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**EFFECTS  
OF  
ALTERATIONS IN POTASSIUM INTAKE  
ON  
BODY FLUIDS AND RENAL FUNCTION  
OF  
MERINO SHEEP**

**by**

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DECLARATION

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ACKNOWLEDGEMENTS

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DECLARATION

I hereby declare that the work presented in  
this thesis has been carried out by myself,  
except where otherwise stated, and that  
this dissertation has not been submitted  
in full, or in part, in any previous  
application for a degree.

D. W. PETER

## PREFACE

This thesis is concerned with physiological aspects of body fluids and renal function of Merino sheep during alterations in potassium intake. The absorption of potassium, sodium and water from the rumen before and after the addition of potassium to the rumen, has been investigated. Studies of the effects of natural hormones and pharmacological agents on the renal excretion of potassium, sodium and water have also been made.

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## ABBREVIATIONS USED

K	Potassium
Na	Sodium
Cl	Chloride
HCO <sub>3</sub>	Bicarbonate
Ac	Acetate

(and other conventional chemical abbreviations)

Isotopes other than the natural isotope are distinguished by a superscript before the symbol, e.g. <sup>42</sup>K. A\* exception to this rule is tritiated water which is designated TOH.

Concentrations in the text, diagrams and figures are expressed by enclosing the chemical symbol in square brackets:- e.g. rumen [K] - rumen potassium concentration.

In referring to pH the hydrogen ion is commonly expressed as H<sup>+</sup>.

### Other abbreviations

m-equiv	milli-equivalent (in text)
mE	milli-equivalent (in tables and figures)
μ-equiv	micro-equivalent (in text)
μE	micro-equivalent (in tables and figures)
m-osmole	milli-osmole (in text)
mOsm	milli-osmole (in tables and figures)

$\mu\text{Osm}$	micro-osmole (in tables and figures)
mU	milli-unit
$\mu\text{C}$	micro-Curie
m $\mu\text{C}$	milli-micro-Curie
g%	grams per 100 millilitres

### Body fluids

ECF	Extracellular fluid
ECV	Extracellular volume
ICF	Intracellular fluid
ICV	Intracellular volume
PV	Plasma volume
TBW	Total body water
% of TBW	percentage of total body water.

### Miscellaneous

Water TO	Water turnover
B.Wt.	Body weight
I.V.	Intravenous
S.A.	Specific activity
Hb	Haemoglobin
PCV	Packed cell volume
RBF or RPF	Renal blood (or plasma) flow
GFR	Glomerular filtration rate

ATP            Adenosine triphosphate

Cyclic AMP    Adenosine-3'5'-monophosphate

ACTH          Adrenocorticotrophic hormone

ADH           Antidiuretic hormone

EACA          6-amino-n-hexoic acid or Epsilon-amino-n-caproic acid

Arg-vasopressin    Arginine-8-vasopressin



## I. INTRODUCTION

### 1. Potassium as an essential body element

Although it is well documented that potassium (K) is required in the diet of animals, the likelihood of K deficiency occurring in a grazing ruminant is extremely low. Moreover, the ingestion of K by the ruminant at pasture is at least two or three times in excess of its optimal requirements and these high K intakes have been implicated, most likely incorrectly, in the development of various metabolic disorders. These high intakes raise the question of how the ruminant tolerates and eliminates this excess and controls K excretion.

"As a plant nutrient, K is the Cinderella of the elements; to it has been attributed the highest and the humblest functions in the plant organism" (Fowler, 1963). This comment may, with some reservations, be applied to the animal as well as to the plant. Most of the functions attributed to K have been described since early last century, following the realization that K is essential for life and growth.

The history of K as an essential element in animal well-being began when Liebig (1847) discovered the contrasting distribution of Na and K in lymph as compared with extravascular tissue. A short time later

von Bunge (1884) claimed that the high K levels of clovers (measured by Boussingault) caused salt craving in animals and he asserted that a lack of Na as compared with K, or an excess of K salts in the diet rather than NaCl, caused Na to be lost in the urine and a salt craving to occur.

In the following years, Ringer (1883) and Locke (1895) contributed largely towards the establishment of K as an essential body element, by their discovery of the antagonism of ions and their elucidation of the role of ionic species including K in maintaining normal function. Meanwhile Loeb's study of Fundulus heteroclitus (minnow) published in "The Dynamics of Living Matter" illustrated the nutritive significance of the inorganic constituents of the diet as contributors to physiological well being (McCollum, 1957).

Osborne and Mendel (1918) were the first, however to study the effects of a low K diet, demonstrating retarded growth in young rats. The rats of Schrader, Prickett and Salmon in 1937 when fed a low K diet showed abnormal distention and cyanosis as well as poor growth. As with Osborne and Mendel's work there was some doubt as to the limitation of the deficiency to K and vitamin B<sub>12</sub> was probably deficient as well. Therefore, the first

absolute demonstration of K being necessary for the growth of animals (rats) was by Orent-Keiles and McCollum (1941).

Quite recently several estimates of the K requirement of growing sheep and cattle have been made. Telle, Preston, Kinter and Pfander (1964) and Campbell and Roberts (1965) estimated that growing sheep require about 0.5 to 0.6% of a balanced ration as K or 1.63 m-equiv/kg body weight/24 hr for maintenance. Similar values were obtained by Roberts and St. Omer (1965) for young steers. St. Omer and Roberts (1967) estimated that the maintenance K requirement of young heifers was 133 m-equiv K daily/100 kg body weight. This was considerably less than the calculated requirement for growth, based on a value of 0.5% dietary K of 287 m-equiv/100 kg body weight. St. Omer and Roberts suggested that this may be due to a suppression of appetite by a low K diet.

In adult lactating cows, only 0.32% of the dry matter of the diet need be K to maintain a milk production of 2 gallons/day (Du Toit, Malan and Groenewald, 1934).

## 2. Potassium and the Ruminant

In comparison to many other inorganic elements of the body (Na - 0.1 to 0.15 g.%, Ca - 0.18 g.%, P - 0.17 g.%, Fe - 0.015 g.%, Co - 0.0011 g.%, Zn - 0.0018 to 0.0033 g.%, I - 0.000005 to 0.00001 g.%, (Underwood, 1966)), a larger fraction of the maintenance diet of sheep needs to be K (0.50 g.%). Owing to the naturally high content of K in most plant species grazed by the ruminant, the K intake usually far exceeds the requirements.

Following the initial discovery by Lawes and Gilbert at Rothamstead (1859) and Knops (1862) that K (potash) was essential for plant growth, fertilizer containing potash or potash alone has been added to both grain and fodder crops and pasture and its use continues to grow. Therefore, the levels of K in plant species grazed by ruminants will probably continue to increase. Table A indicates the levels of K in various foods likely to be encountered by the ruminant.

Although many species of grass may be classified into high, medium or low Na content grasses, no such classification can be found for any groups of plant species for K (Griffiths, Jones and Walters, 1965; Griffiths and Walters, 1966). Furthermore the K content of many plants

varies with the availability of soil K, and this may be further modified by the Na content of the soil (Griffiths and Walters, 1966). Thus, ratios of K to Na may vary over a wide range with no definite relationship between the two nor any absolute level of K.

Referring to Table A again it can be seen that the upper limit of K content is achieved by an arid zone saltbush of Australia (Atriplex lindleyi). Its K content of 1650 m-equiv./kg of dry matter is similar to the Na content of other Atriplex (A. nummularia and A. vesicaria) and Kochia saltbushes which are eaten by grazing sheep (Macfarlane, Howard and Siebert, 1967).

A more common pasture plant which contains large levels of K is alfalfa (Medicago sativa L.) which may reach levels as high as 900-1000 m-equiv./kg of dry matter (personal observation). Many pasture clovers also attain K contents of 750-950 m-equiv./kg of dry matter.

Table A indicates that by grazing any of these species of plant the K intake of the sheep far exceeds the requirements. Therefore it appears likely that the ruminant has developed some mechanism whereby it can regulate the excretion of this excess K load while maintaining normal levels of functions.

TABLE A

POTASSIUM AND SODIUM CONTENT OF SOME FOODSLIKELY TO BE EATEN BY THE RUMINANT

	K		Na.	
	<u>m-equiv</u> kg DM	% DM	<u>m-equiv</u> kg DM	% DM
Alfalfa (vegetative)	175-1055	0.7-4.29	-	-
Alfalfa (hay)	225-1060	1.0-4.30	69-313	0.16-0.72
Barley (vegetative)	610-970	2.44-3.88	-	-
Barley (grain)	160	0.64	-	-
Wheat (grain)	145	0.58	43	0.10
Timothy forage	435	1.74	48	0.11
Clover forage	725	2.90	117	0.27
Clover and mixed grasses	900	3.60	-	-
Rye grass pasture (annual)	365	1.48	-	-
Rye grass pasture (perennial)	475	1.90	-	-
Oats grain	105	0.42	30	0.07
Wild oats (vegetative)	695-720	2.78-2.88	-	-
Corn Kernels	55-230	0.22-0.92	-	-
Corn cobs	165	0.46	-	-
Corn silage	287	1.15	13	0.03

TABLE A cont.

Saltbush <u>Atriplex nummularia</u>	830-980	3.32-3.92	3150-3900	7.25-8.97
Saltbush <u>Atriplex lindleyi</u>	1650	6.6	7	0.016
Red clover hay	466	1.87	-	-
Red clover pasture	430-737	1.72-2.95	100	0.23
Dried Salt bushes (USA)	1255	5.02	-	-

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(Taken from: Personal observations; Biology Data Book - Ed. P.L. Altman and D.S. Dittmer, Federation of American Societies for Experimental Biology, Washington, D.C. (1964); Feeds and Feeding - F.B. Morrison, Morrison Publishing Co., N.Y. (1957); Composition of Cereal Grains and Forages - National Academy of Sciences, National Research Council Publication 585, Washington, D.C. (1958)).

### 3. Postulates

It was thought likely at the beginning of these investigations that the adaption and tolerance of ruminants, sheep in particular, to the ingestion of increasingly large quantities of K would be associated with changes in a number of physiological modalities.

- a. Specialized renal or gut mechanisms whereby the excess K could be excreted might be found (and the sheep does show net secretion of K, at rest, without special K loading).
- b. An increased water turnover as the K intake was increased and an increased urine volume would be likely unless unusual concentration of K occurred in the urine.
- c. There should be alterations in the rate of uptake of K and possibly water and/or Na from the rumen when the K content of the rumen was increased by the ingestion of quantities of K.
- d. Some mechanism stimulating the increased excretion of K without allowing too great a rise in plasma K seemed likely since sheep normally turn over 300-800 m<sub>e</sub>quiv of K daily.
- e. From earlier findings (Kinne, Macfarlane and Budtz-Olsen, 1961) that vasopressin increased K excretion by the kidney there was the possibility that vasopressin as well as other active hormones were involved in regulating K excretion.



Since previous studies (English, 1966; Keynes and Harrison, 1967) have indicated that during the ingestion of 200-800 m.equiv K/day by sheep the fraction of the total K excreted in the urine (relative to urine and faecal loss) remains relatively constant, measurements of the K output in urine and faeces were made when the K intake exceeded 1000 m.equiv /day. From the fact that a large Na intake alters the volumes and concentrations of body fluids as well as water turnover (Macfarlane, Howard and Siebert, 1967) these parameters were also measured at increasing rates of K intake.

To explain the possible changes in water intake associated with an increased K intake, measurements of the changes of rumen and plasma electrolyte concentration and OP were made following the ingestion of varying quantities of K in the food or the direct addition of K salts to the rumen. The time course of changes in urine volume and electrolyte concentration were also followed.

Further, in relation to the addition of K supplements to the rumen, the uptake of water using TOH, K, using  $^{42}\text{K}$  and Na, using  $^{24}\text{Na}$  was followed. Salivary return rates and movement from the blood to the rumen and vice versa with and without saliva were also studied. The uptake rates of TOH,  $^{42}\text{K}$  and  $^{24}\text{Na}$  from other segments of the lower gut were briefly examined.

In attempts to analyse the mechanism of K secretion by the kidney and the possible stimulation of this secretion, the effects of various pharmacological agents and hormones, alone and in combination were observed. Included in these observations was the effect of vasopressin on the renal function of normal, K depleted and K supplemented sheep and sheep undergoing an induced diuresis. The latter investigation was aimed at finding whether the K-excretion effect of this hormone was separate from its antidiuretic action and if so, could vasopressin aid in the regulation of K excretion.

## II. LITERATURE REVIEW

### 1. Body fluids and electrolytes

The total fluid component of mammals (total body water = TBW) in terms of body weight may range from 95% in the human foetus to 30% in a very fat adult pig (Wolf, 1958). In the sheep, TBW can vary from 50-80% of the B.Wt. with a mean of the order of 70%.

The total body fluid of man may be divided into 2 compartments, the extracellular fluid (ECF) and the intracellular (ICF), the former being further subdivided into the transcellular fluid (TF), the interstitial fluid (IF) and the plasma (PV). In ruminants the fluid of the alimentary tract constitutes such a large fraction of the total that it must be considered as a third body fluid compartment. Therefore, in a sheep for example, with a TBW of 70% of the B.Wt. the ECF may occupy 30% of the total body fluid, the ICF 55% and the gut fluid 15%.

There are three major differences between the ECF and the ICF. By far the most important is the predominance of  $K^+$  and to a lesser extent  $Mg^{2+}$  among the cations of the ICF compared with mainly  $Na^+$  in the ECF. Phosphate is the predominant anion of the ICF with  $Cl^-$  and  $HCO_3^-$  as the anions in the ECF. Finally a much greater percentage of the ICF base is neutralized

by protein at pH 6.9 than in the ECF. Several exceptions to the rule that  $K^+$  is the predominant intracellular cation exist, notably in some sheep where the erythrocytes are low in K and high in Na (Evans, 1957).

The roles of electrolytes in the body fluid are quite numerous though they have 3 major functions:

a. Electrolytes and cellular metabolism

The effect of electrolyte concentrations on cellular metabolism is one of the least understood roles of body electrolytes, although the cellular levels of K and Mg are essential for integral function. In the cell, K is combined with protein as an essential ingredient of cytoplasm absorbed on membranes, and along with phosphate is also combined with glucose and glycogen (Harrison, 1954; Kernan, 1965). Cellular K influences the metabolism of fatty acids particularly the increased metabolism to acetoacetate by the liver in the presence of K (Geyer, Meadows, Marshall and Gongaware, 1953), the utilization of fats and proteins added as dietary supplements (Wooley and Michelson, 1954), and the synthesis of protein (Cannon, Frazier and Hughes, 1952).

b. Membrane and cellular potential differences

The electrochemical role of electrolytes is partly concerned with electroneutrality, but the unequal distribution of cations achieved through active transport, between the inside

and outside of cells leads to potential differences across the membrane. Active ion transport depends upon the presence of K extracellularly and Na intracellularly for the function of a specific adenosine triphosphatase (ATPase). Skou (1965) has proposed that this enzyme, located on the cell membrane, is associated with the release of energy for the active transport, by hydrolysis of adenosine triphosphate (ATP). The explanation of the steps involved in supplying this energy are still uncertain (Skou, 1965; Garrahan and Glynn, 1967; Katz and Epstein, 1967a). Other ions and metabolites beside Na and K may also be transported by this Na-K-ATPase system.

The requirement of both K and Na at opposite sides of the membrane for transport involving Na-K-ATPase suggests coupling between the active transport of both these ions (Tosteson, 1966). However, even when Na alone is transported by the frog skin, K is still required (Sharp and Leaf, 1966).

Cardiac glycosides, like ouabain, inhibit this active ion transport probably by interfering with the Na-K-ATPase. Like other ATPases in the cell the Na-K-ATPase has an absolute requirement for  $Mg^{2+}$  but in tissue homogenates both  $Na^+$  and  $K^+$  as well as  $Mg^{2+}$  are required for maximum hydrolytic activity (Katz and Epstein, 1967a). This Na-K specific hydrolytic activity of tissues such as kidney and brain, has been correlated

with their level of active transport estimated from physiological parameters (Bonting, Simon and Hawkins, 1961; Bonting, Caravaggio and Hawkins, 1962). Furthermore, by increasing the glomerular filtration rate and hence tubular reabsorptive work of rat kidneys, by unilateral nephrectomy, by feeding a high protein diet or by administering methylprednisolone, a substantial increase in Na-K-ATPase activity of the tissue has been induced. Other enzymes such as glucose-6-phosphatase and succinic dehydrogenase were unaffected (Katz and Epstein, 1966, 1967b).

A difficulty associated with the correlation of Na reabsorption in vivo and Na-K-ATPase activity of kidney tissue in vitro is the finding that although the 2 diuretics, frusemide and ethacrynic acid are both strong inhibitors of rat kidney Na-K-ATPase activity in vitro, only frusemide inhibits Na reabsorption and causes a natriuresis in vivo in the rat (Hook and Williamson, 1965). Similarly there are other diuretic compounds which inhibit Na reabsorption by the kidney in vitro but have no effect on Na-K-ATPase activity in vivo (Taylor, 1963; Bonting, Canady and Hawkins, 1964; Frazer, 1963). This alone does not rule out any correlation of Na-K-ATPase activity and active transport but it does question the validity of the assumption that a high Na-K-ATPase activity in vitro and a high active transport rate in vivo are associated.

One problem connected with the hypothesis that the Na-K-ATPase is associated with Na reabsorption in the proximal tubule of the kidney is that if Na and K transport are coupled then K should be, but is not, actively transported into the proximal tubule lumen. However, it is thought that the K pump is located on the basal membrane of the renal tubule cell and that if K is actively pumped into the cell it diffuses out (Katz and Epstein, 1967a). It is known that the basal membrane is more permeable to K than the luminal membrane (Malnic, Klose, and Giebisch, 1964).

During active K secretion in the distal tubule and collecting duct, this transport system may operate without back diffusion through the basal membrane.

#### c. Osmotic control of fluid volume

Another role of the electrolytes is to maintain within relatively narrow limits the volume of the ECF and the ICF. This is achieved by control of the fluid OP through changes in electrolyte concentration. Although Na is the major electrolyte of the ECF, and the ECV is presumably first to suffer as the result of any environmental or dietary stress, investigations involving changes in Na and water movement in relation to changes in plasma osmolality show that to accomodate such changes K balance must also be considered (Wynn and Houghton, 1957). Similarly, the osmolality of plasma may be predicted by

a study of total body water and exchangeable Na and K but not exchangeable Na alone (Edelman, Leibman, O'Meara and Birkenfeld, 1958). Thus it can be inferred that intracellular K is osmotically active like the Na of the ECF and plays its part in controlling ECF as well as ICF volume.

Diverging for a moment to consider the plasma separately from the ECF it is seen that the plasma contains significantly more protein than the remainder of the ECF. This protein produces the colloid osmotic or oncotic pressure which prevents the loss of water from the plasma to the interstitial space by the hydrostatic pressure in the capillaries.

Although the ICF and ECF are separated they have 2 major similarities, the first of which is that the total number of cations (m-equiv /l.) balances the total number of anions (m-equiv /l.) plus protein charges and therefore both are nearly neutral. Secondly, the total concentration of electrolytes (+ protein) or osmolarity (m-osmole/l.) of both fluids is very similar; between 150 and 170 m-equiv /l. (Black, 1963).

In the above discussion the ICF and ECF have been considered rather independently, but overall both are in a highly dynamic state with each dependent on the other. This dependence should be greater in sheep than in man since the rumen holds 4-10 l. of alimentary fluids, while 6-15 l./day of saliva and



7-8 l./day of gastric secretions, a total equal to half of the total body fluids, may be poured into the alimentary tract and Lewis, 1959 per day (Annison, Denton, 1957). Hence any changes in these movements as a result of disease, electrolyte deficiency, dietary change, hormone imbalance or stress could result in a complete reshuffle of water and electrolytes throughout the entire body. To prevent any major disturbance of the system a precise control of electrolyte and water movement and excretion is necessary.

## 2. Total and exchangeable electrolytes

The determination of total or exchangeable electrolytes was until the last 15-20 years a tedious post-mortem process involving chemical analysis of whole bodies or body components. Lawes and Gilbert published results in 1859 on the analysis and distribution pattern of the various major inorganic elements including K in domestic animals. Although Liebig in 1847 had discovered the contrasting distribution of Na and K in lymph as compared with extravascular tissue it was not until 1906, that Abderhalden, using human red blood cells, demonstrated that K was maintained at high concentrations as an intracellular cation and Na predominated as an extracellular cation.

Since radioisotopes have become readily available this distribution of K intracellularly and Na extracellularly has been widely investigated in relation to the exchangeable

as well as the total body electrolyte, in an effort to define some relationship between electrolyte and fluid content and body composition. By using  $^{42}\text{K}$ ,  $^{24}\text{Na}$  or  $^{22}\text{Na}$ ,  $^{82}\text{Br}$ ,  $^{131}\text{I}$ -albumin,  $^{52}\text{Cr}$ -labelled red cells and  $^3\text{H}_2\text{O}$  or  $\text{D}_2\text{O}$  measurements of total and exchangeable Na, K and Cl, red cell volume, PV, ECV and  $\text{TBW}_A$  are readily obtainable (Boling, 1963). Whole body counting of the naturally occurring K isotope ( $^{40}\text{K}$ ) is also widely used to obtain whole body K content (Hansard, 1963). These parameters also allow calculations of ICV and electrolyte concentrations to be made.

Total body or total exchangeable K in humans, estimated from  $^{42}\text{K}$  dilution techniques varies with sex and age. Non-obese subjects between the ages of 10 and 30 years contain between 43 and 55 m-equiv/kg in males and 34 and 51 m-equiv/kg in females with means in the order of 45 and 37 m-equiv/kg (Forbes and Lewis, 1956; Threefoot, 1962; Anderson, 1963; d'Deuxchaines, Collett, Busset and Mach, 1961). Expressed on a fat-free basis, these values become much higher as they often do when calculated from whole body counts of  $^{40}\text{K}$ . The difference between total body K estimated by the isotope methods is presumed to be due to a nonexchangeable K fraction which is equilibrated with  $^{40}\text{K}$  but not  $^{42}\text{K}$  but this point is still debatable and difficulties of whole body counting make it hard to resolve (Veall and Vetter, 1958; Miller and Remendrik, 1963).

Levels of total and exchangeable Na in the adult human body, range from 37-47 m-equiv./kg D. Wt, the value for males being greater than that for females (Forbes and Lewis, 1956). Estimates of exchangeable Na using  $^{22}\text{Na}$  or  $^{24}\text{Na}$  are lower than the total Na calculated from whole body analysis.

In sheep the whole body K has been variously estimated by chemical and isotopic means to be of the order of 0.21 to 0.26% of the dressed carcass weight rising to 0.28 - 0.30% on a fat free basis (Spray and Widdowson, 1951; Kirton and Pearson, 1963). Several of these measurements (Kirton and Pearson, 1963; Kulwich, Feinstein and Anderson, 1958) showed that there was considerable K in bone and fat. Mudge (1953) estimated that approximately 5.8% of the whole human body K was in bone and about 70% in muscle while Kirton and Pearson's (1963) measurements on sheep showed 82% of the total body K to be in muscle.

The K and Na content of muscle is not constant and varies between fibre types; red muscle cells containing less K (as low as 153 m-equiv./kg intracellular water) than white muscle (as high as 162 m-equiv./kg intracellular water in rats) with the total number of m-equiv./l. of Na and K remaining relatively constant (Sreter and Woo, 1963).

Since the K of the body is located predominantly in the muscle cells, liver, kidney and brain (lean tissue) correlations between whole body K, TBW, lean body mass and separable fat have been made. Correlation coefficients from -0.72 to -0.89 were found between K content per unit weight and percent separable or excess fat in live lambs using either  $^{42}\text{K}$  or whole body counting (Kirton, Pearson, Nelson Anderson and Schuck, 1961; Judge, Stob, Kessler and Christian, 1962). Using measurements from chemical analysis of pigs, correlation coefficients between whole body K and TBW, ether extract and protein as a percentage of B. Wt. had values of -0.82, -0.83, and 0.71 respectively (Kirton, Graedinger and Pearson, 1962). However, there is a large variability between animals in relation to the K content of muscle and fat. Whole body K determined by chemical analysis has shown better correlations with other parameters than has K content measured by isotopic methods (Kirton and Pearson, 1963).

