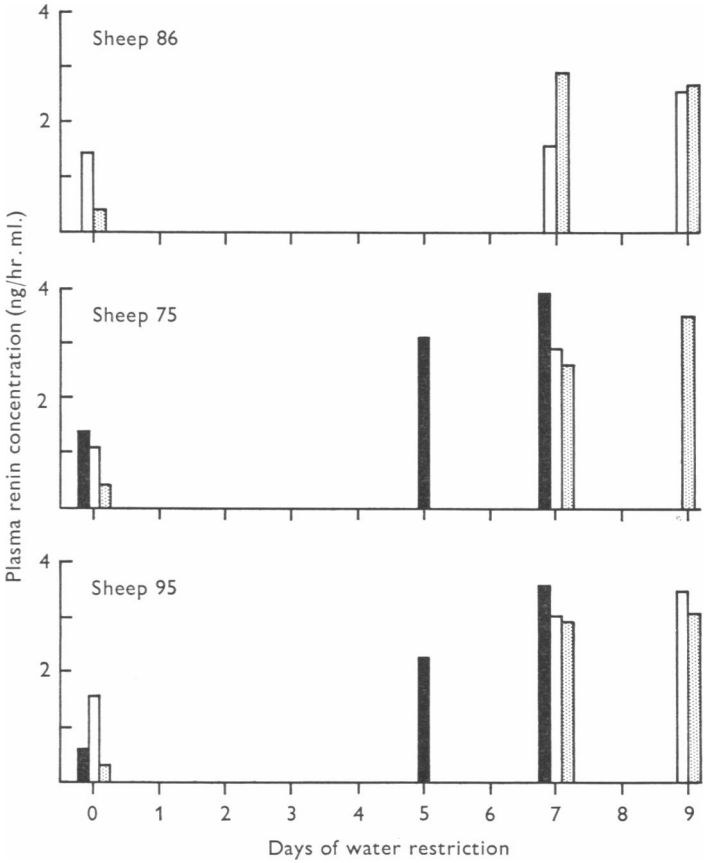

Fig. 1. Plasma renin concentration in three sheep on the day before (day 0) and during water restriction when 500 ml./day of water was given, except on days 6 and 7 of one experiment when no water was allowed. Each bar stands for a single value and each shading indicates a different experiment.