Due in part to the electrolyte content of bone tissue, the turnover of whole body Na and K is relatively slow. In terms of biological half life the turnover of either element will depend on the rate of intake and excretion. As much more Na than K is found in the ECF and is available directly to the kidney, there will be a faster turnover of a greater fraction of total Na than of K. Although the total K turnover/day of a

sheep is high (0.5 - 1.0%/hr) due to the large K intake, relatively small amounts of much of the intracellular K are turned over, since the K (and Na) turnover rate of individual tissues, in particular bone and the red blood cells, is very slow (Potts and Parry, 1964).

### 3. Control of body fluids and electrolytes

Body fluid and electrolyte balance is sustained between the diencephalic regulation of intake on one side and excretion of water and salts by the kidney and gut on the other. The skin, sweat glands, mammary glands and the surface of the lungs and nasal cavity also make significant contributions to water TO depending on the physiological state of the animal. These areas have only minor control functions, however, though some regulation may occur in the sweat glands.

In addition to regulation of total body fluids and electrolytes, there may be changes in the individual fluid compartments where the volume may vary but in which the concentrations of electrolytes are conserved within narrow limits. This was well illustrated by the work of Macfarlane, <sup>Morris,</sup> Howard, McDonald and Budtz-Olsen (1961) when Merino sheep without water in summer lost more than 30% of the PV and ECV but only slight changes occurred in the electrolyte concentration of the plasma.

Electrolytes ingested in food are absorbed from the alimentary fluid into the IF, thence into the plasma. Since this fluid has  $\text{Na}^+$  as its predominant cation any K absorbed in excess of that required to maintain the normal 4 to 6 m-equiv/l. must either pass into cells or be excreted. Although there is evidence that the K content of some mammalian cells may be elevated by raising the ECF  $[\text{K}]$  (Dawborn and Ross, 1967) most of the K absorbed is promptly excreted by the kidney to maintain a balance.

Extracellular  $[\text{Na}]$  and  $[\text{K}]$  may be elevated by the absorption of these electrolytes from the gut or by the loss of ECF water. ECV is also reduced as water is lost by the kidneys or in the sheep by rapid salivation. To restore the ECV and concentration to normal, either in the first instance K and Na must be excreted via the kidney or returned to the gut or water must be absorbed from the cells or the gut. Drinking allows available water in the gut for absorption following which the ECF and ICF volumes and concentrations may be returned to normal by the excretion of any excess water and electrolytes.

Drinking may be initiated directly by the action on hypothalamic receptor cells of an elevated ECF osmolality or indirectly by a decrease in ICF (cell dehydration) following loss of water to the ECF (Wolf, 1958; Stevenson, 1967). Other stimuli and inhibitors of drinking are changed in the ECV,

dryness of the mouth, rate of salivation, a deficit of K in the ICF or gastric volume (Strauss, 1957; Fourman and Leeson, 1959; Epstein, 1967; Adolph, 1967) .

Thus in all the mammalian species studied, the ECV and osmolality and in turn the ICV and concentration are regulated largely, through alterations in the renal excretion of free water, K and Na and to a lesser extent by water and electrolyte excretion in the faeces. These are under hormonal, vascular and nervous control. For example, free-water clearance depends upon the release of vasopressin from the pituitary, while Na excretion is largely controlled by the adrenal secretion of aldosterone and the renin-angiotensin system which originates in the kidney. These hormones are released by various factors which will be enumerated in a following section. The controls of body fluid concentrations of K and its excretion are still, however, largely uncertain.

#### (1) Electrolyte movement in the gut

Besides causing Na retention and in some animals K excretion by the kidney, aldosterone and related mineralocorticoids increase Na reabsorption from the ileum and colon of rats, dogs and man (Edmonds and Marriot, 1967; Levitan, 1967; Shields, Miles and Gilbertson, 1968) while increasing the secretion of K into the colon (Edmonds and Marriot, 1967; Berger, Kanzaki and Steele, 1960; Shields, Mulholland and Elmsie, 1966). As yet there is no information on the effect of aldosterone on

electrolyte movements in the gut of sheep and cattle.

Sodium absorption by the ileum and colon is an active process, against electrochemical gradients (Curran and Schwartz, 1960; Curran and Solomon, 1957; Schultz and Zalusky, 1964a). This active absorption of Na is also related to the active transport of glucose and amino acids (Schultz and Zalusky, 1964b; Schedl and Clifton, 1968) but more importantly water appears to move with the Na in solution isotonic with plasma. Therefore, by increasing reabsorption of Na in the lower gut, aldosterone increases water reabsorption and expands the ECV (Curran and Solomon, 1957; Edmonds and Marriot, 1967).

Although K is secreted into the colon of rats, dogs and man, it is not certain whether a similar secretion occurs in ruminants. Concentration changes in various gut segments of sheep and cattle suggest that K is secreted into the colon though most of the K entering the small intestine is absorbed by the time it reaches the rectum (Horrocks and Phillips, 1964 a, b; Perry, Cragle and Miller, 1967; Van Weerden, 1961). The secretion of K into the colon of rats, dogs and man is a passive process with the ion passing down an existing electrical gradient yet against a concentration gradient (D'Agostino, Leadbetter and Schwartz, 1953; Code, Bass, McClary, Newnum and Orvis, 1960; Cooperstein and Brockman, 1959).



Stimulation of K secretion into the colon of man during primary aldosteronism results in an excess loss of K in the faeces (Shields et al. 1968). This extra K loss can result in a lowered plasma [K] an increased plasma volume, shifts and prolongation of the S-T segment of the electrocardiogram, hypertension and muscular weakness.

Another hormone which influences Na and hence water movement but not K in the gut of man, dog and rat, is vasopressin. Various workers have shown that vasopressin increases the Na and water absorption from the small intestine and colon of dog and rat (Ussing, 1957; Blickenstaff, 1954; Dombradi, Krizsa and Janeso, 1960; Aulsebrook, 1961). Levitan and Mauer (1966, 1968) however, reported decreases of water and Na absorption from the colon of man while Green and Matij (1966) found no effect of Pitressin on Na absorption by the rat ileum or colon in vivo, or by mouse and toad colon in vitro. More recently, Soergel, Whalen, Harris and Geenen, (1968) have shown that presumably endogenous vasopressin released during dehydration as well as exogenous vasopressin, decreased water and Na absorption from an isotonic or hypertonic saline solution perfusing human small intestine in vivo. These workers concluded that circulating vasopressin affects the small intestine of man in one of 2 ways: either by an increased secretion of water and salt into the lumen or by inhibition of an active Na absorbing mechanism.

Overall, the gut, like the kidney, plays an important part in regulating the ICF and ECF volumes and solute concentrations of many animal species but as yet it has to be shown that it can function in the regulation of K metabolism of the ruminant.

#### 4. Kidney function and autoregulation

##### (i) The counter current mechanism and kidney function

The counter-current hypothesis which states "that a small osmotic pressure (OP) difference continuously established between the contents of the limbs of a loop could be multiplied by a counter-current" (Wirz, 1961) is now accepted as an integral part of the renal concentrating mechanism. Good agreement between the mathematically derived explanation of the system (Margitay Kuhn, 1951) and renal function in relation to structure has been found (Gottschalk and Mylle, 1959; Schmidt-Nielsen and O'Dell, 1961).

The major problem still associated with the hypothesis is that no one has demonstrated an active Na pump in the ascending limb of the loop of Henle. Studies on the permeability of the loop of Henle, the vasa recta and the collecting duct, provide observations which are, however, consistent with this idea (Berliner, and Bennett, 1967; Morgan and Berliner, 1968).

Briefly, the mechanisms of kidney tubular function involving the counter-current hypothesis are as follows:-

In the proximal tubule, 70 to 80% of the glomerular filtrate is reabsorbed, the major electrolyte being Na, though at least 70% of the K filtered is also absorbed here. The Na reabsorption is an active process and water is presumed to follow the Na to yield a resorbate isotonic with plasma. How this water movement occurs is uncertain since no osmotic gradient is demonstrable. Two current theories involving the role of intercellular canaliculi as the sites where osmotic gradients are produced and maintained, are the "double-membrane" theory of Curran and MacIntosh (1962) and Dietschy, (1966) and the concept of "local osmosis" now modified and termed the "standing gradient osmotic flow" theory of Diamond and Tormey (1966, a,b) and Diamond and Bossert (1967).

From the proximal tubule, the isotonic filtrate proceeds down the descending limb of the loop of Henle and into the water-impermeable ascending limb. From the ascending limb active Na extrusion occurs causing the fluid of the interstitium, the vasa recta and descending limb to become hypertonic. The vasa rectae may be counter-current exchangers with the limbs of the loop, the multiplier (Berliner and Bennett, 1967). Thus, the fluid entering the ascending limb is truly hypertonic but becomes isotonic or even hypotonic by the time it reaches the first part of the distal tubule.

In the distal tubule, further Na reabsorption occurs with some fraction being exchanged for  $K^+$  and  $H^+$  until the fluid

entering the collecting ducts is isotonic with plasma. If vasopressin is present, the collecting ducts like the distal tubules of some species become more permeable to water, and water is reabsorbed by the osmotic pull of the hypertonic interstitium. Therefore, a hypertonic urine is produced, while in the absence of vasopressin little or no water is reabsorbed in this region and an isotonic or possibly a hypotonic urine results (Gottschalk and Mylle, 1959; Wirz, 1961; Lassiter, Gottschalk and Mylle, 1961; Berliner and Bennett, 1967; Thureau Valtin and Schnermann, 1968).

(ii) Electrophysiology of the nephron and ion movement

Transtubular potential differences of the order of -20 mV, lumen negative, have been measured across the proximal tubules and -35 to -60 mV across the distal tubules of mammalian nephrons. Much smaller potential differences of -1 to -3 mV exist across the thin descending limb of Henle and slightly higher potentials across the thicker ascending limb of the loop and the collecting duct (Windhager and Giebisch, 1965). These values may be only the minimum due to flux asymmetry and it is now doubtful if the potential difference across the proximal tubule is -20 mV. Frömter and Hegel (1966) and Burg, Issacson, Grantham and Orloff (1968) have shown the true potential difference is close to zero, though they have confirmed the existence of a distal transtubular potential of -60 mV in rats.

Potassium transport in the proximal tubule (rats, dogs, rabbits, man) like Na, is apparently active with at least 70% of the filtered K being reabsorbed. Potassium is actively transported

from the lumen or peritubular fluid into tubular cells since the  $[K]$  in tubular fluid is considerably less than expected from passive distribution according to the membrane potentials (Bloomer, Rector and Seldin, 1963; Watson, 1966; Malnic, Klose and Giebisch, 1964; Malnic et al., 1966a,b). From inside the proximal tubule cells the K can diffuse down its concentration gradient into the plasma.

In the distal tubule Na reabsorption must be active since it proceeds against a high electrochemical gradient. In most situations varying degrees of net secretion of K must occur as the concentration increases from below plasma level in the early distal tubule to greater than plasma level at the proximal end of the collecting duct. Comparison of actual  $[K]$  gradients with those calculated on the assumption that K distributes according to the transtubular potential, suggests passive diffusion of this ion from the high cellular  $[K]$  to the lower concentration of the distal tubule. When net reabsorption from the lumen does occur, it should be an active process (Thurau et al. 1968).

For the maintenance of a high intracellular K content which is the source of secreted K (DeRouffignac and Guinnebaux, 1966) an inward K pump at the peritubular cell surface of the distal tubule is likely (Windhager and Giebisch, 1965). The peritubular membrane of rats is selectively permeable to  $K^+$  whereas the luminal membrane is equally permeable to  $K^+$ ,  $Na^+$  and  $Cl^-$ . This means that the transtubular potential difference is dependent on the  $[K]$  of the peritubular fluid. Distal tubular  $[K]$  in

free flow or stationary microperfusion has been found to be lower than that calculated from passive equilibration. Therefore, an active reabsorption of K at the luminal surface is assumed to prevent an electrochemical equilibrium being reached (Malnic, et al., 1966 a, b).

From the high intracellular level of K in distal tubule cells, secretion into the lumen takes place via exchange with  $\text{Na}^+$  and in competition with  $\text{H}^+$  (Berliner, 1961). Disruption of this exchange may occur in K or Na deficiency, when inadequate  $\text{Na}^+$  and K respectively are presented for exchange. However, distal Na reabsorption is always in excess of K secretion and furthermore neither the rate of delivery of Na to the distal tubule nor the [Na] of the tubular fluid appears to alter the K secretion rate (Malnic, et al., 1966 a,b). A one-to-one exchange mechanism is, therefore, very unlikely.

Information about ion movements in the collecting ducts is still rather scanty though it seems that K transport is similar to that in the distal tubule, net secretion being regulated by the transtubular potential difference (Malnic, et al., 1966 a,b).

Chloride is the predominant anion of the urine in non-herbivores, while in herbivores such as the sheep,  $\text{HCO}_3$  is excreted in large quantities to satisfy the large K excretion, which in sheep may exceed 500 m-equiv/day.

Chloride reabsorption from the proximal tubule was, until the time of Frömter and Hegel's (1966) work, considered to be a passive process with Cl moving down its electrochemical gradient. With a net potential difference of zero, active transport of Cl is likely, as work carried out by Clapp, Rector and Seldin (1962) using non-reabsorbable anions has already suggested.

The transtubular potential difference of the distal tubule favours passive Cl reabsorption but Rector and Clapp (1962) have proposed that this too is active or has an active component. Similarly, active reabsorption from the collecting duct is likely (Windhager and Giebisch, 1965). Filtered  $\text{HCO}_3^-$ , the anion which accounts for the alkaline urine of herbivores, is reabsorbed in the proximal tubule. Reabsorption is mediated by exchange with actively secreted  $\text{H}^+$ . The carbonic acid formed in the tubule decomposes to  $\text{CO}_2$  and water which are absorbed. The decomposition of carbonic acid is catalysed by carbonic anhydrase in the luminal membrane.

In the distal tubule a similar  $\text{HCO}_3^-$  reabsorption mechanism is postulated though carbonic anhydrase is absent and a disequilibrium pH develops between the tubular fluid and the plasma (Rector, Carter and Seldin, 1965). The secretion of  $\text{H}^+$  into the distal tubule is in competition with  $\text{K}^+$  so that a high K load leads to decreased  $\text{H}^+$  secretion, decreased  $\text{HCO}_3^-$  reabsorption, the excretion of  $\text{KHCO}_3$  and an alkaline urine (Berliner, Kennedy and Hilton, 1951). This is presumably what

occurs in sheep, whose urine pH may attain values of 8.3 or greater (Macfarlane, et al., 1961; Stacy and Brook, 1964: personal observations). At pH greater than 6.1 no carbonic acid is present in the urine and at pH greater than 8.3, carbonate is formed.

### (iii) Autoregulation

Regulation of urinary excretion and control of body fluids is in turn governed by renal plasma flow (RPF), the glomerular filtration rate (GFR), tubular reabsorption, secretory mechanisms and the action of various hormones. Renal plasma flow and GFR are the 2 factors for which there is evidence of autoregulation (Thurau, et al., 1968) though extra-renal factors may also alter these rates of flow.

Although the RPF may not alter, the distribution of blood and the direction of flow within the cortex and medulla of the kidney may change thus altering water and solute reabsorption and secretion (Thurau, et al., 1968). Normally the blood flow through the medulla is far less than through the cortex. The mean transit time in dog, determined by using  $^{32}\text{P}$ -labelled red cells, was 3.2 sec in the cortex, and 24 sec for the medulla (Grangsjö, Ulfendahl and Wolgast, 1966). The cortical flow rate in rats, like dogs, ranges between 3.84 and 4.90 ml/g of kidney tissue/min (Haining and Turner, 1966). During a mannitol diuresis estimates of cortical blood flow determined from the extraction ratio for p-aminohippurate (PAH) or the uptake of  $^{86}\text{Rb}$ , showed decreases



in cortical flow to 72-74% of the total RPF (Harsing and Bartha, 1966). Thus the mannitol increased the medullary blood flow.

So far, the cortical and medullary blood flow of ruminants has not been estimated, but such investigations may provide insight into the urine concentrating ability of these animals.

There is still uncertainty on what regulates the medullary blood flow independently of the cortical flow and how the total renal blood flow is controlled. Pappenheimer's "cell separation" theory of autoregulation of RPF (Pappenheimer and Kinter, 1956) does not appear very satisfactory (Wirz, 1961; Moffat, 1965) but the "vasoconstrictor" theory of Winton (1956) is more readily acceptable. Various workers have suggested that control of renal vessel dilation or constriction depends on carotid baro-receptors, afferent carotid sinus reflexes and changes in arteriovenous pressure differences. In relation to the latter idea, Scott and coworkers (1965) reported that the concentration of a chemical dilating agent produced in the kidney increased whenever the arteriovenous pressure gradient was reduced. This substance in turn reduced flow resistance by vasodilation. Thureau (1964, 1967) claimed that the substance was vasoconstricting and that its synthesis was inhibited or reduced whenever the perfusion pressure and arteriovenous difference was reduced. He further suggested that this system is associated with the juxta-glomerular apparatus, renin and angiotensin, the controlling factor being the  $[Na]$  of the

intratubular fluid in the distal tubule. The  $[Na]$  at this site is apparently a function of the blood flow since at low blood flow and consequently reduced GFR, the tubular Na load and hence tubular Na reabsorption fall (Lassen, Munck, and Thaysen, 1961; Thureau, 1967). Thureau, therefore, proposed that an uncontrolled increase in RPF causes an increase in the GFR and the post-proximal delivery of Na. This elevated  $[Na]$  activates renin release from the granular cells. Renin then catalyses the formation of angiotensin II from substrate,  $\alpha$ -globulin and angiotensin I. Angiotensin II in turn produces vasoconstriction of the afferent arterioles and a reduction in GFR.

Although such a system may regulate RPF per se it does not necessarily show how medullary blood flow is controlled since the assumption that medullary blood is of post-glomerular origin needs revision (Thureau et al. 1968). Ljungqvist (1965) showed that in some areas of the human cortex there is a shunt between afferent and efferent arterioles and that medullary blood need not necessarily be of post-glomerular origin.

Even though the method of regulation of medullary blood flow is uncertain, it seems that slower blood flow through the medulla (compared with the cortex) is an aid to the counter-current mechanism of concentration. The lower blood flow rate through the vasa rectae of the medulla allows a higher concentration gradient of solutes, particularly Na, to be built up with a resultant hypertonic urine being produced. This high  $[Na]$  or  $[K]$  in

medullary tissue may in itself regulate medullary blood flow since it would produce arteriolar smooth muscle contraction.

Although it has been demonstrated that K secretion does occur in the kidney of the ruminant (McDonald and Macfarlane, 1958) there is no evidence on where or how this secretion takes place or whether K may behave like Na in autoregulation and in the counter-current system.

## 5. Effects of hormones on renal function

In addition to the effects of changes in blood flow discussed in the previous section, hormones may control kidney function by altering tubular reabsorptive and secretory processes.

### (i) Adrenal hormones

Hormones from the adrenal gland may be subdivided into 3 groups on the basis of their normal functions. These groups are the mineralocorticoids, the glucocorticoids (cortical) and the sympathetic catechol amines (medullary).

#### (a) Aldosterone and renin-angiotensin

The 2 primary mineralocorticoids, so named by Selye (1950) for their action on the renal excretion of electrolytes, are aldosterone and deoxycorticosterone (DOC). Aldosterone is the predominant natural hormone and also by far the most effective (Lipsett, Schwartz and Thorn, 1961).

The prime actions of aldosterone on renal functions are the production of an increased Na retention (loss of Na in the absence of the hormone) and in some species an increased excretion of K (Barger, Berlin and Tulenko, 1958; Gaunt, Renzi and Chart, 1955; Lockett and Roberts, 1963). Generally no change in water output has been found during aldosterone administration (Simpson and Tait, 1955; Thorn, Laidlaw and Goldfien, 1955).

The increased retention of Na produced by aldosterone is due to an increased active reabsorption of Na by the distal tubule. How or where it produced an increased K excretion is not yet known (Vander, Malvin, Wilde, Lapidus, Sullivan and McMurray, 1958).

Aldosterone causes the sheep kidney to retain Na and hence it is important in Na deficiency (Blair West, Coghlan, Denton, Goding, Wintour and Wright, 1963). However, aldosterone does not appear to promote K loss in sheep as it does in other species (Kinne, 1963; Macfarlane, 1963).

As well as increasing renal Na retention, aldosterone reduces Na and increases K loss in faeces and sweat of man, dog and rabbit (Duncan, Liddle and Bartter, 1956; Davis, Ball, Bahn and Goodkind, 1959; Dawborn and Ross, 1967) and produces large changes in the salivary composition of sheep (Denton and McDonald, 1957; Goding and Denton, 1956).

It is thought that aldosterone does not stimulate Na reabsorption directly but during a 45 to 60 min latent period following administration or adrenal release, it stimulates production of RNA and protein synthesis (Fanestil and Edelman, 1966; Fimognari, Fanestil and Edelman, 1967). Apparently, this is not the mechanism by which aldosterone produces a kaliuresis (Fimognari, et al., 1967).

By its actions on Na excretion, aldosterone is concerned with regulation of ECV and concentration and hence there have been numerous investigations of the factors controlling its release. One of the most important of these is the renin-angiotensin system previously described (pp 32). When there is a low plasma [Na] or a low ECV, the enzyme renin is released by the receptor cells of the juxta-glomerular apparatus in the kidney (Laragh, Angers, Kelly and Lieberman, 1960; Peart, 1965). Renin release results in the production of the polypeptide, angiotensin II, which stimulates aldosterone release from the zona glomerulosa of the adrenal cortex (Blair-West, Coghlan, Denton, Goding, Munro, Petersen and Wintour, 1962; Ganong and Mulrow, 1962). Small doses of angiotensin II, which is a potent vasoconstrictor, will cause water and Na retention in dogs undergoing a diuresis, but in high doses it is natriuretic. The reverse situation occurs in rat, rabbit and man (Gross, Brunner, and Ziegler, 1965).

The release of aldosterone may also be stimulated by adrenocorticotrophic hormone (ACTH). ACTH produces small

increases in aldosterone secretion in sheep (Blair-West, et al., 1962) in cattle (Kaplan and Bartter, 1962) in dogs (Farrell, Rauschkolb and Royce, 1955) and in man (Crabbe, Reddy, Ross and Thorn, 1959). However, much more ACTH is required to elicit aldosterone secretion than is needed to produce the maximal release of glucocorticoids (Mulrow and Ganong, 1961).

Another factor more pertinent to the subject of this review which directly produces a rise in circulating aldosterone levels is a rise in plasma [K] or a fall in plasma [Na]. In dogs a rise in plasma [K] of 1.3 m-equiv/l. or a fall in plasma [Na] of 14 m-equiv/l. will increase aldosterone secretion (Davis, et al., 1963) though in sheep a much smaller rise in plasma [K] (<0.5 m-equiv/l.) or fall in plasma [Na] (< 5 m-equiv/l.) will release aldosterone (Blair-West, et al., 1963).

Two other factors implicated in the control of the synthesis and release of aldosterone are pulse pressure and arterial pressure. Their action, along with the other factors mentioned, may be mediated through the central nervous system (Denton, 1965).

#### (b) Other corticosteroids

In man, dog and sheep, cortisol is the principal steroid in the adrenal vein blood, though the rat adrenal secretes corticosterone (Hechter and Pincus, 1954).

Although DOC, corticosterone and dehydrocorticosterone always cause salt retention in man (Thorn, Laidlow and Goldfien, 1955) cortisol and cortisone vary, depending on whether the administration is chronic or acute. Chronic administration of cortisol to man stimulates Na retention and increases K loss, leading to hypokalemia (Relman and Schwartz, 1952). The K loss observed is, however, dependent on concomitant Na retention. During acute administration of cortisol in man, a kaliuresis may develop independently of any Na retention. Simultaneously with the kaliuresis is a rise in plasma [K] due to release of intracellular K (Bartter and Fourman, 1957). In the adrenalectomised dog, acute doses of cortisone were found to cause Na retention without K loss (Roberts and Pitts, 1952).

A further effect of both cortisol and cortisone is an increase in GFR which in man, may cause an initial natriuresis prior to Na retention. Furthermore, both these hormones restore the ability of the kidney of adrenalectomised men or of patients with Addison's disease, to excrete a salt load (Burnett, 1950).

In man, dog and rat either cortisol or cortisone is capable of increasing the GFR and water excretion but they both have a mild Na retaining activity. Their prime mineralocorticoid action is a "facilitation of tubular adjustments to salt load" (Lipsett, et al., 1961). There appear to be no reports of the effects of cortisol or cortisone on renal function in the

sheep or cow though Macfarlane (personal communication) found that cortisol had no consistent effect on urinary electrolyte excretion in the sheep.

(c) Adrenal medullary hormones

Adrenalin and noradrenaline, the 2 hormones of the adrenal medulla, probably have their largest effects on kidney function by their vasoconstricting action. Razzach, Hassaballa and Naguib (1964) showed that infusions of noradrenalin elevated the RPF of dogs by 25% while adrenalin decreased RPF by 19%. One would anticipate a decreased RPF and GFR due to vasoconstriction.

The effect of these 2 hormones on electrolyte excretion seems to vary with the species, dose, method of administration, innervation of the kidney and general state of electrolyte and water balance in the animal (Lipsett, et al., 1961). For example, in dogs Na excretion may be reduced by both hormones, in guinea pigs and rats it may be increased while in man the response is variable. Potassium excretion also responds in a variable way though it appears to be independent of Na excretion (Lipsett, et al., 1961). Most frequently, both catechol amines produce a small Na diuresis in dog and man (Green and Sim, 1961) while in sheep both produce a diuresis without any consistent effect on electrolyte excretion (Kinne, Macfarlane, and Budtz-Olsen, 1961; Kinne, 1963). The diuretic effect in dog and man may be



due to the increased blood sugar and increased Tm of glucose produced by both hormones (Wesson, 1961) though it is difficult to conceive that this is the reason in sheep which have a plasma glucose concentration of 50 mg/100 ml.

An extra-renal effect which adrenalin has in relation to K metabolism is its production of a rapid release of hepatic cell K and a transient hyperkalemia. This release of K is related to the stimulation of glycogenolysis with presumably the loss of cell K binding sites during glycogen breakdown resulting in free intracellular K which is then lost to the plasma (Craig, 1958; Craig and Honig, 1963).

#### (ii) Posterior pituitary hormones

The posterior pituitary extract used by Oliver and Schafer in 1895 was found to increase the blood pressure of anaesthetised dogs. A short time later, Magnus and Schafer (1901) demonstrated some effects of this extract on urine flow in the rabbit. Today the term vasopressin, covers an increasing number of polypeptides of relatively similar structure, isolated from the posterior lobe of the pituitary of mammalian and non-mammalian species (Fig. A).

In mammals, 2 vasopressins are found, 8-lysine vasopressin and 8-arginine vasopressin (Fig. A). Lysine vasopressin occurs only among the suborder Suiformes of the

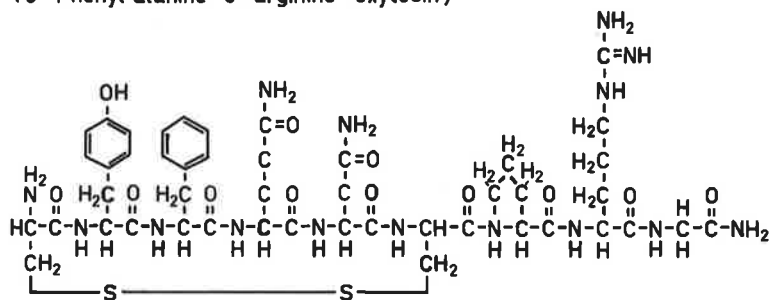
Fig. A. Structure of some naturally occurring neurohypophyseal polypeptides.

## NATURAL HORMONES

8 - Arginine Vasopressin

I

( 3 - Phenyl-alanine - 8 - arginine - oxytocin )



Cysteine	Tyrosine	Phenyl alanine	Glutamine	Asparagine	Cysteine	Proline	Arginine	Glycine amide
1	2	3	4	5	6	7	8	9

(Based on the diagrams  
of Schwartz and  
Livingston (1964))

8 - Lysine Vasopressin

II

( 3 - Phenyl-alanine - 8 - lysine - oxytocin )



Arginine Vasotocin

III

3 - Isoleucine - 8 - arginine - vasopressin

( 8 - Arginine - oxytocin )



Oxytocin

IV

3 - Isoleucine - 8 - leucine - vasopressin

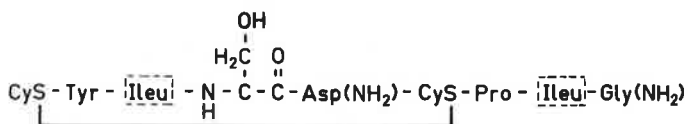


Isotocin

V

3 - Isoleucine - 4 - serine - 8 - isoleucine - vasopressin

( 4 - Serine - 8 - isoleucine - oxytocin )



mammalia and arginine vasopressin is produced in all other suborders. The posterior pituitary lobes of several species of the Suiformes, including those of the European wild boar (Sus scrofa), the warthog (Phaschoerus aethiopicus) the giant forest hog (Mylochoenis angulatus) and the collared peccary (Tayassu angulatus), have been shown to contain lysine vasopressin or both lysine and arginine vasopressin. The posterior pituitary of the white lipped peccary (Tayassu pecari) contains both vasopressins or only arginine vasopressin (Ferguson and Heller, 1965).

A hormone closely allied to vasopressin and apparently bound to the same carrier protein molecule is oxytocin (Fig. A) and Livingston (Schwartz, 1964). Both oxytocin and vasopressin have similar effects on several kidney functions, though the levels of oxytocin required are far in excess of those of vasopressin (Brooks and Pickford, 1958). The amounts of oxytocin required to stimulate milk letdown or uterine contractions are also high.

The prime effect of vasopressin (antidiuretic hormone) is the reduction of high urine flow rates, with varying degrees of effect on electrolyte output, depending on the species. Vasopressins reduce urine flow by allowing the full effect of the high medullary osmotic pressure produced by the counter-current system to be exerted. These hormones increase the permeability to water of the collecting ducts and in some

species of the distal tubule, allowing osmotic equilibration between tubular fluid and the tissue so that a hypertonic urine is produced.

Vasopressin does not alter the electrolyte output of man during a diuresis or at a normal flow rate (Thomson, 1959; Kleeman and Cutler, 1963). Similarly, vasopressin has no effect on the urinary electrolyte output, GFR and RPF of normal or <sup>a,b;</sup> dehydrated dogs (Shannon, 1942, <sub>A</sub> Brooks and Pickford, 1957), but in hydrated dogs small injections of Pitressin increase the Na and Cl and at times the K excretion (Brooks and Pickford, 1957, 1958; Grinnell, Kramer, Duff and Lydon, 1968). Infusions of vasopressin always produce an antidiuresis and increased excretion of Na, K and Cl if initial urine flow rates are high.

Brooks and Pickford (1958), also showed that dogs undergoing a diuresis respond to injections of oxytocin with an increased RPF but with no alteration in electrolyte output, while at low flow rates a marked natriuresis and at times a kaliuresis occurs.

Vasopressin has usually been found to increase the excretion of Na and Cl in rats (Dicker and Heller, 1946; Barnafi, Rosas, de la Lastra and Craxatto, 1960) though Thorn (1959) was unable to find any saluretic effect with doses up to 1 mU. Barnafi, et al., (1960) also found that a delayed increase in K excretion may occur. Infusions of oxytocin in rats produce an

antidiuresis but no changes in electrolyte output (Barnafi et al. 1960). In man, a sustained infusion of oxytocin has been found to lead to excess water retention and water intoxication (Pittman, 1963).

Sheep have several patterns of response to vasopressin. Doses of Pitressin or arginine vasopressin as small as 1 mU (by injection) or 12 mU/hr (by infusion) produce increases in the GFR (transient), K, Na and Cl excretion and are diuretic at urine flows less than 1 to 1.5 ml/min, but they are antidiuretic at flow rates greater than 1.5 to 2.0 ml/min (Kinne, Macfarlane, and Budtz-Olsen, 1961; Cross, Thornton and Tweddell, 1963; Peeters and Debackere, 1963, Gans, 1964). Cross, et al. (1963) claimed that it was the initial urine osmolality which determined whether vasopressin was diuretic or antidiuretic.

The electrolyte intake of the sheep determines whether vasopressin will increase K excretion more than Na. On a high K diet, the increase in electrolyte output produced by vasopressin is mainly due to K while on high Na diet, the reverse situation occurs. (Macfarlane, et al. 1961; Gans, 1964).

Contrary to the findings of Macfarlane and coworkers and others, Stacy and Brook (1964, 1965) oppose the idea that vasopressin may increase electrolyte and water output. In their experiments using water loaded sheep and infusions or injections of vasopressin, they report an antidiuresis, the

production of hypertonic urine and no alteration in total electrolyte output.

The mechanism of action of vasopressin is at present thought to be mediated by the stimulation of the enzyme, adenylyl cyclase, which results in an increased production of 3',5'-cyclic adenosine monophosphate (cyclic AMP) (Orloff and Handler, 1967). Cyclic AMP by some unknown mechanism then alters membrane permeability allowing free water movement. The permeability of isolated toad bladder (Leaf, 1960) and rabbit collecting duct (Grantham and Burg, 1966) is altered in a similar manner by both cyclic AMP and vasopressin. Handler, Butcher, Sutherland and Orloff (1965) have also shown that vasopressin stimulates the production of cyclic AMP by the cells of the toad bladder.

Numerous factors have been implicated in the control of the renal concentrating ability by controlling the release of vasopressin. Two of the most important are the ECV and osmolality. An increased ECF osmolality or a decreased ECV actuates the paraventricular and supraoptic cells of the hypothalamus to release neurophysin granules, from the axon terminals. These granules then pass from the posterior pituitary (Douglas, and Poisner, 1964; Dicker, 1966) to the pituitary veins and thus into the general circulation (Verney, 1947, 1957; Kleeman and Cutler, 1963). Chemical and electrical stimulation of the hypothalamic cells will release vasopressin and it appears that some cortical stimulation of release also occurs (Cross and Green, 1959; Sundsten and Sawyer,

1959; Peeters and Debackere, 1963). Further stimuli for vasopressin release are a decrease in blood pressure and body positional changes. Alterations in blood pressure affect receptors in the right atrium (Share, 1965) while the blood redistribution effected by body positional changes also acts on upper thoracic receptors (Segar and Moore, 1968).

(iii) Other hormones

Two other hormones which have specific actions on renal function are parathormone and calcitonin. These hormones control plasma levels of Ca and phosphate by regulating renal excretion of these ions. The only action these hormones have in relation to K or Na metabolism is that an excess of parathormone, such as occurs in hyperparathyroidism, reduces the concentrating ability of the kidney by reducing Na reabsorption and preventing the establishment of a high medullary interstitial osmotic pressure.

Most of the other hormones of the body have some effect on renal function though whether these effects are of functional significance is unknown. Furthermore, many such effects may be the consequence of a physiological event outside the kidney. For example, glucagon by stimulating gluconeogenesis in rats, may raise the RPF, GFR and glucose excretion rates, urine flow rate and the excretion of Na, K and Ca (Charbon, Hoekstra and Schukink, 1963).



Two further hormones which may have physiological actions on renal function are oestradiol and progesterone. Oestradiol has been shown to complement the action of vasopressin while progesterone increases the excretion of Na, K and Cl in rats, by blocking the Na retaining effect of aldosterone (Thorn and Engle, 1938; Deis, Lloyd and Pickford, 1963; Dance, Lloyd and Pickford, 1959).

#### (iv) Pharmacological Agents

During the past 40 years, various organic compounds have been used to reduce ECV by increasing Na excretion and hence increasing urinary volume. The first "diuretic" used was an organo-mercurial compound commonly known as Salyrgan or Mersalyl acid.

After the discovery and use of sulphonamide by Hörlein (1909) and Domagk (1935) there came a slow realization of its effect on the kidney. This began a new era in diuresis with acetazolamide as the prototype. Disulphonamides followed and in turn the thiazides or heterocyclic disulphonamides and substituted thiazides, which are now in common use. An anthranilic derivate of benzene sulphonamide, frusemide (Lasix) is now one of the most active diuretics. More recently, pteridene (Triamterene) and aryl-oxyacetic acid (ethacrynic acid) derivatives

have been prepared and tested as diuretics. Most of the numerous diuretics may be classified on the basis of chemical composition, structure and action, into 6 groups. These 6 groups are the 1) organo-mercurial compounds 2) thiazides, 3) carbonic anhydrase inhibitors 4) aldosterone inhibitors, 5) osmotic diuretics and 6) other compounds.

The organo-mercurial compounds have the release of free mercuric ions as the basis of their action. These ions bind with sulphhydryl groups (-SH) of the cell proteins of both proximal and distal tubule cells which leads to a reduction in Na reabsorption and increased Na and water loss (Weiner, Levy, and Mudge, 1962; Miller and Farah, 1962).

Like the organo-mercurial compounds, most of the common thiazides such as chlorothiazide, hydrochlorothiazide and cyclothiazide largely reduce distal but also proximal tubular Na reabsorption. Many of thiazides increase K and  $\text{HCO}_3$  excretion as well as Na. Chlorothiazide stimulates distal K secretion in rabbits (Foulkes, 1965) while hydrochlorothiazide increases the length of the collecting duct of rats which secretes K (Sullivan and Pirch, 1966). This effect is partially independent of any carbonic anhydrase inhibitory activity which these compounds possess. Many of the thiazides, like the organo-mercurial diuretics, also increase 17-hydroxycorticosteroid production, these steroids in turn having a supplementary effect on water excretion and renal function.

The anthranilic derivative of benzene sulphonamide, frusemide, appears to inhibit Na reabsorption from the loop of Henle rather than in the proximal or distal tubules (Malnic, Vieira; Enokibara, 1965). This inhibition results in a failure of the counter-current system and prevents the production of a high medullary osmotic gradient necessary for the formation of hypertonic urine. By this means, frusemide is a very effective diuretic causing a large Na and water loss, with a smaller increase in K excretion than is produced by most diuretics (Malnic et al. 1965; Suki, Rector and Seldin, 1965). Frusemide may also increase the RPF and GFR (Vorbürger, 1964; Hook, Ludens, Brody and Williamson, 1966), and alter the distribution of blood flow in the kidney (Birtch, Zakheim, Jones and Barger, 1967).

Ethacrynic acid, an aryl-oxyacetic acid derivative also appears to block Na reabsorption in the loop of Henle and only slightly inhibit proximal and distal solute reabsorption (Gussin and Cafruny, 1966; Earley and Friedler, 1964). It has been suggested that ethacrynic acid like the mercurial diuretics, acts by binding with -SH groups (Komorn and Cafruny, 1965) though there is the alternative suggestion that both frusemide and ethacrynic acid inhibit the Na-K-ATPase found in renal tubules.

By inhibiting carbonic anhydrase activity diuretics such as acetazolamide and dichlorophenamide prevent  $\text{HCO}_3^-$  reabsorption and also increase K and water loss (Maren, 1967).

The osmotic diuretics do not, however, inhibit reabsorption per se but as they are not readily reabsorbed they maintain a high tubule fluid to plasma OP ratio which prevents water reabsorption. Similarly, aldosterone inhibitors such as spironolactone, do not directly retard Na reabsorption but prevent the stimulation of the reabsorption produced by aldosterone and related mineralocorticoids. This in turn results in a greater Na and water loss.

### III. INVESTIGATIONS

#### 1. Materials and methods

##### (i) Animals

Merino ewes and wethers selected from the Waite Institute flock were used in all experiments. These sheep ranged in age from 1 to 5 years, and their weight varied from 25 to 45 kg.

During 1966 and 1967 the sheep were housed in semi-open pens while not being used for experimental purposes but in 1968 and 1969 were maintained indoors. Experiments were performed in small indoor laboratories or in a constant temperature room.

Eleven of the sheep possessed chronic rumen fistulae, 3 had chronic oesophageal fistulae while 4 others had fistulae in other segments of the gut.

##### (ii) Experimental procedures and techniques

During the majority of experiments, animals were kept in pairs in metabolic cages. Pairing was used to reduce the stress of isolation in pens. If necessary these cages allowed the measurement of food and water intake and the collection of urine and faeces for weighing and sampling.

##### (a) Blood sampling and the injection or infusion of fluids

For single blood samples, blood was obtained from the jugular vein using a heparinised syringe and needle. When serial

blood sampling was necessary, commercially obtainable plastic cannulae comprising a plastic tube fitting closely to a hollow trochar (Branula, Braun) were inserted into the jugular vein and retained in position by suture through the skin. In experiments of more than 1-2 hr duration, a nylon obturator was inserted through the cannula to prevent blockage and to prevent the cannula slipping out of the mobile blood vessel.

Blood samples were taken using syringes containing either dried heparin or heparin in a few drops of saline. Samples for whole blood analysis or for TON determinations were stored in bottles in a refrigerator at 0 to 5°C until needed. If plasma was required, the blood was centrifuged within 2 hr of sampling and the plasma separated from the red cells and stored at -5°C until analysed.

Injections of drugs and hormones intravenously were made through the cannula inserted for blood sampling but when infusions were carried out a second cannula was inserted into the jugular on the opposite side of the neck, and solutions were infused through this cannula.

Infusions were given using a Sigma constant volume finger infusion pump with a sensitive variable control of flow rate.

(b) Short-term urine collection

For urine collection over short intervals a self-retaining indwelling catheter (Foley type: Warne) of 3 to 5 ml

bulb capacity was inserted into the bladder. Catheters were inserted at least 2 hr prior to beginning collection as were jugular cannulae. These catheters were left in position for varying periods of time ranging from 10 to 60 hr depending on whether or not the sheep showed any sign of reaction to the devices.

Urine from the catheter was passed by means of a 1 mm (ID) polythene tube either directly to a LKB fraction collector attached to a LKB Radi-Rac electronic timer or to a cell containing a combined pH-calomel-electrode (Radiometer or Tektron), a K electrode and a Na electrode (Tektron) in series. From the electrode cell urine flowed between the electrodes of a conductivity meter (Radiometer) and then to the LKB fraction collector.

The pH, the K and the Na electrodes were connected to a single Radiometer pH meter which in turn was connected to a Philips multi-channel recorder. The conductivity meter was also attached to the recorder.

The use of a single pH meter for all glass electrodes was made possible by using a series of 3 gV relays activated by a clockwork mechanism driven by the recorder (constructed by myself).

Urine samples were collected in tubes containing 3 to 4 drops of 10% thymol in butanol as a preservative. As soon as possible after collection the urine volume was measured and a

subsample taken and stored at + 5°C until analysed.

(c) Rumen supplements and sampling of rumen and saliva

Two methods of adding solutions to the rumen were employed depending on the volume to be added and the speed at which the operation had to be carried out. When volumes in excess of 100 ml were added such as in the addition of 400 m-equiv. of K salts in solution or during  $^{42}\text{K}$  or  $^{24}\text{Na}$  experiments, the solution was usually poured into the rumen from a cylinder which fitted closely to the rubber opening of the rumen cannula. At other times, when rapid mixing was required, the solutions were injected into a number of sites in the rumen fluids using a syringe fitted with a very long needle. The latter method was normally employed when adding phenol red to measure the volume of the rumen. As the sheep with oesophageal fistulae did not have rumen fistulae, solutions were added to the rumen via a tube inserted through the oesophageal fistula. Endotracheal cannulae were found to be very satisfactory for adding solutions to the rumen of oesophageal fistulated sheep since by inflating the balloon regurgitation was prevented. These cannulae were therefore retained in position throughout the course of an experiment and they allowed saliva to be returned or rumen samples to be taken with minimal disturbance.

Rumen sampling from rumenostomised sheep was carried out using a 30 to 40 cm long, 1 to 1½ cm glass or hard polythene



tube attached to a large rubber suction bulb. Samples were taken from at least 3 sites within the rumen to provide a sample volume of at least 25 ml.

Saliva samples were collected by restraining the movement of the sheep with a head halter and allowing free flow of saliva into stainless steel or polythene trays.

Estimates of salivary flow rate were made from samples collected over periods of 1 or 2 hr. These estimates were only used for volume measurement while samples for analysis were spot samples taken at the end of each period. This prevented any contamination by the rumen fluid which may have been forced past the "tracheal cannula" bulb. Samples were frozen until analysed. Saliva from each collection period was returned to the rumen via the oesophageal tube at the end of each 1 or 2 hr period.

(d) K supplementation experiments

Initially a trial experiment involving 3 ewes was run. One sheep was maintained as a control at all times while the other 2 received varying quantities of KCl,  $\text{KHCO}_3$  or KAc solutions, added daily to a basic ration of 900 g of lucerne chaff. Total body water, water TO, ECV and PV were measured once a week.

In the main experiment, 4 sheep were used, 2 of which had chronic rumen fistulae. The 3 to 6 periods of K supplementation extended for 25 to 30 days and involved supplements of 0, 600 and 1200 m-equiv of K salts as an equimolar mixture of KCl,  $\text{KHCO}_3$  and KAc.

The basic diet of all sheep was 900 g of lucerne chaff which was supposedly of uniform composition. For supplementation periods a large quantity of chaff was mixed with the appropriate quantity of K salts in a mechanical mixer and each sheep was fed 900 g of chaff/day plus the respective quantity of K salts.

During the first 17 to 22 days of any period, the sheep were kept in pens in a controlled temperature room (19 to 22°C) and during the last 7 to 8 days placed in metabolic cages in the same room.

A 10 to 15 day interval at the beginning of each period was allowed so that the sheep became accustomed to the diet. At the end of this period, TBW, ECV and PV were estimated. Food and water intakes were then measured for the next 7 to 8 days before sheep were placed in metabolic cages where urine and faecal measurements and sampling continued to the end of the supplementation period. At the end of this period TBW, ECV and PV were redetermined. The next supplementation period did not necessarily commence immediately.

During the period in which the sheep were in metabolic cages, rumen samples were taken thrice daily from the 2 rumen fistulated sheep. Samples were taken prior to feeding, 4 to 6 hr and 8 to 10 hr after eating began.

Following examination of the results obtained using the 2 rumen-fistulated sheep, a further 2 sheep with oesophageal fistulae were placed on K supplements. Spot saliva samples as well as rumen samples were then taken at the 3 periods just outlined.

(e) K restriction experiments

Two sheep were fed a mixture containing 68% dried brewers grain, 17% crushed maize seed, 12% hammermilled washed cardboard, 1.5% animal tallow, with vitamin A and D supplements. Both sheep were also allowed continuous access to a urea-mineral block containing not less than 30% urea, 32 % NaCl, + trace elements and less than 28 m-equiv/kg of K. The total moisture of the food was in the order of 2 to 3% but prior to feeding the mixture was dampened so that the paper stayed mixed with the grain.

Five hundred g of mixture was fed each sheep daily, but as K deficiency developed the appetite of both sheep fell and the daily ration was finally reduced to 250 g/day. Both sheep were weighed daily.

(iii) Experimental measurements

Measurements made during the various experiments were as follows:-

- (a) Total body water (TBW) measured by the dilution of tritiated water (TOH).
- (b) Extracellular volume (ECV) measured by the dilution of sodium thiocynate (NaSCN).
- (c) Plasma volume (PV) measured by the dilution of dye T-1824 (Evans blue).
- (d) Water turnover (TO) estimated by measurement of the specific activity (SA) of TOH from the blood or plasma over a period of from 7 to 16 days.
- (e) Urine flow rate was measured by directly recording urine volume from a catheter over a timed interval.
- (f) Urine pH was determined automatically on urine passing directly from a catheter to a combined pH-calomel electrode connected to a Radiometer pH meter which was in turn attached to a Philips multi-channel recorder.
- (g) Urine [Na] and [K] (comparative only) measured by Na and K sensitive glass electrodes coupled in series in a cell with the combined pH electrode. By using a series of 3 gV relays activated by a clockwork mechanism fitted in the recorder only a single pH meter was required to allow recording of pH, [Na] and [K] in turn.