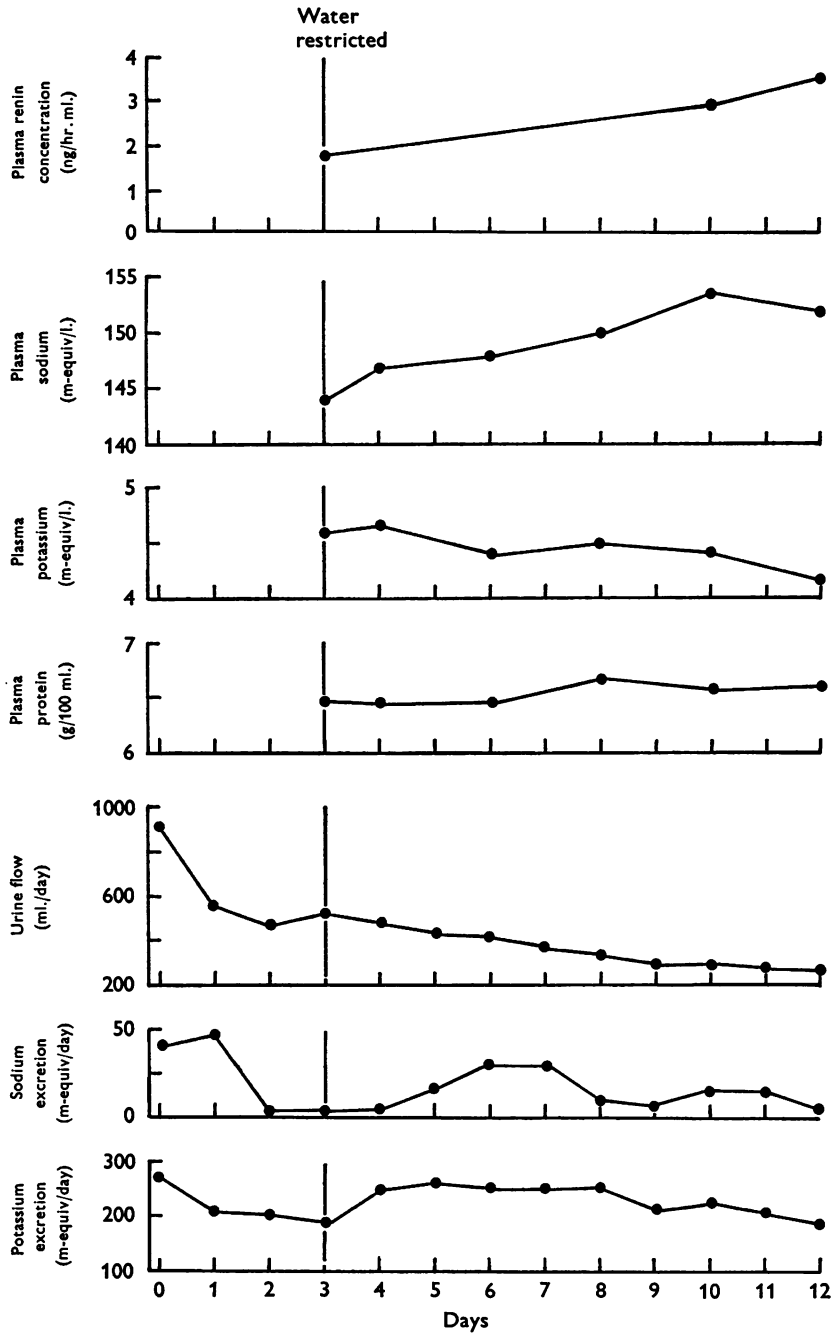

Fig. 2. Effects of restricting water intake of a sheep to 500 ml./day on plasma concentrations of renin, sodium, potassium and protein, on urine flow rate and on urinary sodium and potassium excretion.

ment in Fig. 2. The daily urinary loss of sodium was variable without a regular trend. Urinary potassium excretion was maintained at about 250 m-equiv/day for the first 5 days, and then fell to 180 m-equiv/day. Food intake started to fall on day 5 of water restriction and was only 400 g/day on day 9. Urine flow rate declined steadily to about 200 ml./day. Plasma sodium concentration rose from 144 to 154 m-equiv/l. on the seventh day of water restriction. Plasma potassium levels, however, fell from a control value of 4·5 m-equiv/l. to 4·2 m-equiv/l. by the end of the dehydration period. Plasma protein concentration had risen slightly by the fifth day and then remained stable. Not shown in the figure are the faecal losses of sodium and potassium and the systolic blood pressure. Faecal sodium excretion was not affected by water restriction and ranged from 3 to 16 m-equiv/day during control and experimental periods. Similarly the range of potassium excretion was 11–37 m-equiv/day, the only apparent trend being a fall in excretion on the last two days of water restriction. Systolic B.P. fluctuated randomly between 75 and 85 mm Hg. Results of the other seven experiments were essentially similar, with the following exceptions: the rise in plasma protein concentration was generally greater, the mean increase and range being 0·65 % (0·36–1·08 %), and in one sheep plasma potassium concentration remained unchanged during each of the two periods of water restriction.

In the eight experiments the animals lost between 3·2 and 3·9 kg in weight after 6–9 days of water restriction.

Rehydration. The animals were offered water *ad libitum* at the end of water restriction, and the effects were followed on one occasion for 6 hr and on another occasion for 24 hr in each of the three animals. They drank 4·0–5·6 l. within 4 min, and no more until they were fed at the end of the experiments. The results are shown in Figs. 3 and 4. In each animal PRC had about doubled after 6 hr, and had risen three- to fourfold after 12 hr. After 24 hr, PRC was still substantially higher than before the animals had drunk. Plasma sodium concentration fell from about 150 to 130 m-equiv/l. after 24 hr, most of the fall having occurred in the first 6 hr. Plasma potassium concentration had fallen by about 1 m-equiv/l. after 12 hr. This fall did not start until 3 hr after drinking and was sometimes preceded by a transient rise (Fig. 3). Plasma protein concentration had fallen by about 1 % within 12 hr and then stabilized. Urine flow rate remained low after drinking, rising only from about 0·2 ml./min before drinking to 0·5–0·8 ml./min 24 hr later. Renal excretion of sodium fell to about 2 μ-equiv/min in less than 2 hr and remained at that level during the 24 hr of observation. Urinary potassium excretion diminished by about 100 μ-equiv/min within 3 hr of drinking. There were no consistent changes in systolic B.P. On the first occasion the mean of two B.P. determinations