- (h) Glomerular filtration rate (GFR) estimated from the clearance of inulin from plasma into the urine.
- (i) Renal plasma flow (RPF) estimated from the clearance of sodium-para-aminohippurate (PAH) from plasma into the urine.
- (j) Electrolyte excretion [Na], [K] and [Cl] and osmolar concentrations were measured in urine, plasma, whole blood, saliva, rumen fluid and faeces.
- (k) Packed cell volume (Haematocrit, PCV) determinations by centrifuging whole blood for 60 min at 3000 rpm at a radius of 12 cm in precision bore (0.7 mm) tubes graduated to 100 divisions.
- (l) Plasma protein concentration calculated from the plasma refractive index by the formula of Sunderman (1944) which was derived from measurements on human serum. The absolute values are therefore probably not correct but the method is adequate for comparative purposes.
- (m) Rumen volume calculated from the dilution of a known quantity of phenol red in the rumen.
- (n) Salivary flow measured directly by collecting saliva from oesophageal fistulae in stainless steel or polythene trays.
- (o) TCE,  $^{42}\text{K}$  and  $^{24}\text{Na}$  distributions measured by counting samples of various body fluids taken at regular intervals after the initial addition of one or more of these isotopes

to different body fluids.

- (p) Hormones and pharmacological agents action measured by calculation of water and electrolyte output after I.V. injection or infusion of the compound concerned.

Hormones used were vasopressin (Pitressin - Parke Davis), d, 1-aldosterone (Aldocorten - CIBA) and Cortisol (Effcortelan - Glaxo Laboratories, England).

Pharmacological agents tested were, acetazolamide sodium (Diamox - Lederle), cyclothiazide (Boehringer), frusemide (Lasix - Australian Hoechst Ltd.), ethacrynic acid (Merck, Sharp and Dohme), 6-amino-n-hexoic acid (Epsilon-amino-caproic-acid - BDH) and L-lysine (Sigma).

#### (iv) Laboratory methods

##### (a) Total body water and turnover measurements

The suitability of using tritiated water for the measurement of TBW has been well documented by various workers (Prentice, Siri, Berlin, Hyde, Parson, Joiner and Lawrence, 1952; Richmond, Langham and Trujillo, 1962; Hansard, 1963). Morris, Howard and Macfarlane (1962) showed that at least 6 hr was necessary for equilibration in sheep following intramuscular injection of TOH, during which time the animals were deprived of food and water. Six or more hr were allowed for the mixing of TOH with H<sub>2</sub>O in all experiments.

Tritiated water as TOH was obtained in 1 Curie quantities from the Radiochemical centre, Amersham, England. This was diluted in 0.85% saline to a concentration of 100  $\mu\text{C}/\text{ml}$  and sufficient was injected to give a measurable activity in body fluids for 2 weeks. Doses of 100 to 300  $\mu\text{C}$  per sheep were used.

Samples of blood, urine and (during TOH uptake studies) rumen fluid, were sublimed to dryness to permit the collection of free water. Either 2 ml of sublimed water was added to 14 ml of scintillation fluid or 1 ml to 10 ml of scintillation fluid for counting. The scintillation fluid contained 100 g naphthalene, 250 mg 1-4-bis-2-(4 methyl-5-phenyl-oxazoly1) benzene, 10 g 2-5-diphenyloxazole and dioxane to 1 l. (Werbin, Chaikoff and Imada, 1959). Samples were counted in a Packard Tri-Carb Spectrometer.

(b) Extracellular volume measurement

A sterile solution of NaSCN (15 mg/kg) was injected intravenously and allowed to distribute in the ECF for 20 min. Thiocyanate concentrations were measured in 20 min plasma samples by the method of Bowler (1944).

(c) Plasma volume measurement

The dye, Evans blue (0.3 mg/kg) was injected intravenously and allowed to equilibrate for 5 min before a blood

sample was taken. The extinction in a spectrophotometer at 620 m $\mu$  of the 5 min plasma allowed determination of the dye concentration and estimation of plasma volume.

(d) Inulin clearance

A similar method to that of McDonald and Macfarlane (1958) was employed. Following a priming injection of 15 g of inulin as a 10% sterile solution of pyrogen free inulin, an infusion of 2% inulin at the rate of 1 ml/min was continued for 2 to 6 hr. Plasma and urine samples were analysed by the method of R $\ddot{c}$ , Epstein and Goldstein (1949) following appropriate dilutions. Plasma protein was first precipitated using 0.3 N perchloric acid.

Since inulin clearance appears to measure GFR (Shannon, 1942a) the GFR was calculated according to the equation of Smith (1951).

(e) PAH clearance

Inulin and PAH clearances were always carried out together so the stock solution contained both. The PAH concentration was 6% and the priming injection contained 0.9 g of PAH. A constant infusion of 1.2% at 1 ml/min followed the primer. This rate of infusion produced plasma concentrations low enough for PAH clearance to be considered to be the effective renal plasma flow (Smith, 1951). PAH concentration was estimated in diluted plasma filtrate and urine samples by the method of Smith, Finklestein,



Aliminosa, Crawford and Garber (1945).

(f) Electrolyte measurements

Food and faeces samples were dried at 100°C for 24 hr then weighed for dry matter determinations (DM). A random sample of the dried material was homogenised and a further 1 g random sample of homogenate was taken for water extraction or ashing. Ashing was carried out at 550°C for 24 hr followed by the addition of 2 to 3 drops of concentrated nitric acid. This solution was then diluted to the appropriate electrolyte concentrations (0.1 to 2.0 m-equiv/l.) for measurement.

In the water extraction, the homogenized material was placed in a flask with 50 ml of water and shaken for 3 to 4 hr in a water bath at 80°C. Dilution of this solution was then made as with the ashed samples.

Measurements of the [Na] and [K] of urine, blood rumen or saliva samples and food or faecal extracts were made using an EEL flame photometer or a Unicam SP. 90 absorption spectrometer attached to a Unicam log recorder, following dilution of the samples according to their electrolyte content. Dilutions were initially made using volumetric flasks but during the latter half of experimentation a Hook and Tucker automatic diluter was used. The osmolality of all fluid samples was measured by freezing point depression using a model G Fiske osmometer.

(g) Haemoglobin concentration was calculated from the absorption at 540 mμ of a 1/500 dilution of blood in a solution containing cyanmethaemoglobin. Spectrophotometers were calibrated in terms of a standard cyanmethaemoglobin solution provided by Ethnor-Ortho Diagnostics.

(h) Rumen volume measurement

A saturated solution of phenol red was prepared and filtered. Quantities ranging from 50 to 75 ml were added to the rumen as previously described. Hourly rumen samples were taken and centrifuged to obtain the supernatant. Phenol red concentrations in the rumen liquor were determined according to the method of Hecker, Budtz-Olsen and Ostwald(1964) with the modification of Ternouth (1967). The log of the concentrations of phenol red was plotted against time and a line drawn through these points by visual observation and extrapolated to zero time. The rumen volume was then calculated from the zero time concentration and the quantity of phenol red added.

(i)  $^{42}\text{K}$  and  $^{24}\text{Na}$  distribution measurements

Samples of whole blood, rumen and saliva fluid were counted in 8 ml aliquots in a Packard auto-gamma spectrometer. Plasma was counted in 5 ml of plasma when sampling was frequent. The same samples were later sublimed to provide water for TOH measurements when necessary.

The electrolyte concentrations of all samples were also measured to allow calculation of the specific activities.

(v) Analysis and Presentation of Results

Linear relationships between K intake and a number of dependent variables presented in Section 2 (i) were calculated by the standard method of least squares regression analysis and are based on pooled data from 4 sheep. Where a sheep has received differential treatment e.g. periods of water restriction, the results from it have been plotted in the figures but excluded from the analyses.

The calculated regression lines have been drawn in most of the figures only when the regression coefficient "r" achieved significance at the 5% level or better, that is, when the slope of the regression line was significantly different from zero. The level of significance of "r" is shown in the figures by \* =  $P < 0.05$ ; \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ .

Water turnover is presented not only as l./day but also as  $l./kg^{0.82}/day$ . This expression derives from the slope (0.82) of the interspecific line relating log water turnover (l./day) to log body weight (kg) (Macfarlane, 1965), and has been used to give a common expression for sheep differing in body weight and to provide a figure for comparison with other ruminants.

## 2. Results

### (1) Effects of a high K intake on body fluids and electrolytes

#### (a) Pilot experiment

The ingestion of large quantities of K in the diet is common in grazing sheep. Since the effects of increasing the K intake on body fluids and water TO were uncertain, a pilot experiment was run using 3 sheep. Potassium supplements, in the form of one of the 3 salts, KCl, KAc or  $\text{KHCO}_3$  were added to 900 g of lucerne chaff and TBW, ECV, PV and water TO were measured before and during supplementation. One sheep was used to determine whether there was any difference in effect due to the anion. Results obtained using this sheep have been included in the tables and figures, but as there was no control period the effects of K intake on body fluids and water TO were determined only for the 2 other sheep.

Table 1 and Fig. 1 show the relationship between B.Wt., measured body fluid volumes and water TO in relation to K. intake. There was no significant change in B.Wt. or TBW though both increased slightly throughout the experiment. Similarly, over the range of K intakes used there were no regular changes in ECV or PV alone or as a percentage of TBW.

TABLE 1\*

## EFFECTS OF K INTAKE ON BODY FLUIDS AND WATER TURNOVER

<u>Sheep B</u>		K salts	O	O	KCl	KAc	KHCO <sub>3</sub>	KAc
K Intake	mE/day	667	667	921	935	946	981	
B.Wt.	kg	36.25	37.40	34.50	36.45	32.80	31.00	
TBW	l.	26.4	24.8	24.1	24.9	23.4	23.2	
TBW % B.Wt.	%	72.9	66.3	69.9	68.2	71.5	74.7	
ECV	ml	8640	8400	9010	8720	8450	8990	
ECV % TBW	%	32.7	33.9	37.4	35.1	36.0	38.8	
PV	ml	1961	1721	2024	1926	1387	1548	
PV % TBW	%	7.4	6.9	8.4	7.8	5.9	6.7	
TO	l./day	4.58	3.71	5.20	5.48	6.49	7.48	
TO	l./kg <sup>0.82</sup> /day	0.241	0.190	0.285	0.286	0.371	0.448	
<u>Sheep I</u>		K salts	O	O	KAc	KHCO <sub>3</sub>	KCl	KCl
K Intake	mE/day	600	721	946	981	1067	1067	
B.Wt.	kg	30.65	31.90	31.00	30.30	31.35	31.40	
TBW	l.	23.0	21.8	22.5	23.9	24.5	24.1	
TBW % B.Wt.	%	74.9	68.4	72.5	79.0	78.0	76.9	
ECV	ml	8440	8910	7060	8920	8120	8970	
ECV % TBW	%	36.8	40.9	31.4	37.3	33.2	37.2	
PV	ml	1994	2003	1322	1655	1816	1729	
PV % TBW	%	8.7	9.2	5.9	6.9	7.4	7.2	
TO	l./day	4.47	3.50	5.33	7.04	6.23	5.58	
TO	l./kg <sup>0.82</sup> /day	0.270	0.250	0.319	0.430	0.370	0.331	

\* K intakes do not necessarily follow a natural time sequence

..... continued

TABLE 1 continued

Sheep F

K intake	K salts mE/day	KHCO <sub>3</sub>		KCl		KAc	
		981	1063	1000	1121	1067	1067
B.Wt.	kg	31.80	33.90	32.25	34.20	35.45	34.90
TEW	l.	23.9	27.0	25.7	25.0	25.3	25.5
TEW % B.Wt.	%	75.3	79.6	79.6	73.0	71.4	73.0
ECV	ml	11330	8940 <sup>a</sup>	10080	11500	10800	10190
ECV % TEW	%	47.3	33.1 <sup>a</sup>	39.3	46.0	42.7	40.0
PV	ml	1938	1158 <sup>a</sup>	2286	2293	2590	2051
PV % TEW	%	8.1	4.3 <sup>a</sup>	8.9	9.2	10.2	8.1
TO	l./day	4.36	4.57	4.86	3.39	4.59	3.90
TO	l./kg <sup>0.82</sup> /day	0.256	0.254	0.281	0.187	0.246	0.212

<sup>a</sup> The values of ECV and PV are too low apparently due to some technical problem.

TABLE 2

EFFECTS OF K INTAKE ON WATER AND ELECTROLYTE METABOLISM\*SHEEP B

K Intake	m-equiv/day	765	1177	1500	1950	1730	570	545	1935
								R <sup>a</sup>	R
Na Intake	m-equiv/day	120	129	126	139	142	263	181	155
B.Wt.	kg	36.30	36.25	38.32	38.97	41.02	40.44	39.5	38.90
		36.10	38.00	39.32	41.37	41.22	38.70	38.8	39.45
TBW	l.	23.84	23.27	30.38	28.14	28.80	28.20	25.43	28.09
		24.31	28.11	30.03	30.08	30.29	25.90		26.44
TWB % B.Wt.	%	65.67	64.19	79.28	72.21	70.21	69.73	65.54	72.20
		67.34	73.97	76.37	72.71	73.48	66.92		67.02
ECV	ml	8494	9898	8904	9817	9249	9178	10243	9582
		9698	8648	9249	10150	9321	8774		9431
ECV % B.Wt.	%	23.40	27.30	23.24	25.19	22.55	22.59	26.39	24.63
		26.85	22.77	23.52	24.53	22.61	22.67		23.90
ECV % TBW	%	35.63	42.53	29.31	34.89	32.11	32.55	40.27	34.11
		39.82	30.76	30.80	33.74	30.77	33.87		35.67
PV	ml	1951	1718	1687	1693	1805	1839	1805	1707
		1794	1641	1751	1717	1821	1878		1834

\* Electrolyte intake in water is not included nor is loss in suint. K intake in water ranged from approximately 1.0 to 7.0 m-equiv/day and Na intake varied from 2.0 to 16.0 m-equiv/day depending on water intake. continued.....

<sup>a</sup> R represents a period during which water intake was restricted to the value shown.

TABLE 2 cont.

PV % B.Wt.	%	4.38 4.97	4.74 4.32	4.40 4.45	4.34 4.15	4.40 4.42	4.58 4.85	4.65	4.39 4.65
PV % TBW	%	6.67 7.38	7.38 5.84	5.55 5.83	6.02 5.71	6.27 6.01	6.52 7.25	7.10	6.08 6.94
Plasma [K]	$\frac{\text{m-equiv}}{\text{l.}}$	4.50 4.65	4.80 5.20	5.15 4.85	5.25 5.20	4.75 4.80	5.10 4.80	5.37 5.40	4.70 5.40
Plasma [Na]	$\frac{\text{m-equiv}}{\text{l.}}$	137.5 137.2	131.7 137.9	138.2 136.0	140.5 136.0	136.8 131.7	142.7 134.5	135 135	140.5 140.5
Plasma [Cl]	$\frac{\text{m-equiv}}{\text{l.}}$	104 105	103 106.5	104.5 104.5	103.5 110.5	110 106	103 112	110 108	108 107.5
Plasma OP	$\frac{\text{m-osmole}}{\text{l.}}$	292 293	292 294	294 290	284 290	294 285	309 289	302 299	303 304
Plasma Protein	$\frac{\text{g}}{100 \text{ ml}}$	6.15 5.99	5.94 5.76	6.02 5.92	6.04 5.52	5.87 5.66	5.66 5.97	6.24 6.93	5.65 6.12



TABLE 2 (cont)

SHEEP B.

Whole blood [K]	$\frac{\text{m-equiv}}{\text{l.}}$	7.9 8.1	8.5 8.95	8.0 8.45	8.77 8.55	8.25 10.0	9.35 8.07	8.3 7.5	8.85 9.1
Whole blood [Na]	$\frac{\text{m-equiv}}{\text{l.}}$	121.5 122.5	119 117.5	117.5 123.5	125.5 120.5	124 130	126.3 127.3	126.5 125	130 131.5
Water Intake	$\frac{\text{l.}}{\text{day}}$	3.35	4.24	5.42	6.99	6.69	3.51	2.0	5.0
Water TO	$\frac{\text{l.}}{\text{day}}$	4.15	4.78	6.18	7.53	7.23	4.07	2.41	5.42
TO/kg <sup>0.82</sup>	$\frac{\text{l.}}{\text{day}}$	0.219	0.242	0.303	0.356	0.343	0.201	0.119	0.262
Urine volume	$\frac{\text{l.}}{\text{day}}$	2.01	2.62	3.84	5.05	4.60	1.97	1.27	3.16
Urine [K]	$\frac{\text{m-equiv}}{\text{l.}}$	359	445	410	388	390	265	415	606
Urine K/day	$\frac{\text{m-equiv}}{\text{day}}$	719	1159	1567	1924	1767	537	527	1908
Urine [Na]	$\frac{\text{m-equiv}}{\text{l.}}$	47	37	28	24	28	131	168	47
Urine Na/day	$\frac{\text{m-equiv}}{\text{day}}$	94	97	107	121	128	266	213	149
Urine OP	$\frac{\text{m-osmole}}{\text{l.}}$	1355	1455	1215	1025	1121	1456	1762	1793
Urine <del>solute</del>	$\frac{\text{m-osmole}}{\text{day}}$	2713	3803	4627	5072	5084	2827	2241	5665
Faeces wet wt.	kg	0.55	0.58	0.65	0.66	0.69	0.57	0.49	0.63

TABLE 2 cont.

SHEEP B

% DM in faeces	%	44.4	44.7	41.5	42.0	41.0	50.4	46.8	44.1
Faecal water	l.	0.31	0.32	0.38	0.38	0.40	0.28	0.26	0.35
DM digest. %	%	69.9	67.1	66.2	64.8	64.1	63.3	70.0	64.8
Faecal K content	<u>m-equiv</u> kg	67	58	59	64	59	28	30	62
Faecal water [K]	<u>m-equiv</u> l.	23.6	25.0	23.7	28.9	25.0	28.6	26.9	25.7
Faecal K loss	<u>m-equiv</u> day	7	8	9	11	10	8	7	9
Faecal Na content	<u>m-equiv</u> kg	25	20	30	37	32	34	21	33
Faecal water [Na]	<u>m-equiv</u> l.	51.6	46.9	42.1	47.3	42.5	35.5	19.2	60.0
Faecal Na loss	<u>m-equiv</u> day	16	15	16	18	17	10	5	21

TABLE 2 cont.

SHEEP F

K Intake	m-equiv/day	663	1009	1719	1811	570
Na Intake	m-equiv/day	207	191	196	205	268
B.Wt.	kg	34.30 35.67	34.29 34.58	36.38 36.65	37.17 37.24	35.90 35.10
TBW	l.	24.00 25.00	26.05 23.95	28.05 24.97	25.66 25.58	24.72 23.29
TWB % B.Wt.	%	69.97 70.09	75.97 69.26	77.10 68.13	69.03 68.76	68.86 66.35
ECV	ml	10894 11212	10494 11082	13089 10848	11662 11405	9620 9898
ECV % B.WT.	%	31.76 31.43	30.60 32.05	35.98 29.60	31.37 30.62	26.79 28.20
ECV % TBW	%	45.39 44.85	40.28 46.27	46.66 43.44	45.44 44.58	38.91 42.50
PV	ml	1746 1885	1938 2004	1931 2017	2028 2064	1947 2030
PV % B.WT.	%	5.09 5.28	5.65 5.80	5.31 5.50	5.46 5.54	5.42 5.78
PV % TBW	%	7.28 7.54	7.44 8.37	6.88 8.08	7.90 8.07	7.88 8.72

TABLE 2 contSHEEP F

Plasma [ K ]	$\frac{\text{m-equiv}}{\text{l.}}$	5.45 5.32	5.47 4.90	5.25 5.40	5.35 5.18	4.80 4.37
Plasma [ Na ]	$\frac{\text{m-equiv}}{\text{l.}}$	140.7 142	137 141.5	132 143.5	138.5 140.5	140.5 137.5
Plasma [ Cl ]	$\frac{\text{m-equiv}}{\text{l.}}$	109 112	104 113	108 111	107 110	106 105
Plasma OP	$\frac{\text{m-osmole}}{\text{l.}}$	287 291	284 298	289 290	288 291	298 290
Plasma protein	$\frac{\text{g}}{100 \text{ ml}}$	5.55 6.24	6.24 7.30	6.72 7.50	6.50 7.20	6.20 5.76

TABLE 2 cont

SHEEP F

Whole blood [ K ]	<u>m-equiv</u> <u>l.</u>	6.00 6.75	6.20 5.70	7.00 8.10	6.75 7.40	6.35 6.50
Whole blood [ Na ]	<u>m-equiv</u> <u>l.</u>	127.5 127.5	126.5 128.5	123 127	125.5 126.5	126 128
Water intake	<u>l.</u> <u>day</u>	2.62	2.38	3.91	4.26	2.75
Water TO	<u>l.</u> <u>day</u>	3.02	2.78	4.42	4.65	3.50
TO/kg <sup>0.82</sup>	<u>l.</u> <u>day</u>	0.161	0.152	0.231	0.243	0.190
Urine Volume	<u>l.</u> <u>day</u>	1.64	1.49	2.81	2.85	1.14
Urine [ K ]	<u>m-equiv</u> <u>l.</u>	491	689	651	625	480
Urine K/day	<u>m-equiv</u> <u>day</u>	734	930	1829	1781	559
Urine [ Na ]	<u>m-equiv</u> <u>l.</u>	125	122	76	72	228
Urine Na/day	<u>m-equiv</u> <u>day</u>	206	182	214	205	245

TABLE 2 cont

SHEEP F

Urine OP	<u>m-osmole</u> l.	1561	1973	1688	1555	2030
Urine <del>solute</del> day	<u>m-osmole</u> day	2560	2940	4743	4431	2319
Faeces wet wt.	kg	0.69	0.49	0.74	0.71	0.65
Faeces dry wt.	kg	0.28	0.21	0.29	0.27	0.29
% DM in faeces	%	40.9	43.3	38.5	37.6	44.2
Faecal water	l.	0.41	0.28	0.46	0.44	0.36
DM digest %	%	63.4	71.8	63.1	66.4	62.1
Faecal K content	<u>m-equiv</u> kg	25	46	41	44	48
Faecal water [K]	<u>m-equiv</u> l.	17.1	32.1	21.7	27.3	25.0
Faecal K loss	<u>m-equiv</u> day	7	9	10	12	9
Faecal Na content	<u>m-equiv</u> day	26	28	33	11	33
Faecal water [Na]	<u>m-equiv</u> l.	19.5	21.4	19.6	6.8	38.8
Faecal Na loss	<u>m-equiv</u> day	8	6	9	3	14

TABLE 2 contSHEEP N

K intake	m-equiv/day	545	1270	1918
K intake	m-equiv/day	228	206	216
B.Wt.	kg	25.85 25.83	26.70 26.20	28.33 28.43
TBW	l.	- 19.78	20.85 19.19	22.58 22.98
TBW % B.Wt.	%	- 76.58	78.09 73.24	79.70 80.83
ECV	ml	- 7797	8079 9126	9228 9625
ECV % B.Wt.	%	- 30.18	30.26 34.83	35.27 33.86
ECV % TBW	%	- 39.42	38.75 47.56	40.87 41.88
PV	ml	- 1717	1603 1486	1684 1658
PV % B.WT.	%	- 6.65	6.00 5.67	5.94 5.83
PV % TBW	%	- 8.68	7.69 7.74	7.46 7.21

TABLE 2 cont

SHEEP N

Plasma [K]	<u>m-equiv</u> <u>l.</u>	4.90 4.75	4.05 4.25	4.25 4.15
Plasma [Na]	<u>m-equiv</u> <u>l.</u>	140 151.5	135.5 142.5	141.5 136.5
Plasma [Cl]	<u>m-equiv</u> <u>l.</u>	102.4 104.8	101.6 104.6	102.7 101.9
Plasma OP	<u>m-osmol</u> <u>l.</u>	284 270	269 279	275 261
Plasma protein	<u>g</u> <u>100 ml</u>	5.15 4.84	5.56 5.69	5.94 6.08
Whole blood [K]	<u>m-equiv</u> <u>l.</u>	6.35 6.40	6.15 6.15	6.00 6.05
Whole blood [Na]	<u>m-equiv</u> <u>l.</u>	137.5 143	124.5 127.5	125 122
Water intake	<u>l.</u> <u>day</u>	2.30	3.32	4.50
Water TO	<u>l.</u> <u>day</u>	2.88	4.07	4.58
TO/kg <sup>0.82</sup>	<u>l.</u> <u>day</u>	0.2000	0.279	0.294



TABLE 2 cont.

SHEEP N

Urine volume	<u>l.</u> day	1.34	2.38	3.90
Urine [ K]	<u>m-equiv</u> <u>l.</u>	372	510	478
Urine K/day	<u>m-equiv</u> day	498	1214	1864
Urine [ Na]	<u>m-equiv</u> <u>l.</u>	152	82	70.5
Urine Na/day	<u>m-equiv</u> day	204	195	275
Urine OP	<u>m-osmole</u> <u>l.</u>	1975	1457	1148
Urine <u>solute</u> day	<u>m-osmole</u> day	2640	3475	4741
Faeces wet wt.	kg	0.61	0.61	0.83
Faeces dry wt.	kg	0.25	0.23	0.33
% DM faeces	%	42.0	38.1	36.5
Faecal water	l.	0.36	0.38	0.53
DM digest %	%	66.8	70.8	63.0
Faecal K content	<u>m-equiv</u> kg	46	43	41.5
Faecal water [ K]	<u>m-equiv</u> <u>l.</u>	31.9	26.3	23.6

TABLE 2 cont.

SHEEP N

Faecal K loss	<u>m-equiv</u> day	11.5	10	12.5
Faecal Na content	<u>m-equiv</u> kg	32	37.5	30
Faecal water [ Na ]	<u>m-equiv</u> l.	22.2	23.7	17.0
Faecal Na loss	<u>m-equiv</u> day	8	9	9

TABLE 2 cont.

SHEEP O

K intake	m-equiv/day	545	1270	1918
Na intake	m-equiv/day	228	206	216
B.Wt.	kg	27.00 26.98	27.55 29.35	28.99 29.09
TBW	l.	18.45	19.46 21.98	22.47 22.69
TBW % B.Wt.	%	68.38	70.64 74.89	77.51 78.00
ECV	ml	7290	7965 8105	9018 8676
ECV % B.Wt.	%	27.02	28.91 27.61	31.10 29.82
ECV % TEW	%	39.51	40.93 36.87	40.13 38.24
PV	ml	1593	1511 1658	1611 1524
PV % B.Wt.	%	5.90	5.48 5.65	5.56 5.24
PV % TEW	%	8.63	7.76 7.54	7.17 6.72

TABLE 2 cont

SHEEP 0

Plasma [ K]	<u>m-equiv</u> l.	5.40 4.90	4.00 4.00	4.65 4.30
Plasma [ Na]	<u>m-equiv</u> l.	143 154	133.5 155.5	147 129.5
Plasma [ Cl]	<u>m-equiv</u> l.	109.3 102.6	102.1 110.7	106.5 102.2
Plasma OP	<u>m-osmole</u> l.	285 285	267 294	286 257
Plasma protein	<u>g</u> 100 ml	5.00 5.33	5.33 5.15	6.10 5.53
Whole blood [ K]	<u>m-equiv</u> l.	8.50 8.80	7.75 7.50	8.05 8.45
Whole blood [ Na]	<u>m-equiv</u> l.	130.5 133.5	125.5 127	126 125.5
Water intake	<u>l.</u> day	2.39	3.52	4.41
Water TO	<u>l.</u> day	2.94	4.07	4.99
TO/kg <sup>0.82</sup>	<u>l.</u> day	0.197	0.255	0.314

TABLE 2 cont.

SHEEP 0

Urine volume	$\frac{\text{l.}}{\text{day}}$	1.54	2.20	3.57
Urine [K]	$\frac{\text{m-equiv}}{\text{l.}}$	317	550	530
Urine K/day	$\frac{\text{m-equiv}}{\text{l.}}$	488	1210	1892
Urine [Na]	$\frac{\text{m-equiv}}{\text{l.}}$	140	86.5	59
Urine Na/day	$\frac{\text{m-equiv}}{\text{day}}$	216	190	210
Urine OP	$\frac{\text{m-osmole}}{\text{l.}}$	1695	1600	1310
Urine <del>solute</del> <sup>osmole</sup> day	$\frac{\text{m-osmole}}{\text{day}}$	2587	3531	4676
Faeces wet wt.	kg	0.70	0.67	0.75
Faeces dry wt.	kg	0.27	0.23	0.27
% DM faeces	%	39.3	34.3	36.0
Faecal water	l.	0.43	0.44	0.48
DM digest %	%	64.3	70.8	67.1
Faecal K content	$\frac{\text{m-equiv}}{\text{kg}}$	44	35.5	89
Faecal Water [K]	$\frac{\text{m-equiv}}{\text{l.}}$	28.0	18.1	50

TABLE 2 cont.

SHEEP 0

Faecal K loss	<u>m-equiv</u> day	12	8	24
Faecal Na content	<u>m-equiv</u> kg	40	32	7.5
Faecal water [ Na ]	<u>m-equiv</u> l.	25.5	17.0	23.0

There was a significant rise in water TO and TO/kg<sup>0.82</sup>/day as the K intake/day was increased and a significant regression for TO/day ( $y = 0.358 + 0.0058x$ ,  $r < 0.01$ ) was calculated for sheep B and I. Sheep F which received K supplements throughout had a significantly lower TO than sheep B and I for equivalent K intakes but the different anions had no detectable effect on any parameter.

(b) Main experiment

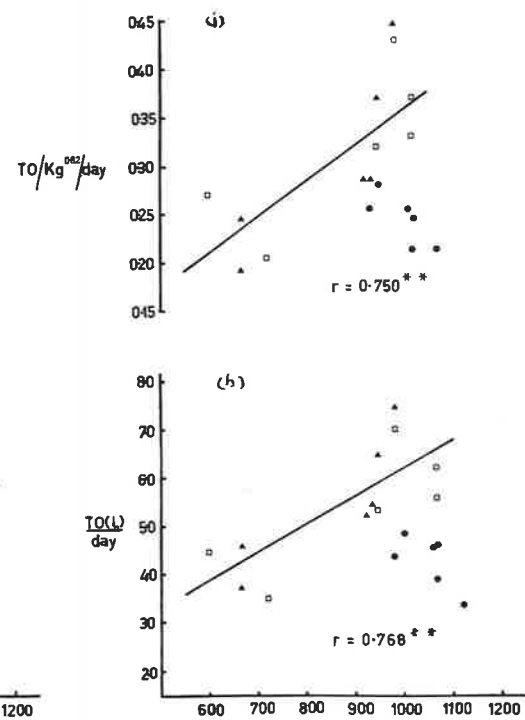
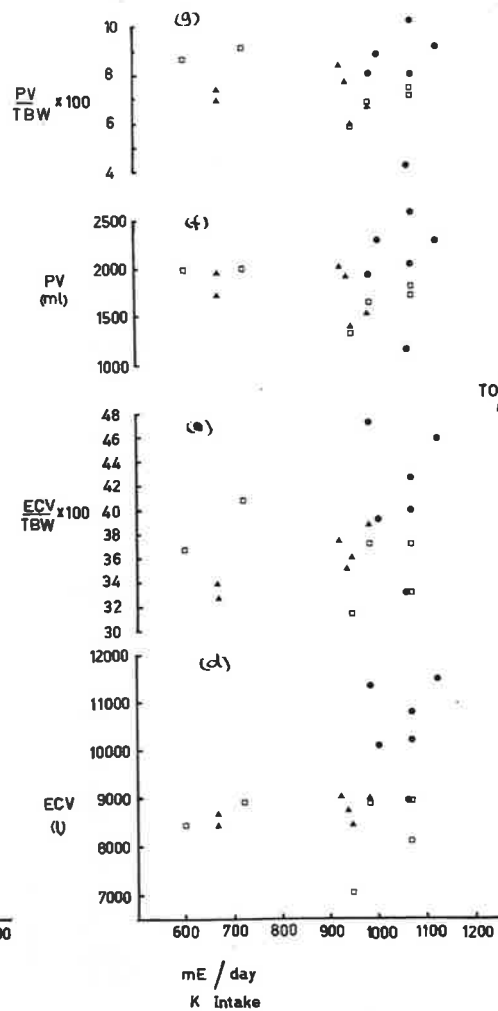
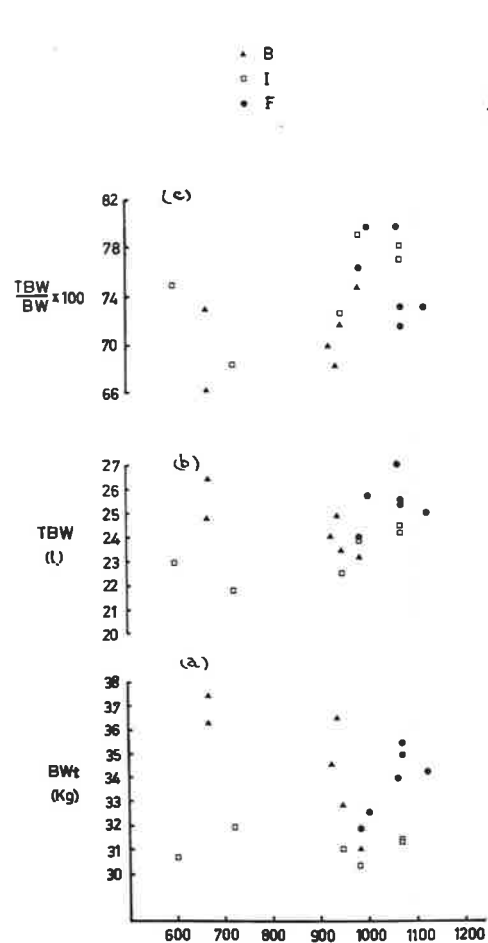
From the results obtained in the pilot experiment, it was decided to repeat the experiment using 4 sheep in an attempt to remove some of the interfering factors and also to examine urine and faecal losses of K and the levels of blood and rumen electrolytes. The following sections describe the results obtained.

(1) Body fluids

The TBW, ECV and PV of all 4 sheep were determined by the methods and at the various times described. These results are summarised in Table 2 and shown graphically in Fig. 2. Significant regressions were calculated for TBW and TBW as a percentage of B.Wt. against K intake. Body weight also tended to increase, but this was shown to be non-significant when

Fig. 1. Effects of daily supplements of KCl,  $\text{KHCO}_3$  and KAc on body fluid volumes and water turnover of Sheep B, I and F. The diet of sheep F was continually supplemented and values obtained for the different parameters were not included in the calculations of the regressions.





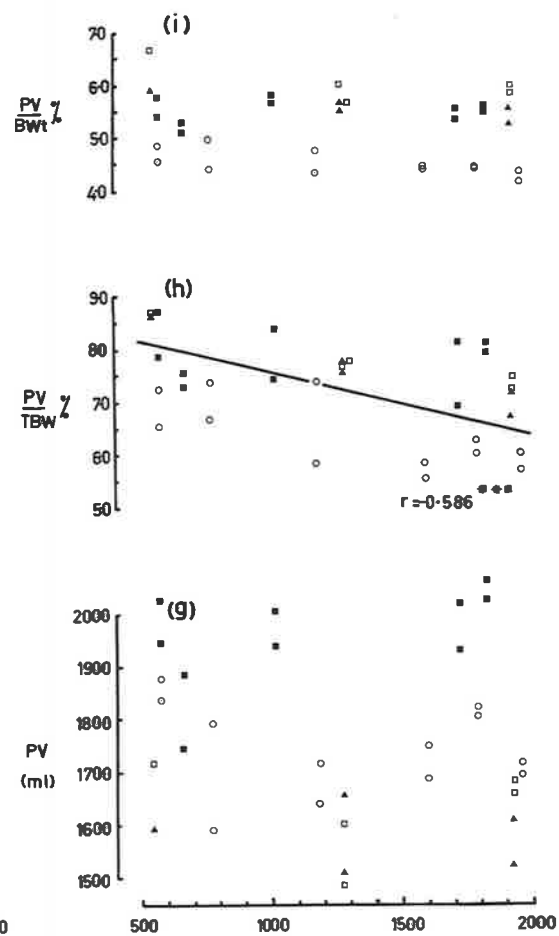
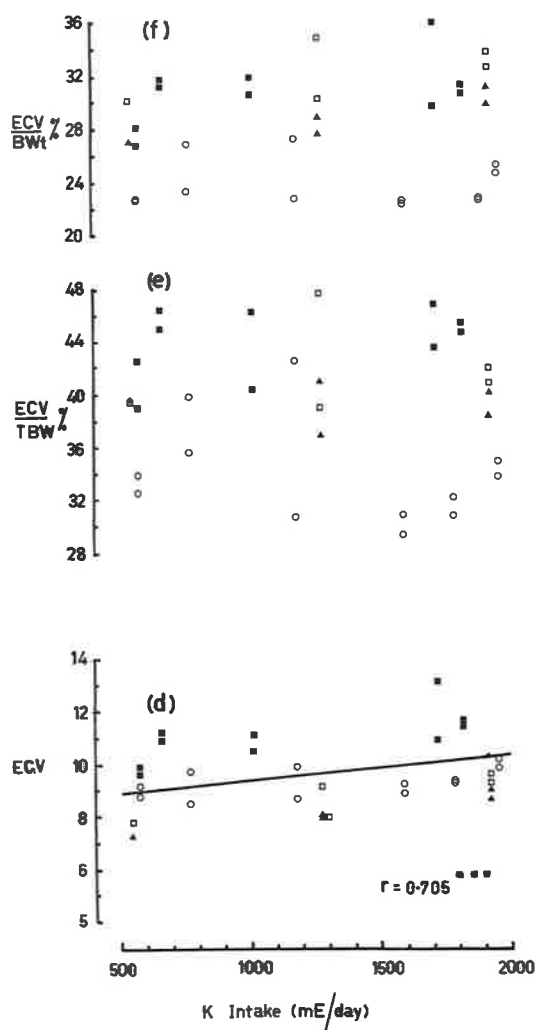
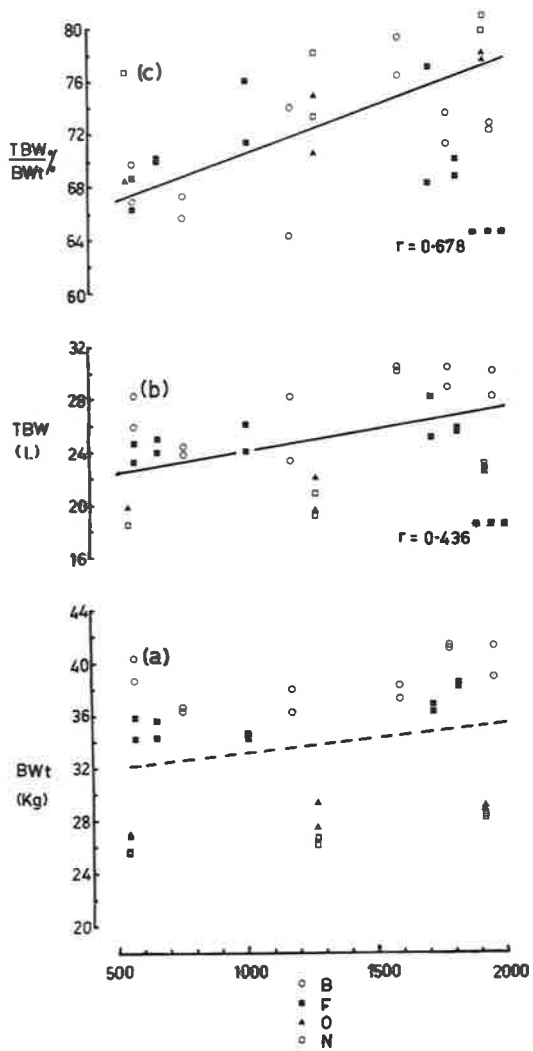
results from all 4 animals were grouped. Since TBW as a percentage of B.Wt. increased as the K intake rose, any increase in B.Wt. which did occur was likely to be due to an increase in water retention rather than in body solids.

There were considerable variations in measurements between sheep and between times of measurement of all parameters at any particular K intake in the same sheep. In several cases (Table 2) the increase in TBW as measured 3 to 10 days after the beginning of a supplementation period was not sustained at the end of the period. Nevertheless the second measurement of TBW was usually still greater than during the previous period of lower K intake.

As anticipated the ECV rose and the TBW increased with increasing K intake. In a like manner to TBW, ECV as a percentage of B.Wt. and TBW varied greatly. There was no significant relationship between ECV as a percentage of either B.Wt. or TBW and K intake though in a couple of instances individual sheep showed a tendency to have slightly higher percentages on the highest K intakes.

Overall, PV did not change as the K intake rose and hence there was a significant reduction in PV as a percentage of TBW. Again the sheep performed as individuals with sheep

Fig. 2. Effects of varying the daily intake of K on B.Wt.,  
TBW, ECV and PV alone and as percentages of B.Wt  
and/or TBW for sheep E, F, N and O in a constant  
environment.



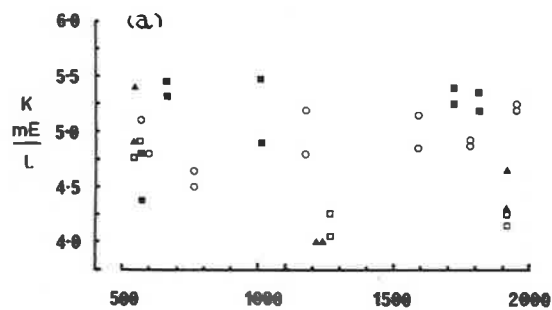
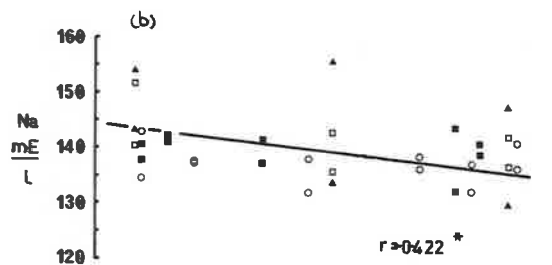
B and O maintaining a relatively constant PV, sheep F increasing and sheep N decreasing then increasing its PV as the level of K supplementation increased.

The changes in body fluid volumes brought about by increasing the K intake were reversed within a month of the cessation of K supplements. In this time, TBW and ECV returned to close to initial presupplement levels or if not to the actual values at least to similar values as fractions of the B.Wt. Thus in summary, the effects of increasing the K intake from 545-765 to 1780-1950 m-equiv/day were a resultant increase in TBW, TBW as a percentage of B.Wt. and ECV with PV unchanged though reduced as a percentage of TBW and B.Wt. Much of the increase in TBW was the result of the expansion of the ECV though it is impossible to exclude some alterations in ICF and gut volume.

## (2) Plasma and whole blood electrolytes

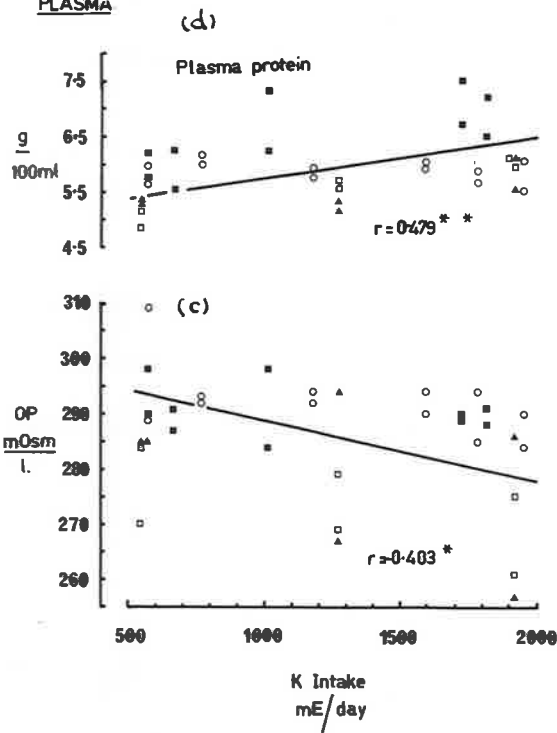
Variations in the plasma [K] were pronounced but irregular. Overall, there was no alteration of the plasma [K] with changes in K intake but there was a significant reduction in plasma [Na] (Fig. 3-a,b) as the K intake rose. The plasma [K] of sheep B did increase with increasing K intake but that of sheep F was variable and sheep N and O decreased then increased their plasma [K].

Fig. 3. Effects of varying the daily intake of K on plasma  
and whole blood concentrations of sheep E, F, N and O.

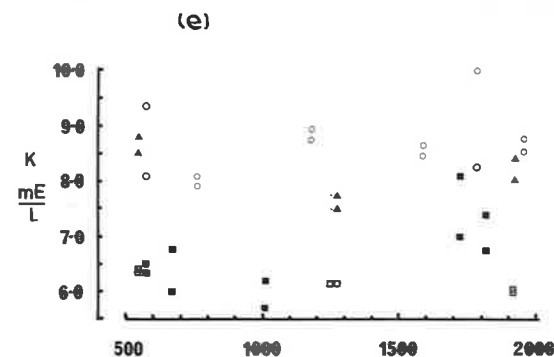
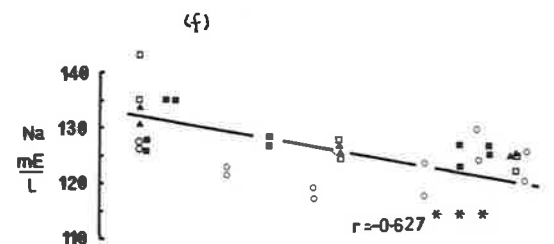


○ B  
■ F  
□ N  
▲ O

PLASMA



WHOLE BLOOD



Like the plasma  $[Na]$ , the plasma OP fell as the quantity of K ingested rose, particularly in sheep N and O (Fig. 3-c). Conversely, plasma protein concentration increased significantly from the lowest to the highest K intake by approximately 1g% (Fig. 3-d).

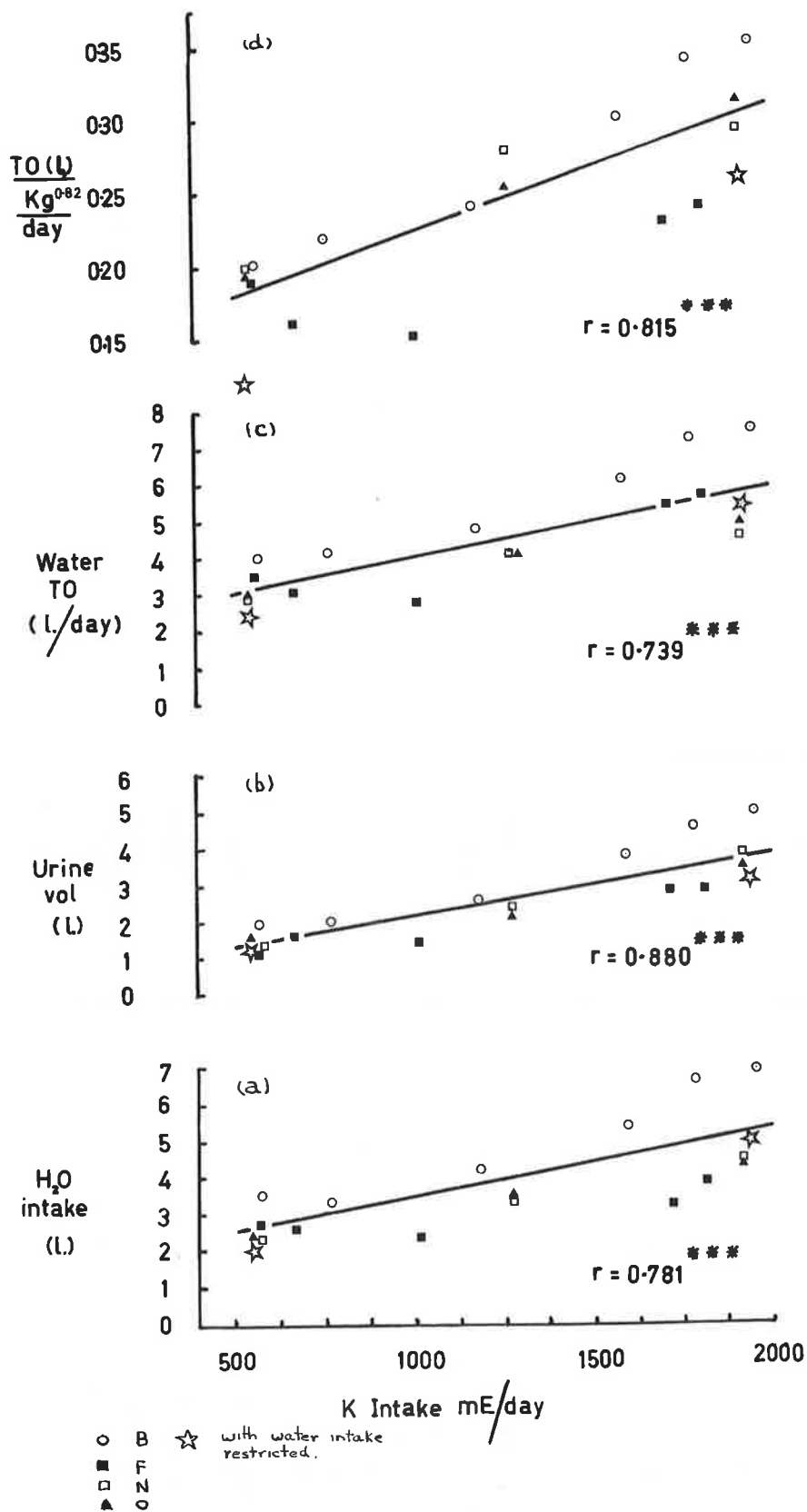
The individuality of all 4 animals was again expressed in terms of the whole blood  $[K]$  and although the alterations in K intake produced no overall change, there were possible rises in whole blood  $[K]$  of sheep B and F at the upper levels of K intake (Fig. 3-e).. As with the plasma  $[Na]$  the whole blood  $[Na]$  fell as the K intake rose (Fig. 3-f).