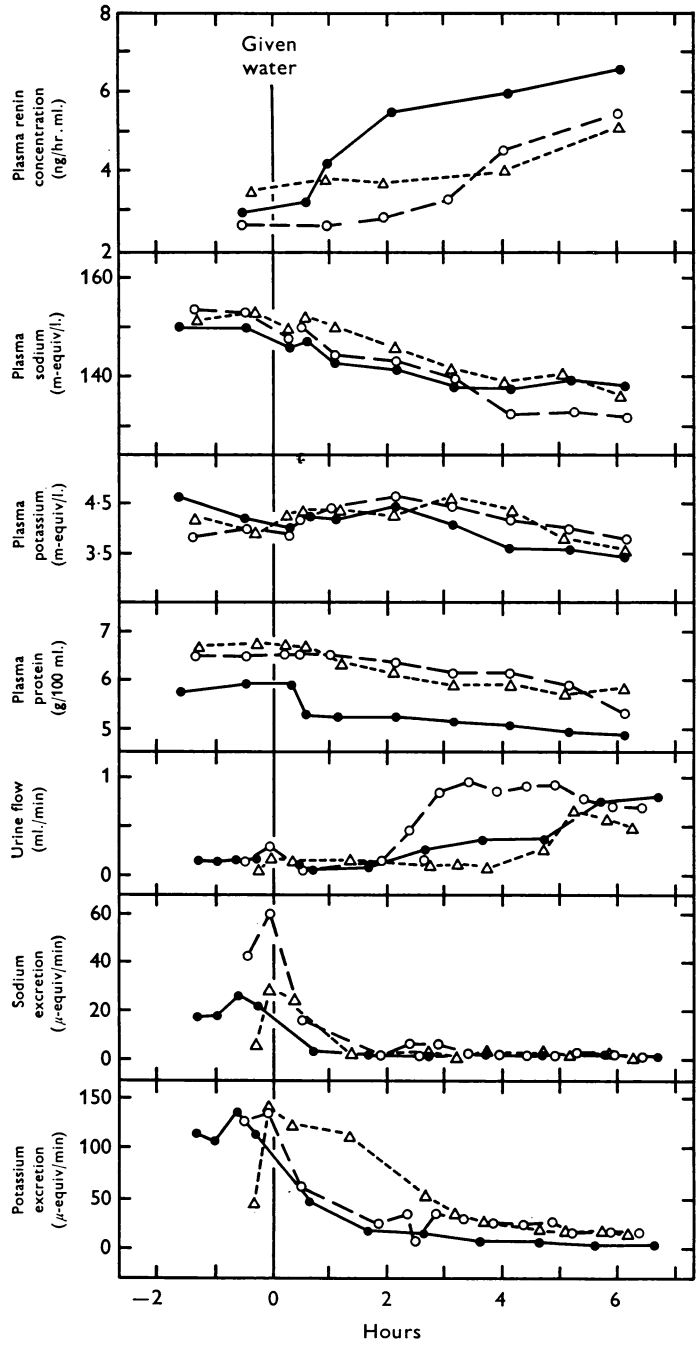

Fig. 3. Plasma and urinary parameters before and for 6 hr after rapidly drinking 4·0–5·6 l. water. Sheep 75 (●), 86 (○) and 95 (△). Sheep 75 had been restricted to 500 ml./day of water for 7 days, and sheep 86 and 95 to 500 ml./day for 9 days.

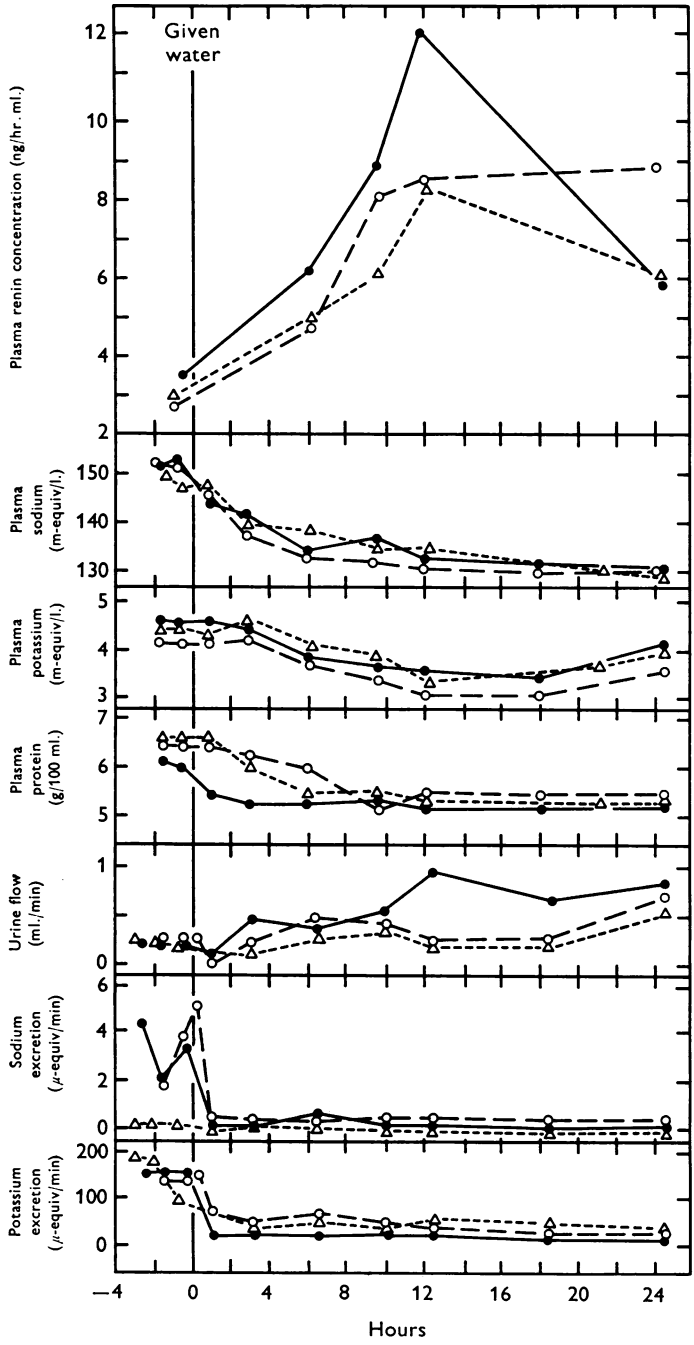

Fig. 4. Plasma and urinary parameters before and for 24 hr after rapidly drinking 4·3–5·6 l. water. Sheep 75 (●), 86 (○) and 95 (△). Water intake had been restricted to 500 ml./day for the previous 9 days.

made within 1 hr before drinking and the mean of two measurements taken 5–6 hr after drinking were, respectively, for sheep 75: 91 and 90 mm Hg; sheep 86: 84 and 93 mm Hg; sheep 95: 87 and 90 mm Hg. On the second occasion the mean of two B.P. determinations made within 1 hr before rehydration and the mean of two determinations made 10 and 12 hr after drinking (when PRC values were highest) were, respectively, for sheep 75: 86 and 100 mm Hg; sheep 86: 85 and 91 mm Hg; sheep 95: 84 and 80 mm Hg.

Relation between weight loss and water intake at rehydration. The sheep were last weighed on the day before rehydration. The immediate volume of water drunk and the measured weight losses were as follows: sheep 75:

TABLE 1. Blood concentrations of corticosteroids and plasma concentration of renin-substrate in sheep before and during water restriction, and after the animals were rehydrated

Animal	Period	Aldosterone (ng/100 ml.)	Corticosterone (μg/100 ml.)	Cortisol (μg/100 ml.)	Plasma renin-substrate (ng/ml.)
Sheep 75	Control*	6·0	0·010	0·15	170
	Water restriction†	3·5	0·060	0·64	200
Sheep 86	Control*	12·3	0·012	0·16	200
	Water restriction†	1·8	0·012	0·46	200
	Rehydration‡	16·5	0·021	0·28	—
Sheep 95	Control*	9·7	0·032	0·43	200
	Water restriction†	§	0·024	0·98	140
	Rehydration‡	4·0	0·025	0·08	—

* One determination on last control day.
† After 7 days (sheep 75) and 9 days (sheep 86 and 95) of water restriction.
‡ 6 hr after drinking.
§ Not detectable.