### (3) Water intake, TO and urine volume

There were significant increases in water intake of all 4 sheep as the K intake rose (Fig. 4-a). Sheep F did not increase water input until the K intake exceeded 1009 m-equiv/day while sheep B had a water intake greater than that of the other 3 sheep. This was subsequently expressed in B by the highest TO and urine volume (B, TO at maximum K intake 81% greater than initial TO, cf. F, 63%; N, 59%; O, 70%.



Fig. 4. Alterations in water, intake, TO and urine volume  
produced by changes in the daily K intake of Sheep  
B, F, N and O.



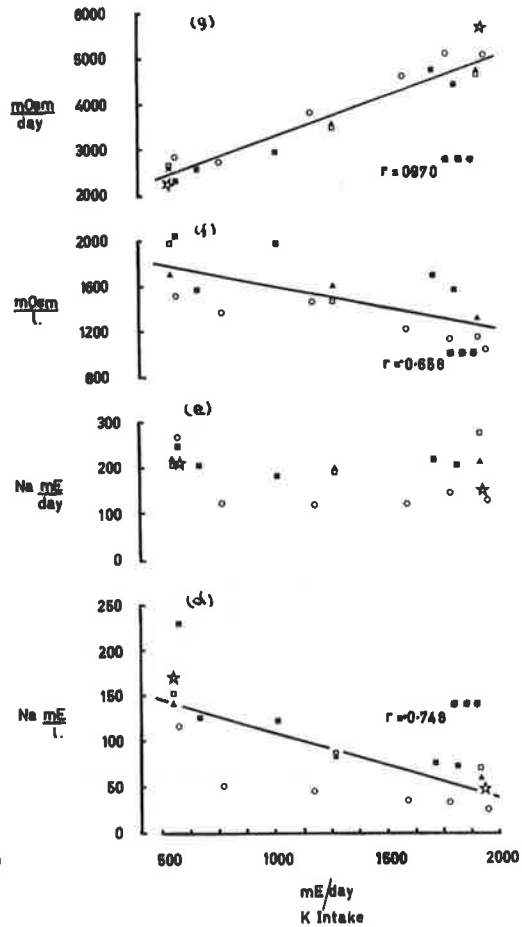
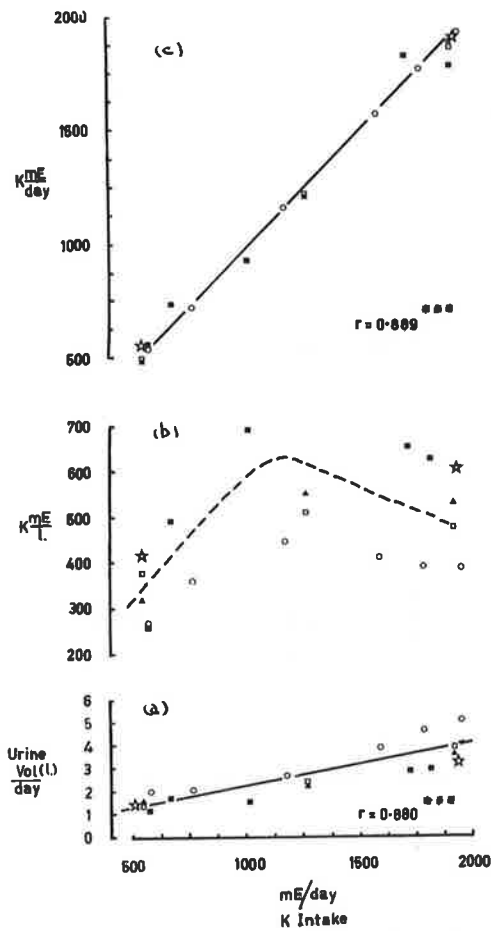
All 4 sheep also showed significant increases in water TO,  $TO/kg^{0.82}$  and urine volume with increasing K supplementation. The similarity of the slopes of the regressions of water ingested and TO versus K intake, indicate that the changes in daily K input had no effect on metabolic water production and that the major part of the extra water ingested on the higher K intakes was excreted in the urine.

#### (4) Urinary and faecal electrolytes

Fig. 5 shows the relationship between urine volume, urine [K] and [Na], urine K and Na outputs/day, osmolar concentration and output/day and the intake of K/day.

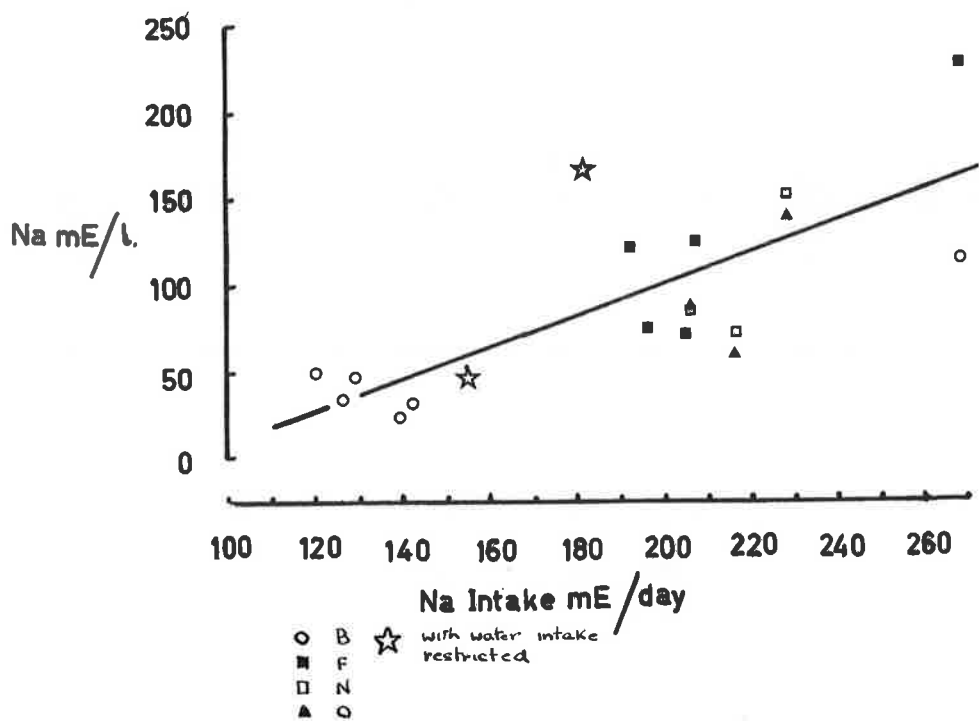
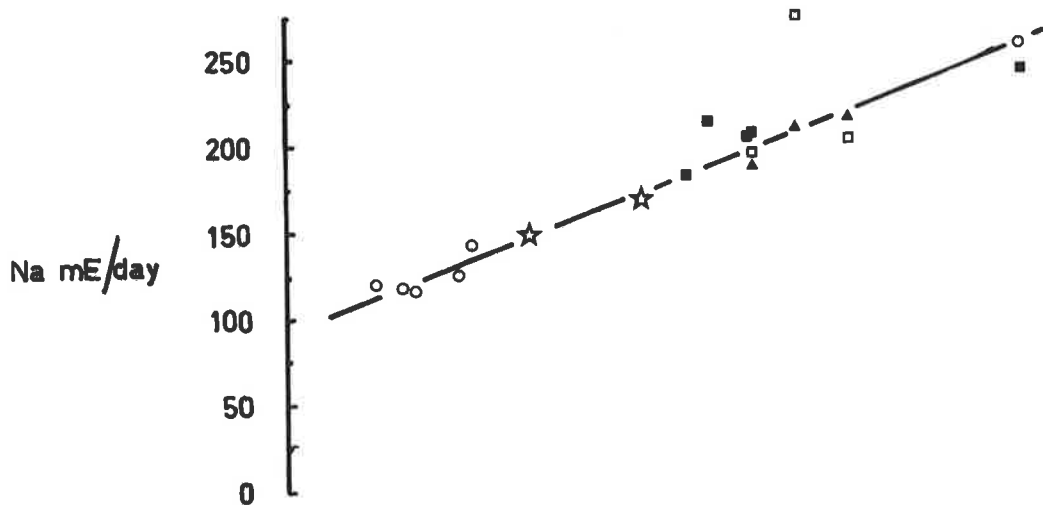
Initially as the K intake increased and despite a rise in urine volume, the [K] of the urine rose reaching a maximum with the ingestion of 1100 to 1300 m-equiv K/day. Above this K intake, the urine [K] decreased though still remained above unsupplemented levels. As expected the sheep with the lower water intakes and urine volumes had a proportionally higher urine [K]. The highest urine [K] measured (690 m-equiv/l.) represented a concentration 130 times that of plasma [K]. The linearity of the relationship of urinary K loss and K intake indicates that there was a directly proportional relationship between K intake and urinary K excretion. Since the slope of this

**Fig. 5.** Effects of alterations in K intake on urine electrolyte concentrations and excretion of sheep B, F, N and O.



O B  
 □ F  
 ▲ N  
 ☆ with water intake restricted.

Fig. 6. Relationship of urine [Na] and excretion per day and daily Na intake during variations of daily K intake.



regression is very close to unity, for each m-equiv of K ingested, a m-equiv was excreted in the urine.

The [Na] of the urine declined with increasing K intake though it is not certain whether the relationship was directly linear. A difficulty associated with relating urinary [Na] or Na loss/day to K intake is the fact that the Na intake/day was not constant throughout. Thus, the graph of urinary Na loss/day against K intake cannot be used to examine whether urinary Na loss decreased while K input and urinary excretion increased. Table 3 shows the balances of K and Na inputs in food and Na and K loss in urine and faeces. This indicates that at the higher K intakes there was a tendency for total Na loss in some instances to exceed the input causing the sheep to be in a negative Na balance. Sheep F on a K intake of 663 and 1719 m-equiv/day was also in an apparent negative K balance. A highly positive K balance occurred when the daily K intake was raised from 663 to 1009 m-equiv and this may explain the unexpectedly low water intake, TO and urine volume at this K intake.

Urine osmolar concentration for sheep B followed the same trend as the [K] but for sheep N and O decreased throughout as the K intake was increased. Sheep F was somewhat erratic (Fig. 5-f). However, the total urinary osmolar loss was proportional to the K input for all 4 sheep (Fig. 5-g).



TABLE 3

## BALANCES OF POTASSIUM AND SODIUM AT VARYING POTASSIUM INTAKES

	K intake* (m-equiv/day)	Loss of K in faeces and urine (m-equiv/ day)	Balance of K	Na Intake* (m-equiv/day)	Loss of Na in faeces and urine (m-equiv/day)	Balance of Na
<u>SHEEP B</u>	570	545	+ 25	268	276	- 8
	765	726	+ 39	120	112	+ 8
	1177	1167	+ 10	129	112	+ 17
	1590	1576	+ 14	126	123	+ 3
	1780	1777	+ 3	142	139	+ 3
	1950	1935	+ 15	139	145	- 6
	545 R	534	+ 11	181	218	- 37
	1935 R	1917	+ 18	155	170	- 15
<u>SHEEP F</u>	570	568	+ 2	268	250	+ 9
	663	741	- 78	207	214	- 7
	1009	939	+ 70	191	190	+ 3
	1719	1839	-120	196	223	- 27
	1811	1793	+ 18	205	208	- 3
<u>SHEEP N</u>	545	509	+ 36	228	212	+ 16
	1270	1224	+ 46	206	204	+ 2
	1918	1876	+ 42	216	264	- 68
<u>SHEEP O</u>	545	500	+ 45	228	227	+ 1
	1270	1218	+ 52	206	197	+ 9
	1918	1916	+ 2	216	221	- 5

\* Electrolyte intake in water is not included.

R = water intake restricted

Since the Na input of each sheep was relatively constant for all periods, though different from each other, Fig. 6 shows that each sheep followed its individual pattern. There is some indication that the urinary [Na] rises with increasing Na input and that the Na output/day followed a linear relationship with the Na input/day.

A plot of the sum of the urinary [K] and [Na] against the urinary osmolar concentration (Fig. 7) shows the distinctive urinary concentrating ability of each sheep. Naturally as the sum of the urinary [K] and [Na] increased so did the osmolar concentration (concentrations from other periods of measurement are included in Fig. 7). The wide spread of points indicates that there are numerous osmotically active substances other than Na and K contributing to the osmolar concentration.

In terms of total (Na + K) output/day and osmolar output/day there is a clear linear relationship which is common to all sheep (Fig. 8).

A significant positive regression was calculated for faeces wet wt. in relation to K intake/day (Fig. 9) but individual sheep did not always follow this trend completely. Whereas sheep B increased both its wet and dry faeces wt. with increasing K intake, F, N, and O initially decreased both weights

Fig. 7. Comparison of total urinary (Na + K) concentration and urine OP during variations in the daily K intake.

Values obtained during several periods of K supplementation of the diets of Sheep B and F for measurements of short term variations in urine and plasma parameters are included.

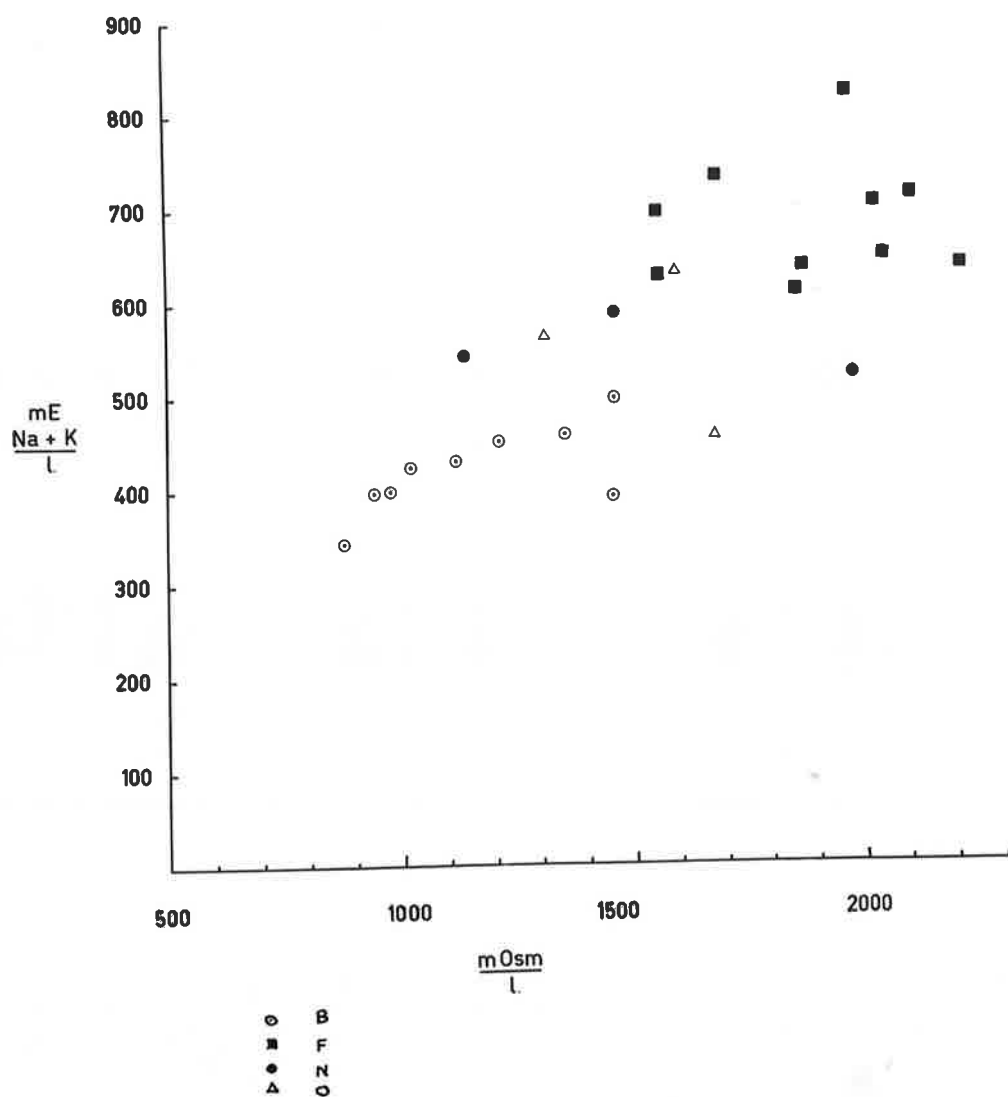


Fig. 8. Comparison of total urinary (Na + K) excretion and urinary solute loss per day during variations in the daily K intake.

Values obtained during several periods of K supplementation of the diets of Sheep B and F for measurements of short term variations in urine and plasma parameters are included.

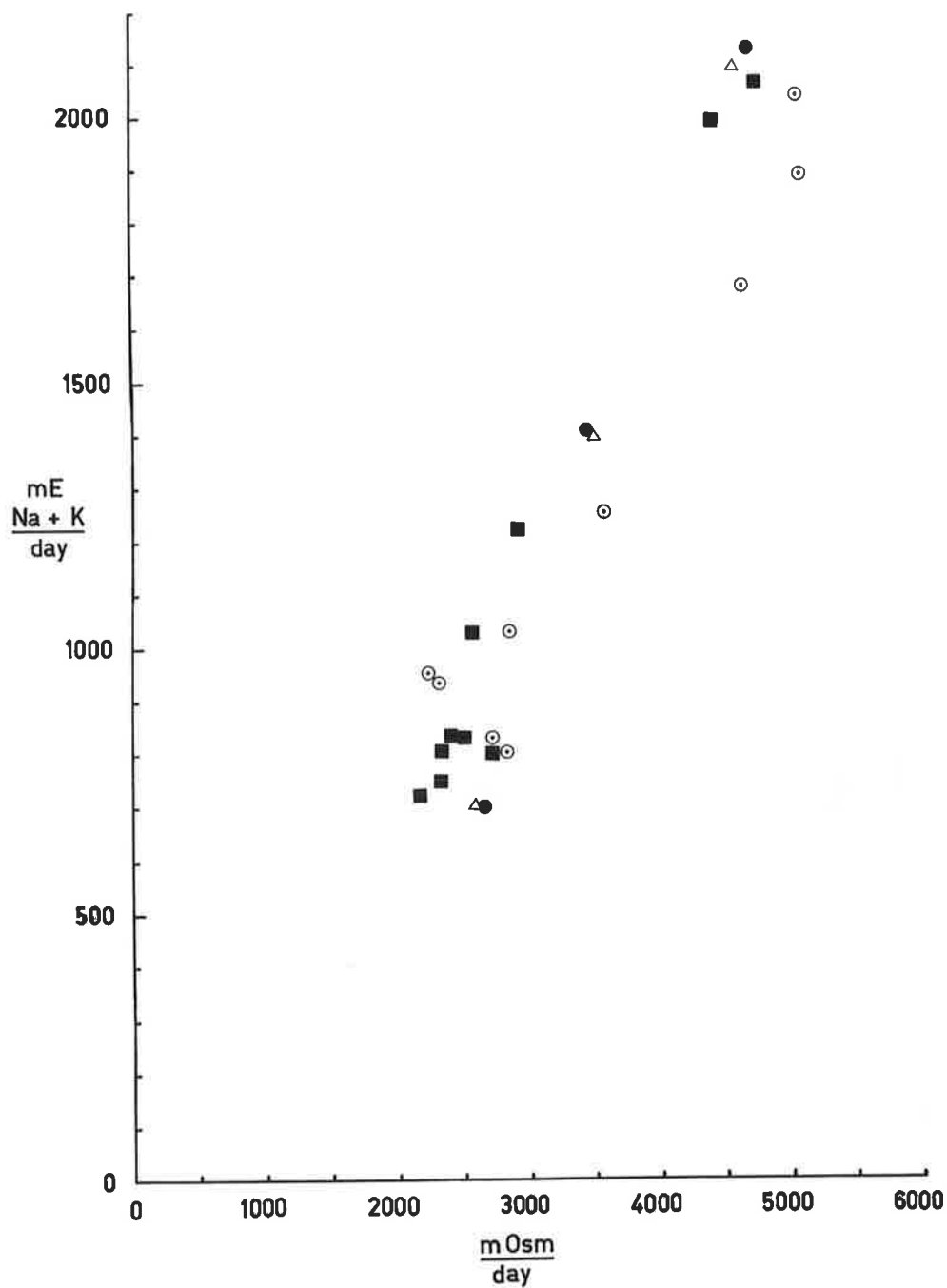
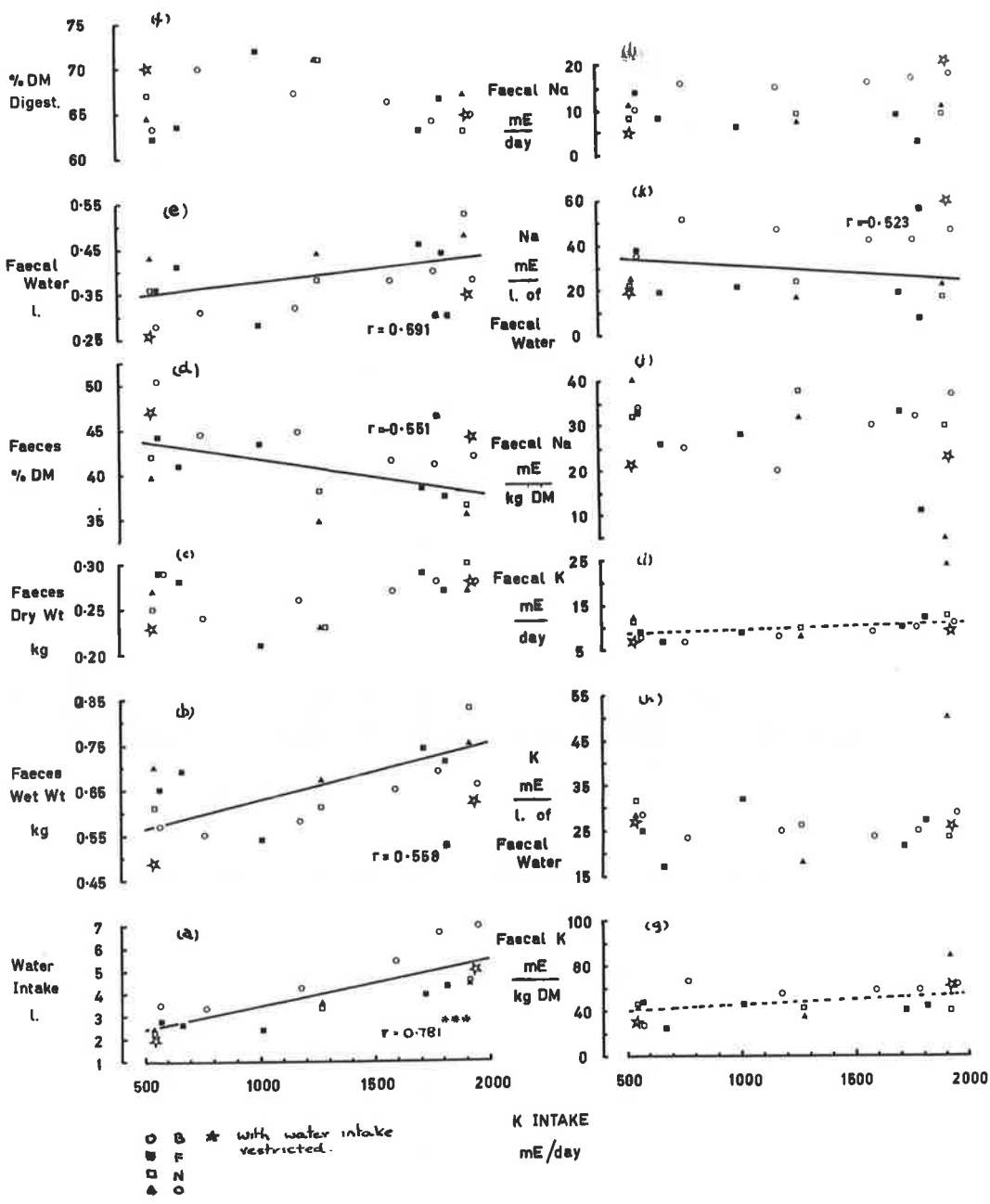


Fig. 9. Effects of alterations in daily K intake on the daily faecal excretions of sheep B, F, N and O.





then increased them at the maximum levels of K supplementation. Even though no significant relationship between faecal dry wt. and K intake could be found the percentage of DM in the faeces declined as the K intake rose (Fig. 9-d). This subsequently resulted in an increase in faecal water loss/day from the initial, of between 7 to 47 % at the maximum K supplement (Fig. 9-e).