4·0 l. (3·9 kg), 4·3 l. (3·9 kg); sheep 86: 5·6 l. (3·3 kg), 5·3 l. (3·9 kg); sheep 95: 5·0 l. (3·2 kg), 5·5 l. (3·7 kg). Water intake always exceeded weight loss and it is unlikely that the extra weight lost between the last weighing and rehydration the next day could have accounted for discrepancies of greater than 0·4 l.

Effects of water restriction and rehydration on blood corticosteroids. Blood concentrations of aldosterone, cortisol and corticosterone were measured in three sheep at the end of a control period and at the end of a period of water restriction. In two of the animals, corticosteroid levels were also determined 6 hr after rehydration. The results are shown in Table 1. Blood aldosterone concentration had fallen during water restriction and had risen again 6 hr after rehydration. Blood corticosterone concentration was unchanged during these experiments, but blood cortisol concentration rose during water restriction.

Effects of water restriction on plasma renin-substrate concentration. Plasma renin-substrate concentration was measured in six experiments at the end of the control period and at the end of the period of water restriction. Results of three experiments are shown in Table 1. The levels were not substantially or consistently changed during water restriction. Results in the other three experiments were similar, with a substrate concentration (mean and range) of 215 (150–250) ng/ml.

DISCUSSION

The principal findings of the present study were that water restriction caused an increase in PRC, and that after subsequent rehydration PRC increased even further and remained elevated for at least 24 hr. The levels of PRC after 7–9 days of water restriction were quantitatively similar to the plasma renin activity after water deprivation in man (Maebashi & Yoshinaga, 1967) and rat (Rosenthal *et al.* 1969). They were also similar to PRC observed in sheep during rapid feeding (Blair-West & Brook, 1969), or after one day of sodium depletion by a parotid fistula (Blair-West, Cain, Catt, Coghlan, Denton, Funder, Scoggins & Wright, 1971*a*). The peak levels of PRC after rehydration were similar to the high levels seen in sheep with more severe sodium depletion (Blair-West *et al.* 1971*a*). It is interesting that 23 yr ago Kenney (1949) considered the possibility that the renin mechanism is stimulated by water deprivation.

Hypotheses on factors controlling renin release include: changes in sodium transport at, or load to, the macula densa (Vander, 1967; Nash, Rostorfer, Bailie, Wathen & Schneider, 1968; Vander & Carlson, 1969), renal baroreceptor mechanisms (Skinner, McCubbin & Page, 1964; Blaine & Davis, 1971) and sympathetic nervous activity (Vander, 1965; Gordon, Küchel, Liddle & Island, 1967; Mogil, Itskovitz, Russell & Murphy, 1969). These factors, singly or conjointly, are thought to mediate the renin response to haemorrhage (Hodge, Lowe & Vane, 1966; Bunag, Page & McCubbin, 1966), to low sodium intake (Brown, Davies, Lever, Robertson & Peart, 1964), and to sodium depletion by diuretics (Veyrat, de Champlain, Boucher & Genest, 1964; Vander & Luciano, 1967) or by parotid fistula (Blair-West *et al.* 1971*a*).

In the present experiments the findings during water restriction are consistent with the proposition that PRC was increased by a reduction of vascular volume without a change of external sodium balance. This proposition is, however, not tenable for the augmentation of PRC after acute rehydration since the concentrations of protein and sodium in plasma fell, indicating an expansion of vascular volume, which in turn must have led to an expansion of extracellular and intracellular fluid volumes. Again, sodium balance was unaffected during this period.

Mechanisms involving increased sympathetic nervous activity or renal baroreceptors cannot be excluded as causes of renin release during rehydration, because they are likely reactions to hypovolaemia. However, these mechanisms probably did not account for the increase in PRC after rehydration, since fluid volumes were expanding and B.P. remained steady throughout. The renin response to both water restriction and rehydration is, however, consistent with the macula densa hypothesis of Vander (1967) and Vander & Carlson (1969). During water restriction plasma oncotic pressure was raised and G.F.R. was probably reduced (Kenney, 1949). The raised plasma oncotic pressure may have increased sodium reabsorption from the proximal tubule (Brenner, Falchuk, Keimowitz & Berliner, 1969), thus both factors would tend to reduce sodium load to the macula densa and may consequently have stimulated renin release. After rehydration, the renal retention of sodium began within 30 min of drinking and became maximal in less than 2 hr. This rate of change of sodium excretion was too rapid to be attributed to the action of aldosterone (Barger, Berlin & Tulenko, 1958; Blair-West, Coghlan, Denton, Scott & Wright, 1968), suggesting that the retention of sodium occurred at sites proximal to the macula densa, e.g. reduction of filtered sodium load and/or increased reabsorption in the proximal tubule. Irrespective of the mechanism acting proximally to retain sodium during the decline of plasma sodium concentration, the large increase in PRC may have been due to reduced sodium load to the macula densa site.

There is also the possibility that an interaction between antidiuretic hormone (ADH) and renin played a part in the rise of PRC after drinking. Bunag, Page & McCubbin (1967) and Vander (1968) have reported that high levels of ADH can inhibit renin release. It can be reasonably inferred that ADH levels were reduced after rehydration, which may thus have removed the presumptive inhibition to renin release.

Plasma renin-substrate concentration in twelve normal sheep was found by Simpson & Blair-West (1971) to have been (mean and range) 309 (200–480) ng/ml. The levels in the present experiment fluctuated around 200 ng/ml. and were not affected by water restriction. This is in contrast to the finding by Rosenthal *et al.* (1969) that in dehydrated rats substrate concentration was markedly reduced.

Sodium excretion during control and experimental periods was similar, indicating that total body sodium had not changed during water restriction. The handling of sodium during dehydration and rehydration by the sheep can therefore be viewed as the response of an animal well adapted to periodic shortages of water and faced with a chronic scarcity of sodium in its diet. Water can be lost and then regained rapidly, but sodium is retained during dehydration with consequent hypernatraemia, to be titrated back

to normal after water becomes available. Thus body fluid volume, principally that of the circulation, is safeguarded and maintained at the expense of transient changes in plasma sodium concentration and osmolality.

The pattern of urine flow after dehydration is of considerable interest. The low plasma osmolality (reflected by the hyponatraemia) and the expanding extracellular fluid volume, as indicated by the fall in plasma concentrations of protein, sodium and potassium, might have been expected to suppress the release of ADH with a resultant water diuresis. Yet the rates of urine flow of the three sheep were only between 0·5 and 0·8 ml./min even 24 hr after rehydration. It appears inescapable that ADH was active after rehydration although vascular volume was expanding and plasma osmolality must have been of the order of 260 m-osmole/kg. One possible mechanism for the maintenance of ADH levels appropriate to such urine flow rates may be that the high renin levels stimulated ADH release, as was shown by Bonjour & Malvin (1970) to occur in the dog.

Blood levels of corticosteroids were in the normal range during water restriction and after rehydration, although blood cortisol concentration rose when water was restricted. It therefore appears that neither water restriction nor rehydration had a regular effect on ACTH release. Normal blood concentration (mean and range) of corticosterone is 0·032 (0·01–0·15 μg/100 ml. (n = 304), and of cortisol 0·53 (0·1–1·5) μg/100 ml. (n = 297) (J. P. Coghlan & B. A. Scoggins, unpublished). Blood aldosterone concentration during the control period was for no known reason above the usual range of 0–4 ng/100 ml. (n = 47) (J. P. Coghlan & B. A. Scoggins, unpublished) found in sodium replete sheep. During dehydration blood aldosterone concentration was reduced to low and even undetectable levels, demonstrating again a situation in which PRC and aldosterone are poorly correlated (Blair-West *et al.* 1971*a*). The low concentration of aldosterone in the presence of a raised PRC may have been due to suppression of aldosterone secretion by the high plasma sodium concentration (Blair-West, Coghlan, Denton, Goding, Wintour & Wright, 1966; Blair-West *et al.* 1971*b*). The increase of aldosterone after rehydration may be ascribed to the withdrawal of sodium inhibition as plasma sodium concentration fell to about 130 m-equiv/l., or to the very high renin levels attained after drinking. Possibly both factors were involved.

The present study shows that PRC may increase without consistent relation to any one extrarenal parameter such as sodium balance, intravascular or extravascular volumes, systemic blood pressure or plasma sodium concentration. If there was a single stimulus for renin release during water restriction and rehydration, it seems likely that the mechanism was activated by intrarenal events such as reduced sodium load to,

or transport at, the macula densa, as proposed by Vander (1967) and Vander & Carlson (1969).

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