As the K intake was increased the DM digestibility of the food decreased for sheep B but in F, N and O there was an initial rise followed by a fall.

Since the DM intake (excluding K supplement) varied from sheep to sheep and period to period, the relationship of faeces wet and dry wt, % DM, and K intake should not be considered directly. However, when these values were expressed as a fraction of the DM intake the relationships shown in Fig. 9 were unaltered. Furthermore, the linearity of the relationships as shown was not improved.

Electrolyte excretion in the faeces was very low in all sheep and did not alter significantly as the K load was increased. There was a slight tendency for the K content of the dry faeces to increase without increasing K intake and hence K output/day also tended to increase but not significantly. The Na content and Na output were unaffected by changes in K input.

When the K and Na content of the faeces were expressed as m-equiv/l. of faecal water (Table 2, Fig. 9-k) the [Na] fell significantly as the daily K intake rose but the [K] was unaltered.

The percentage of K ingested each day that was excreted in the faeces ranged from 0.56 to 0.91% for sheep B; 0.58 to 1.58% for F; 0.65 to 2.11 % for N and 0.63 to 2.20% for O. Similar values for Na were 11.6 to 13.6% for B; 1.46 to 5.22% for F; 3.51 to 4.37 % for N and 3.64 to 5.09% for O. This meant that over 97% of the total urinary plus faecal K loss occurred in the urine.

#### (5) Water restriction

To ascertain whether the urinary concentrating power of a given sheep kidney was more limited than that of others and thus a cause of the difference between the water intake, urine volume and concentrations of individual animals, sheep B was placed on a restricted water intake. Water intake at both the maximum K supplement level and with no K supplement was restricted to 70% of the volume which it was anticipated would be drunk with water ad lib. The results of these restricted periods have been included in Table 2 and in several instances represented in the figures using a star symbol. A more direct comparison is given in Table 4 where the values obtained during

TABLE 4

EFFECTS OF WATER RESTRICTION ON  
WATER AND ELECTROLYTE METABOLISM OF SHEEP B  
AT VARYING POTASSIUM INTAKES

		No K		+ 1200 m-equiv K	
		Normal	Restricted	Normal	Restricted
K Intake	<u>m-equiv</u> day	570	545	1950	1935
Na Intake	<u>m-equiv</u> day	268	181	139	155
B.Wt.	kg	39.7	39.1	40.2	39.2
TBW	l.	24.1	25.4	29.1	27.3
$\frac{TBW}{B.Wt.} \times 100$	%	66.5	65.5	72.4	69.6
ECV	ml	9096	10234	9984	9506
$\frac{ECV}{TBW} \times 100$	%	37.7	40.3	34.3	34.9
PV	ml	1872	1805	1705	1770
$\frac{PV}{TBW} \times 100$	%	7.03	7.10	5.86	6.51
Plasma [K]	<u>m-equiv</u> l.	4.55	5.40	5.20	5.00
Plasma [Na]	<u>m-equiv</u> l.	137.5	135	138	140.5
Plasma [OP]	<u>m-osmole</u> l.	292	301	287	303
Plasma protein	g%	6.07	6.58	5.78	5.89
Water Intake	<u>l.</u> day	3.51	2.00 (57% of unrestricted)	6.99	5.00 (72% of unrestricted)

TABLE 4 cont.

Water TO	<u>l.</u> day	4.07	2.41	7.53	5.42
TO/kg <sup>0.82</sup>	<u>l.</u> day	0.201	0.119	0.356	0.262
Urine volume	<u>l.</u> day	1.97	1.27	5.05	3.16
Urine [K]	<u>m-equiv</u> <u>l.</u>	265	415	388	606
Urine K/day	<u>m-equiv</u> day	537	527	1924	1908
Urine [Na]	<u>m-equiv</u> <u>l.</u>	131	168	24	47
Urine Na/day	<u>m-equiv</u> day	266	213	139	149
Urine OP	<u>m-osmole</u> day	1456	1762	1025	1793
Urine <u>Solutes</u> day	<u>m-osmole</u> day	2827	2241	5072	5665
Faeces Wet wt.	kg	0.57	0.49	0.66	0.63
Faeces Dry wt.	kg	0.29	0.23	0.28	0.28
% DM in faeces	%	50.4	46.8	42.0	44.1
Faecal water	<u>l.</u>	0.28	0.26	0.38	0.35
DM digest %	%	63.3	70.0	64.8	64.8
Faecal K content	<u>m-equiv</u> kg	28	30	64	62
Faecal water [K]	<u>m-equiv</u> <u>l.</u>	28.6	26.9	28.9	25.7
Faecal K loss	<u>m-equiv</u> day	8	7	11	9
Faecal Na content	<u>m-equiv</u> kg	34	21	37	33
Faecal water [Na]	<u>m-equiv</u> <u>l.</u>	35.5	19.2	47.3	60.0
Faecal Na loss	<u>m-equiv</u> day	10	5	18	21

the restricted periods are compared with those at similar K intakes but with water offered ad lib.

At both the levels of K supplementation plasma [K], [Na] and OP rose above normal. Whole blood [K] and [Na] also rose with the high K supplement but were unaltered with no K supplement. Unfortunately, accurate determinations of PCV were not made so it cannot be determined whether these changes were due to alterations in the circulating red cell volume.

With the high K intake plasma protein did not change but was significantly increased during the period without extra K.

Water restriction brought about reduction in urine volume to values almost proportional to the anticipated reductions in water intake. These decreases resulted in pronounced increases in the urinary [K], [Na] and OP at both levels of K intake. Total K and Na outputs were, with one exception, similar to those found at equivalent K intakes but with no water restriction. The exception was the Na output with no K supplement when urinary Na output exceeded the intake by some 32 m-equiv/day. Coupled with a faecal loss of 5 m-equiv/day this resulted in a negative Na balance of 37 m-equiv/day. This loss of 5 m-equiv of Na/day in the faeces represented the smallest fraction of Na intake lost in the faeces (2.75%) of all supplement periods for sheep B -

next lowest value was 11.6%. A 13.5% faecal loss occurred during water restriction at the high K intake.

The dry faeces weights and DM digestibility were unaffected by restriction of water intake and were similar to values for equivalent K supplementation periods with water offered ad lib. but the faeces wet wt., % DM and faecal water were significantly reduced. These reductions were proportional to the reduction in water intake. In summary (Table 4) the restriction of available water produced a decrease in ECV and rises in plasma electrolyte concentrations. There was a reduction in urinary volume which necessitated concentration of K and Na in the urine by the kidney so that the excretion of the excess K and Na ingested in the diet could be maintained. To conserve water which could presumably be used to maintain body fluid volumes and aid in urinary excretion the amount of water lost in the faeces was reduced. Faecal electrolyte losses, with one exception, were unaltered and faecal electrolyte output was not increased to prevent concentration of the urine.

(6) Changes in rumen and salivary electrolyte concentrations

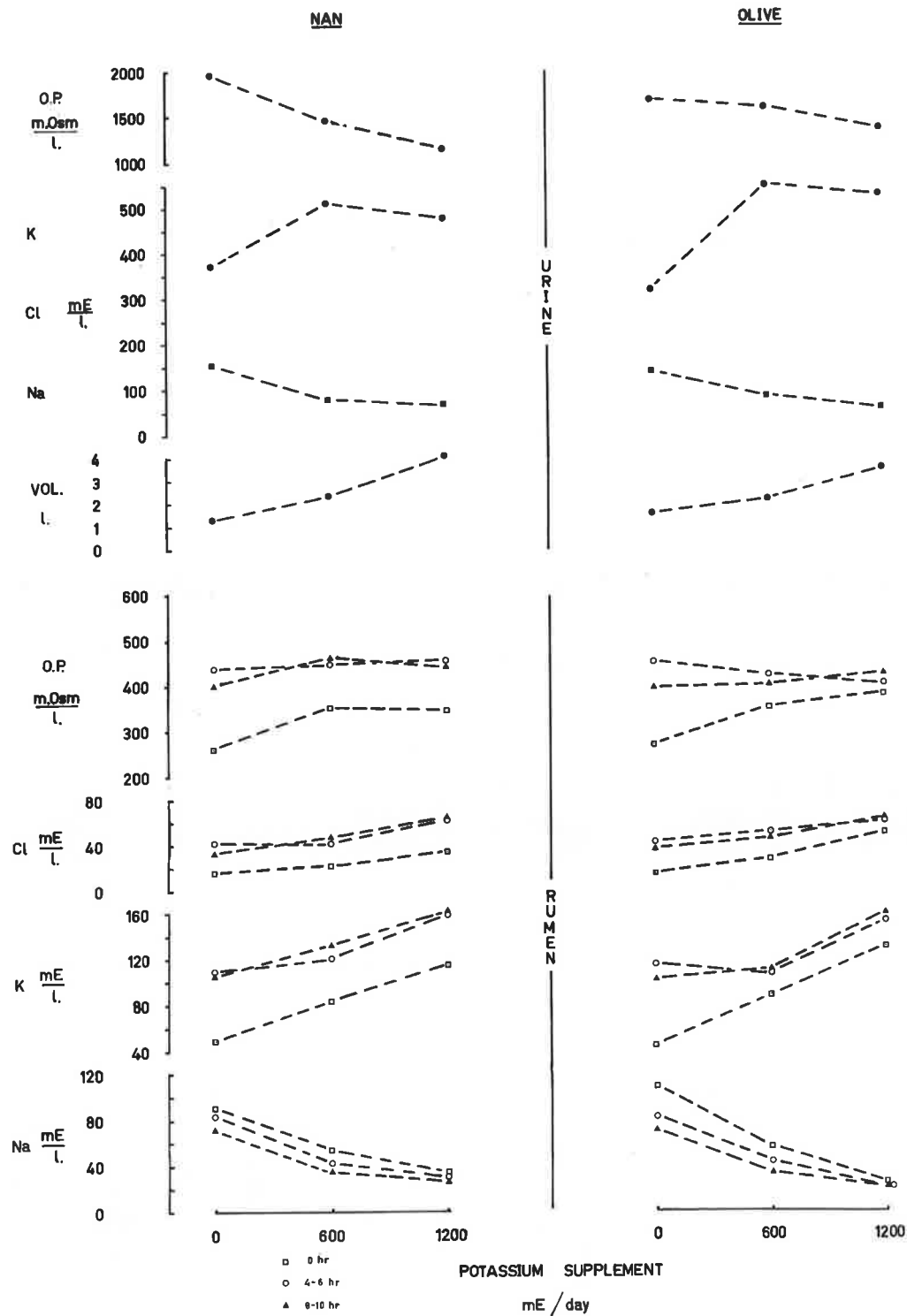
Alterations in the K intake of sheep N and O, produced considerable changes in rumen electrolyte concentrations

(Fig. 10). Similar changes occurred in sheep U and V (Fig. 11) but they were smaller and less regular, probably because of leakage from oesophageal fistulae. Furthermore, sheep U was allowed free access to a NaCl lick throughout and this could well have modified the end results.

At any particular supplement level samples were taken, thrice daily: before feeding (prefeeding), 4 - 6 and 8 - 10 hr after feeding began. As the K intake rose, there was an increase in the prefeeding rumen [K] and a decrease in rumen [Na] of sheep N and O. Rumen [K] rose from 44 - 50 m-equiv/l. with no K supplement to 110 - 116 m-equiv/l. on the maximum K supplement. There was a simultaneous decrease in rumen [Na] from 98 - 110 m-equiv/l. to 24 - 35 m-equiv/l. The rumen [K] at 4 - 6 hr and 8 - 10 hr was always greater than the prefeeding level while the rumen [Na] was always less. This increase in rumen [K] following feeding was reduced as the K intake rose. Thus there was a rise of between 60 - 72 m-equiv/l. with no K supplement but a rise of only 13 - 23 m-equiv/l. with maximum K supplement. Similarly the decline in rumen [Na] which occurred after feeding was progressively reduced as the K intake rose, changes being reduced from 19 - 38 m-equiv/l. with normal lucerne chaff to 4 - 8 m-equiv/l. when 1200 m-equiv of K was added. From these values it can be seen that the daily fluctuations

Fig. 10. Changes in rumen electrolyte concentrations of sheep N and O during the addition of varying quantities of K to the diet. Samples were collected at 0, 4-6 and 8-10 hr after eating began. Mean daily concentrations of urinary electrolytes are also shown.





in rumen [K] were greater than those of [Na] regardless of K intake.

Rumen [Cl], like [K], rose at each of the 3 daily sample periods as the quantity of K in the diet was raised. These increases were less pronounced than those in [K]. The maximum rumen [Cl] attained was between 60 and 65 m-equiv/l. in the post-feeding rumen fluid on the maximum supplement.

Changes in rumen OP were much the same as those in rumen [K], with considerable rises in the prefeeding levels as the K intake rose. This resulted in the prefeeding rumen OP on the maximum K supplement being well in excess of the plasma OP. Rumen OP also exceeded the plasma OP at 4 - 6 and 8 - 10 hr after feeding began at all levels of K intake. Rises in rumen OP following feeding were greatest with no K supplement and were of the order of 180 m-osm /l. As the K intake rose the post-feeding OP rises became smaller and did not follow any consistent pattern. The maximum rumen OP recorded was 453 m-osm/l. for the 8 - 10 hr sample with a daily K supplement of 600 m-equiv.

It is of interest to observe that the rumen electrolyte concentrations of sheep U during a period of Na depletion while being fed normal chaff, were somewhat similar to those found

during feeding of a 600 m-equiv K supplement. The rumen [K] was between 70 to 90 m-equiv/l. and the [Na] between 50 to 60 m-equiv/l. (Fig. 11). This may have been due to the NaCl available to sheep U.

In sheep V increasing the K intake caused an increase in saliva [K] from 7.5 - 8.5 m-equiv/l. with no K supplement to 25 - 30 m-equiv/l. with a daily K supplement of 600 m-equiv. With a K supplement of 1200 m-equiv. saliva [K] at all 3 periods was still greater than that with no supplement but less than that with a 600 m-equiv supplement - 16 to 18 m-equiv/l. Changes in saliva [Na] of U were directly opposite to those of [K], [Na] falling from 188 - 197 m-equiv/l. with no supplement to 133 - 139 m-equiv/l. with a daily K intake of 600 m-equiv and then increasing to 157 - 165 m-equiv/l. on the maximum K supplement. Prefeeding salivary Na:K ratios for the 3 levels of K supplementation in sheep V, 0, 600 and 1200 m-equiv/day, were 24.4, 5.2 and 8.7 respectively (Table 5). In sheep U the prefeeding salivary Na:K ratios were not altered greatly except at the maximum K intake. The latter fall was due to a increase in the saliva [K] rather than a decrease in [Na]. Some slight increases in [K] were found at both the post-feeding sample periods.

Fig. 11. Changes in rumen and saliva concentrations of sheep V and U, during the addition of varying quantities of X to the diet. Both rumen and saliva samples were collected at 0, 4-6 and 8-10 hr after feeding.

Sheep V did not have access to NaCl during the period of this experiment whereas U did. Rumen and saliva electrolyte concentrations for a period during which sheep U was slightly Na deficient are shown.

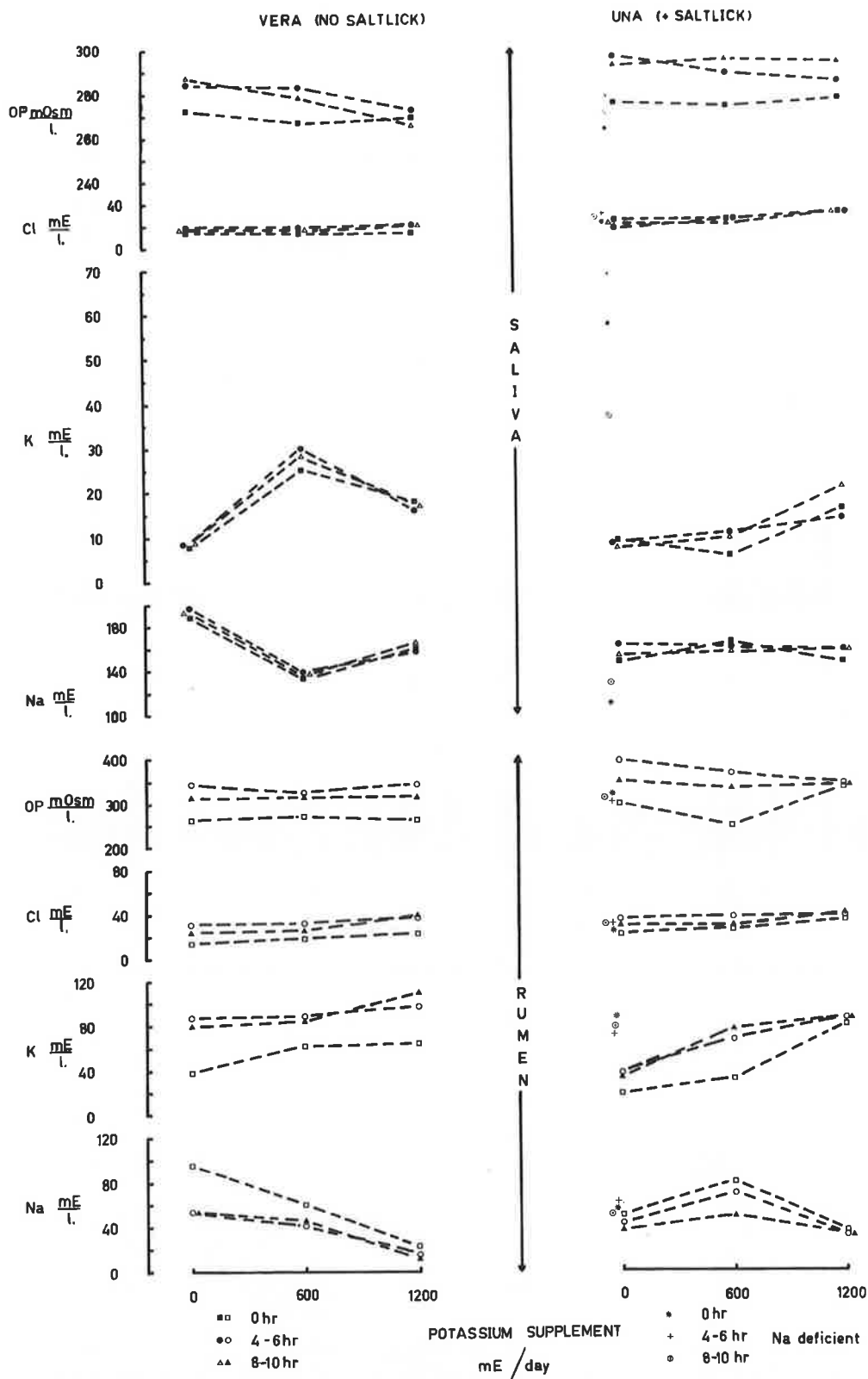


TABLE 5

SODIUM:POTASSIUM CONCENTRATION RATIOS OF

RUMEN FLUID AND SALIVA OF

SHEEP U AND V DURING ALTERATIONS IN POTASSIUM INTAKE

<u>UNA</u> K supplement (m-equiv/day)	<u>Na:K ratio</u>	
	Rumen Fluid	Saliva
0	1*	2.63
	2*	1.16
	3*	1.
600	1	2.58
	2	1.03
	3	0.64
1200	1	0.45
	2	0.37
	3	0.37
O-Na deficient	1	0.63
	2	0.83
	3	0.64
<u>VERA</u>		
0	1	2.50
	2	0.62
	3	0.68

TABLE 5 cont.

VERA (cont)

600	1	0.97	5.32
	2	0.47	4.61
	3	0.53	5.11
1200	1	0.36	8.74
	2	0.15	9.69
	3	0.11	10.12

---

\* 1 = 0 hr.

\* 2 = 4-6 hr.

\* 3 = 8-10 hr.

(ii) Potassium restriction

Because there was no control diet containing adequate K, it was difficult to ascertain whether the following results were due to K deficiency or a low level of DM intake.

Body Wt. and TBW both decreased during the 3 months period that sheep M and P were on the low K diet. However, TBW of M as a percentage of B.Wt. increased significantly to values of 79 to 84% (Table 6). Too few estimates of ECV and PV were made to determine the real effect the low K diet had on these parameters though they did decrease from the start to the end of the experiment.

Plasma [K] decreased during the period of low K intake, falling to as low as 2.96 m-equiv/l. in one instance. Such a low concentration was not maintained and the plasma [K] was normally in the range 3.5 to 4.6 m-equiv/l. While plasma [K] decreased, the [Na] rose to levels of 165 to 189 m-equiv/l. No regular measurements of rumen electrolyte concentrations were made. From a few samples of rumen fluid which were analysed there did appear to be a rise in rumen [Na], values reaching 135.0 m-equiv/l. The rumen [K] in these samples ranged from 15 to 35 m-equiv/l., concentrations which were found in normal sheep after 36 to 48 hr starvation.



TABLE 6

EFFECTS OF K DEPLETION

		Normal	4 wk	11 wk	15 wk
<u>SHEEP M</u>					
B.Wt.	kg	26.8	23.4	20.8	20.2
TBW	l.	21.1	18.5	17.5	17.0
$\frac{TBW}{B.Wt.} \times 100$	%	78.7	79.0	84.1	84.0
ECV	ml	8146			6175
$\frac{ECV}{B.Wt.} \times 100$	%	28.6			29.7
$\frac{ECV}{TBW} \times 100$	%	38.4			36.2
PV	ml	1708			1597
$\frac{PV}{B.Wt.} \times 100$	%	5.99			7.68
$\frac{PV}{TBW} \times 100$	%	8.06			9.37
Plasma [K]	$\frac{m-equiv}{l.}$	4.45- 5.25	3.52- 5.04	4.40- 5.30	3.50- 4.20
Plasma [Na]	$\frac{m-equiv}{l.}$	135.5- 155.5	157.0- 170.0	170.0- 189.0	147.0- 165.5

Mean values (per day) after 15 weeks of low K diet measured over a period of 7 days.

* K intake in food	$\frac{m-equiv}{day}$	3.98
Food intake (wet wt)	g	309 (150-450)
Food intake (dry wt)	g	147 (78-235)
Water drunk	l.	0.57 (0.32-1.02)
Total water intake	l.	0.73

TABLE 6 cont

SHEEP M cont

Urine volume	l.	0.45 (0.32-0.55)	
Urine [K]	<u>m-equiv</u> l.	1.71 (1.30-2.25)	
Urine K/day	<u>m-equiv</u> day	0.75 (0.62-0.99)	
Urine [Na]	<u>m-equiv</u> l.	34.6 (17.1-50.0)	
Urine Na/day	<u>m-equiv</u> day	15.2 (8.9-26.5)	
Urine [Cl]	<u>m-equiv</u> l.	39.4 (36.8-44.4)	
Urine Cl/day	<u>m-equiv</u> day	17.8 (13.9-24.1)	
Urine OP	<u>m-osmole</u> l.	620 (545-701)	
Urine OP/day	<u>m-osmole</u> day	273 (212-310)	
Faecal Wet wt.	kg	0.125	
Faecal Dry wt.	kg	0.065	
% DM in faeces	%	53.0	
Faecal Water	l.	0.06	
DM digest	%	55.1	
Faecal K content	<u>m-equiv</u> kg	127	
Faecal K loss	<u>m-equiv</u> day	8.3	
Faecal Na content	<u>m-equiv</u> kg	100	
Faecal Na loss	<u>m-equiv</u> day	6.5	
Total K intake in food and water (m-equiv)		4.71	
Total K loss in faeces and urine (m-equiv)		9.05	
Balance			-4.34

TABLE 6 cont.

		0 wk	4 wk	11 wk	15 wk
<u>SHEEP P</u>					
B.Wt.	kg	29.0	27.2	24.4	22.9
TBW	l.	20.5	20.2	18.0	16.3
$\frac{TBW}{B.Wt.} \times 100$	%	70.5	74.3	73.9	71.1
ECV	ml	7913			6518
$\frac{ECV}{B.Wt.} \times 100$	%	27.3			28.5
$\frac{ECV}{TBW} \times 100$	%	38.6			30.0
PV	ml	1599			1569
$\frac{PV}{B.Wt.} \times 100$	%	5.51			6.84
$\frac{PV}{TBW} \times 100$	%	7.8			9.61
Plasma [K]	$\frac{m-equiv}{l.}$	4.00- 5.50	3.45- 4.05	4.42- 4.93	3.30- 3.80
Plasma [Na]	$\frac{m-equiv}{l.}$	137.5- 157.5	164.0- 171.0	174.0- 181.0	163.0- 174.0

Mean values (per day) after 15 weeks of low K diet measured over a period of 7 days).

K intake in food	$\frac{m-equiv}{day}$	11.0
Food intake (wet wt)	g	783 (690-980)
Food intake (dry wt)	g	385 (316-477)

TABLE 6 cont

SHEEP P cont

Water drunk	l.	0.881 (0.59-1.33)
Total water intake	l.	1.27
Urine volume	l.	0.74 (0.52-0.90)
Urine [K]	<u>m-equiv</u> l.	1.67 (1.15-2.25)
Urine K/day	<u>m-equiv</u> day	1.25 (0.65-1.75)
Urine [Na]	<u>m-equiv</u> l.	20.5 (13.1-35.5)
Urine Na/day	<u>m-equiv</u> day	15.1 (9.1-18.9)
Urine [Cl]	<u>m-equiv</u> l.	32.6 (19.4-52.4)
Urine Cl/day	<u>m-equiv</u> day	23.6 (16.2-39.8)
Urine OP	<u>m-osmole</u> day	543 (431-649)
Urine OP/day	<u>m-osmole</u> day	401 (270-535)
Faeces wet wt.	kg	0.30
Faeces dry wt.	kg	0.14
% DM in faeces	%	46.6
Faecal water	l.	0.16
DM digest	%	64.1
Faecal K content	<u>m-equiv</u> kg	95
Faecal K loss	<u>m-equiv</u> day	15.3
Faecal Na content	<u>m-equiv</u> kg	114
Faecal Na loss	<u>m-equiv</u> day	16.0

TABLE 6 cont.

SHEEP P cont.

Total K intake in food and water (m-equiv)	Total K loss in faeces and urine (m-equiv)	Balance
12.14	16.97	-4.83

\* Sodium intake not measured as sheep had access to NaCl-urea blocks.

The most interesting aspects of the "low K" experiment were the marked reductions which occurred in the urinary [K] and loss/day despite maintenance of the urinary volume at between 400 and 1000 ml/day. Urinary [K] fell to as low as 1.15 m-equiv/l. and over a weekly period of measurement near the end of the experiment the mean [K] for sheep M and P were 1.61 and 1.71 m-equiv/l. respectively. These low urinary [K] resulted in daily urinary K outputs of down to 0.5 m-equiv/day respectively. Urinary [Na] also decreased appreciably despite the ready access to NaCl at all times.

Faecal electrolyte excretion was surprisingly maintained at levels similar to those of normal or K supplemented sheep. Faecal K excretion averaged 12 m-equiv/day while that of Na averaged 11 m-equiv/day. The total K loss in urine and faeces allowed the sheep to stay close to a K balance since the daily K intake ranged from 3 to 15 m-equiv (Table 6).

(iii) Changes in plasma and rumen electrolyte concentrations and urinary electrolyte and water loss during and after feeding.

(a) Plasma Electrolytes

Plasma samples were taken from sheep B and F at varying intervals, before, during and after eating 900 g of

lucerne chaff to which varying amounts of K were added. In these instances the sheep ate quite normally to finish eating their ration within 6 hr of being fed.

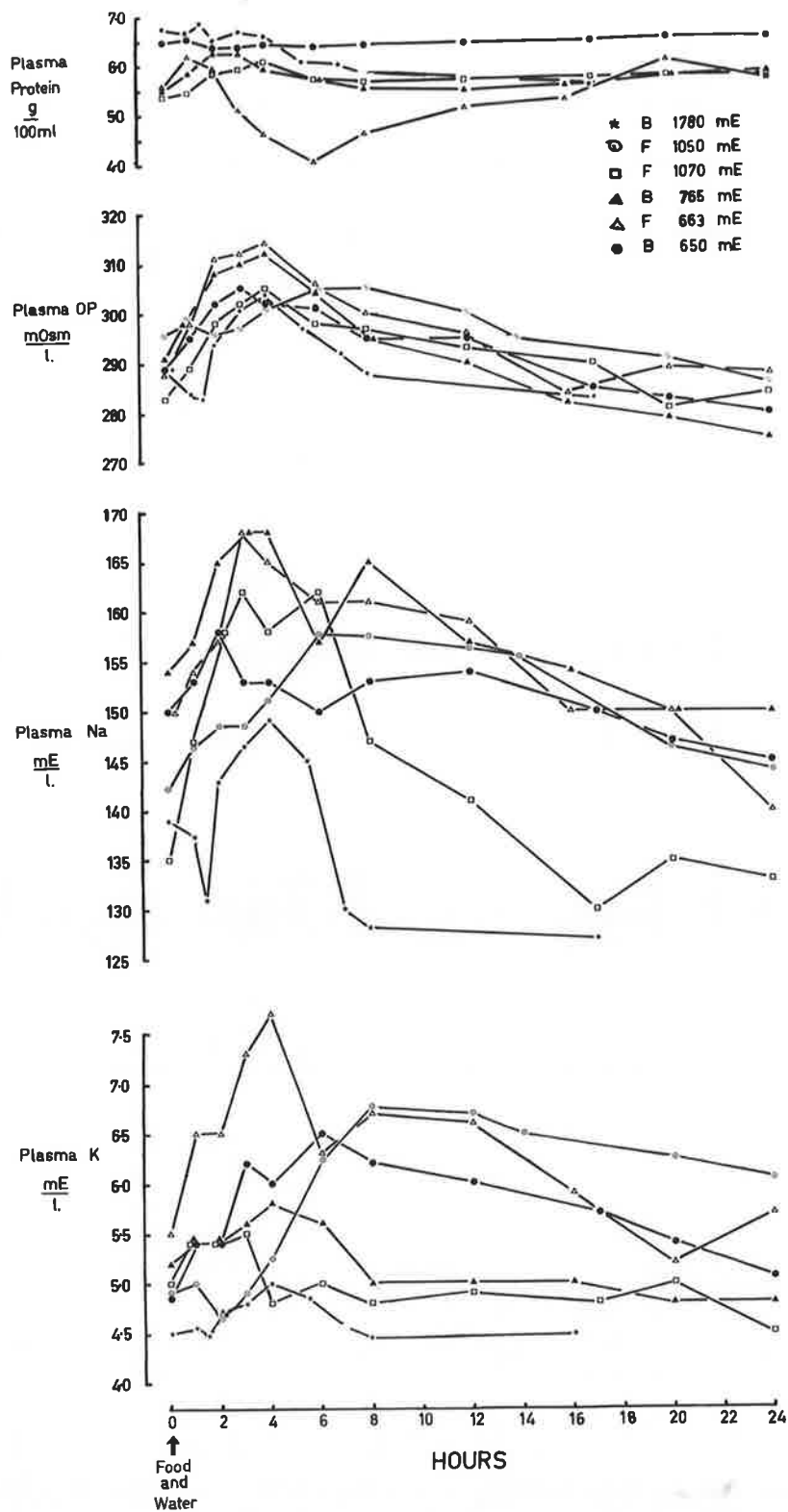
There was a rise in plasma [Na], [K] and OP after feeding with the highest concentrations occurring from 2 to 8 hr after feeding began. In other experiments when sheep were catheterised for urine collection and rumen samples were taken, these peaks did at times occur after 8 hr, particularly if the K intake was high (Fig. 14, 15).

The magnitude of plasma [Na], [K] and OP following feeding was unrelated to the quantity of K ingested. In fact the highest values for these 3 parameters were in sheep F without a K supplement. Then the [K] reached 7.7 m-equiv/l., the [Na] 166 m-equiv/l. and the OP 314 m-osmol/l.

Increases in the plasma [K] ranged from 0.5 to 2.2 m-equiv/l., in plasma [Na] from 8 to 27 m-equiv/l. and in OP from 9 to 26 m-osmol/l. The rise in OP was mainly due to the increase in [Na]. Although the absolute rise in plasma [Na] was far more than that of [K] the percentage increase was much less (Mean [Na] rise 10.8%, max. 20%; mean [K] rise 23.7%, max. 40%; mean OP rise 6.3%, max. 9.0%)

Fig.12. Post-feeding changes in plasma [Na], [K], OP  
and protein concentration of sheep B and F  
ingesting varying quantities of K in the diet.





Plasma electrolyte concentrations returned to very nearly the prefeeding levels within 24 hr.

Changes in plasma protein concentrations after eating always followed a similar pattern but the magnitude of alterations was quite variable (Fig. 12). Plasma protein concentration rose to a maximum 1 to 4 hr after eating began after which it declined to below pre-feeding levels. This decline continued until 6 to 12 hr when the concentration once again commenced to rise reaching values close to the prefeeding ones at 18 to 24 hr. The start of the decline in plasma protein concentration was usually associated with drinking. Animals rarely drank in the first hr of eating and it was not until the second to third hr that a large volume of water was consumed.

(b) Rumen and abomasal electrolytes

An extension of the results already described in Section ii-d was a more detailed analysis of changes in rumen electrolytes following feeding (Fig. 13,14,15). Usually feeding periods were restricted to 3 hr for these experiments to allow a better study of the changes which proceeded after eating.

With a meal of normal or K supplemented lucerne chaff there was a rapid rise in rumen [K] and OP in the first hr after feeding began (Fig. 13,14). The magnitude of this rise depended

largely on the initial rumen  $[K]$  and the quantity of K ingested, though the largest changes usually occurred with no K supplement. Rumen  $[Na]$  usually rose slightly in the first hr of feeding before it began to fall, which it continued to do until 10 to 11 hr after eating began.

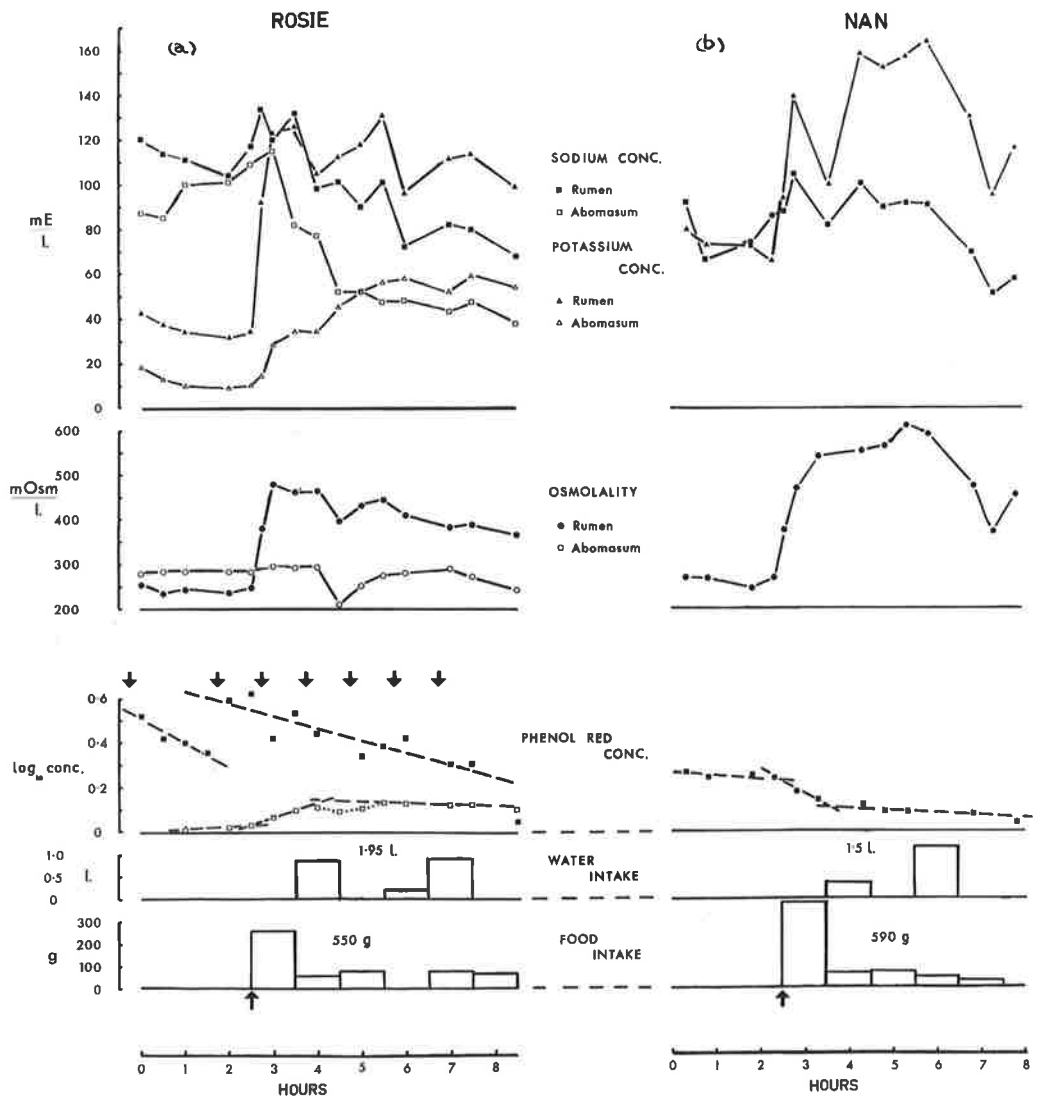
As pointed out in Section 1-d the maximum K supplementation produced a prefeeding rumen  $[Na]$  of as low as 28 m-equiv/l. Hence the post feeding decline in rumen  $[Na]$  when sheep ate K supplemented chaff was not as great as when eating normal chaff. On normal chaff rumen  $[Na]$  rarely declined below 50 m-equiv/l. during and after feeding but the actual fall represented a decrease of between 40 and 70 m-equiv/l.

After the initial rise in rumen  $[K]$  and OP these values tended to fluctuate before beginning to fall quite sharply. The maximum rumen  $[K]$  recorded was 175 m-equiv/l. (Sheep N - Fig. 13),  $2\frac{1}{2}$  hr after eating began. This concentration also corresponded to the highest OP measured of 610 m-osmol/l. Normally the maximum rumen  $[K]$ , regardless of K intake was between 100 and 120 m-equiv/l. and the equivalent OP was between 375 and 475 m-osmol/l. These values illustrate the large osmotic gradient sustained between the blood and rumen fluid 1 to 3 hr after feeding commenced. Rumen OP was still greater than that of the plasma up to 15 hr after food intake

Fig. 13. Alterations in plasma, rumen and abomasal electrolyte concentrations during and after eating of normal lucerne chaff and drinking.

Sheep R - the first arrow on the phenol red graph, represents the addition of 30 ml of phenol red to the rumen. Subsequent arrows represent the addition of 6 ml of phenol red. During the period of feeding 360 m-equiv of K and 90 m-equiv of Na were ingested.

Sheep N - 20 ml of phenol red added to the rumen at time 0. During the period of feeding 354 m-equiv of K and 103 m-equiv of Na were ingested.



in non-supplemented sheep and with eating restricted to 3 hr, this difference was often present at 7 to 8 hr. With daily K supplements of 600 and 1200 m-equiv the rumen fluid was in most instances always hypertonic to the plasma.

Part of the rise of rumen OP must have arisen from electrolytes, other than K and Na, and from such substances as urea and volatile fatty acids (VFA). In one experiment, the VFA concentrations in rumen fluid of 2 sheep were measured using gas chromatography (Packard Gas Chromatograph). This was done in an attempt to explain the sudden change in rumen fluid pH shown by the colour change of phenol red used for rumen volume measurements. It was found that the concentration of acetic, propionic and butyric acids increased by as much as  $2\frac{1}{2}$  to 3 times the resting levels in the first hr after feeding began. The major change was in acetic acid concentrations. Such an increased production of VFA could contribute up to 40 m-osmol/l. to the rise in rumen fluid OP.

Abomasal concentrations of electrolytes and OP showed a remarkable constancy during feeding until drinking occurred. Prior to drinking the abomasal OP was almost constant and close to plasma levels - 270 to 280 m-osmol/l. (Fig. 13-a). Both the abomasal [K] and [Na] were less than their rumen equivalents

though like the rumen [K] and [Na], both changed in the same direction as eating began. The abomasal [K] rose steeply to as high as 70 m-equiv/l. while [Na] fell to as low as 45 m-equiv/l. In contrast with the rumen, however, the abomasal fluid OP did not rise greatly during feeding and in 4 experiments it did not exceed 300 m-osmol/l.

Some of the changes induced in abomasal fluid by drinking are illustrated in Fig. 13-a. Part of the water from an initial drink apparently by-passed the rumen and passed directly through into the abomasum. This caused a sharp transitory fall in the abomasal OP (to 210 m-osmol/l.) and phenol red concentration. Subsequent intakes of water did not cause such a large fall though there were reductions in rumen fluid [K] and [Na] with smaller falls in OP.

Abomasal phenol red concentrations showed that the rate of movement of rumen fluid to the abomasum increased during eating. Furthermore, the rate of gastric secretion was greater than the rate of fluid entry from the rumen since the phenol red concentration in the abomasum was always less than half the rumen concentration (observed in 3 experiments though phenol red was not added continually as in the experiment of Fig. 13-a).

(c) Urinary excretion

The urinary [K] and output rose during the first 1 to 2 hr after eating began (Fig. 14,15,16). This change occurred regardless of the K intake. Excretory rates of K reached a peak in from 2 to 11 hr after the start of feeding. This also was independent of the levels of K intake and varied with the feeding pattern.

There was also an increase in urine [Na] and output as well as of K after eating but the rise was normally secondary to the rise of K. Nevertheless the peak of Na output was attained at various times relative to the maximal K output (Fig. 14,15,16). As expected the peak in urinary [Na] and excretion was always exceeded by that of K. The maximum K output attained in any experiment tended to depend on the individual animals and on the K intake. Sheep whose daily water intake,  $T_O$  and urine volume were low usually produced a concentrated urine, though they did not necessarily have as high an electrolyte excretory rate as sheep with higher daily water intakes and urine volumes. As the K intake increased so the maximum rate of urine K loss increased. Several sheep reached K excretory rates as high as 1200  $\mu$ -equiv/min when on the maximum K supplement. Maximum urinary concentrations of K were in the



Fig. 14. Post-feeding changes in plasma and rumen electrolyte concentrations and in urinary excretion of Sheep J after being feed 900 g lucerne chaff to which 600 m-equiv of K had been added. The period of eating was restricted to 4 hr, during which time some 560 g of lucerne chaff containing 868 m-equiv of K and 95 m-equiv of Na was consumed.

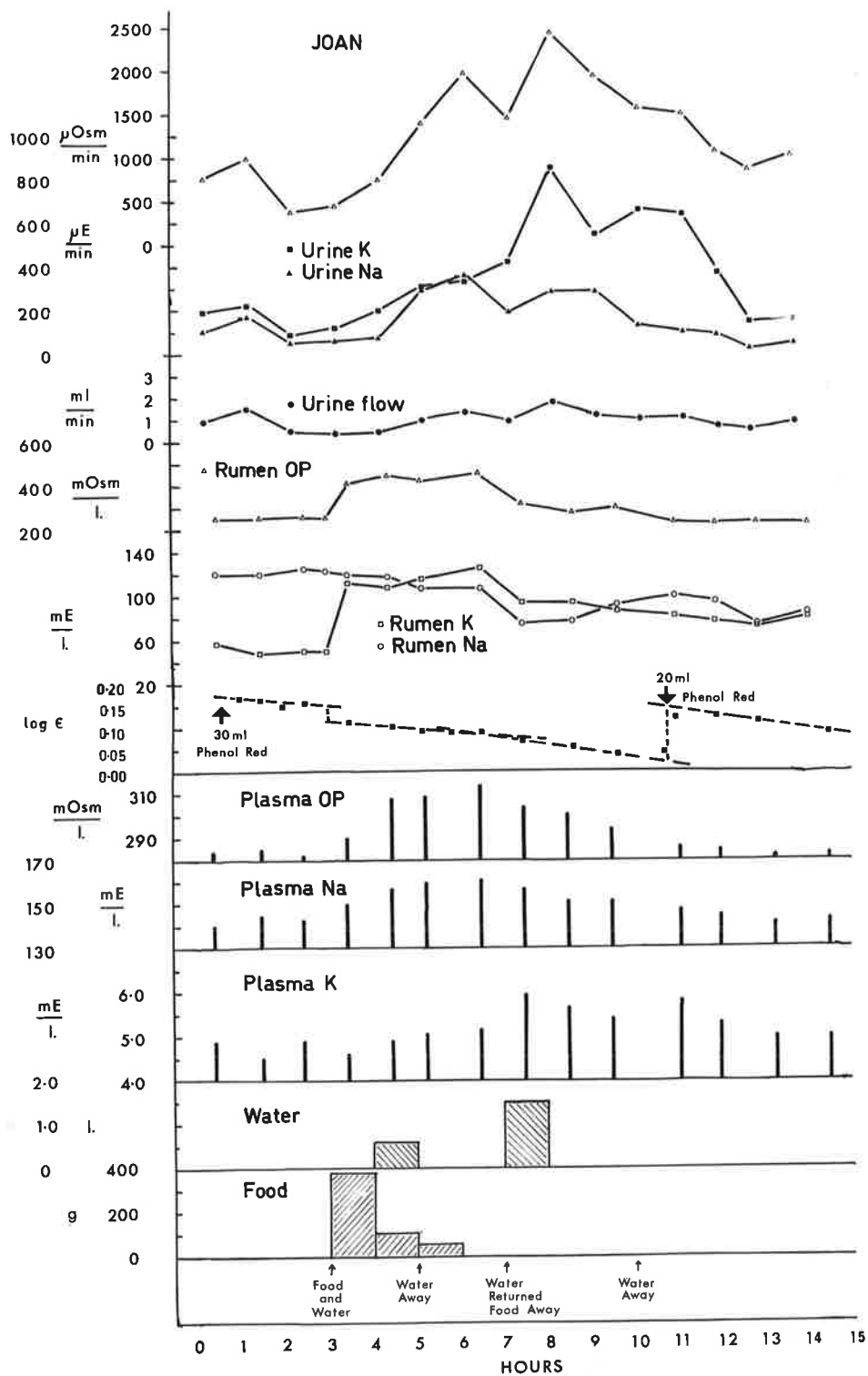


Fig. 15. Pre- and post-feeding changes in plasma and rumen electrolytes and in urinary excretion. Sheep P was fed 1000 g of lucerne chaff to which 1200 m-equiv of K had been added, and in a period restricted to 4 hr consumed 520 g containing 1320 m-equiv of K and 110 m-equiv of Na. Water was removed at 4 hr, returned at 6 hr and removed again at 9 hr.

The first arrow of the phenol red graph represents the addition of 20 ml of phenol red to the rumen and the second arrow 10 ml of phenol red.

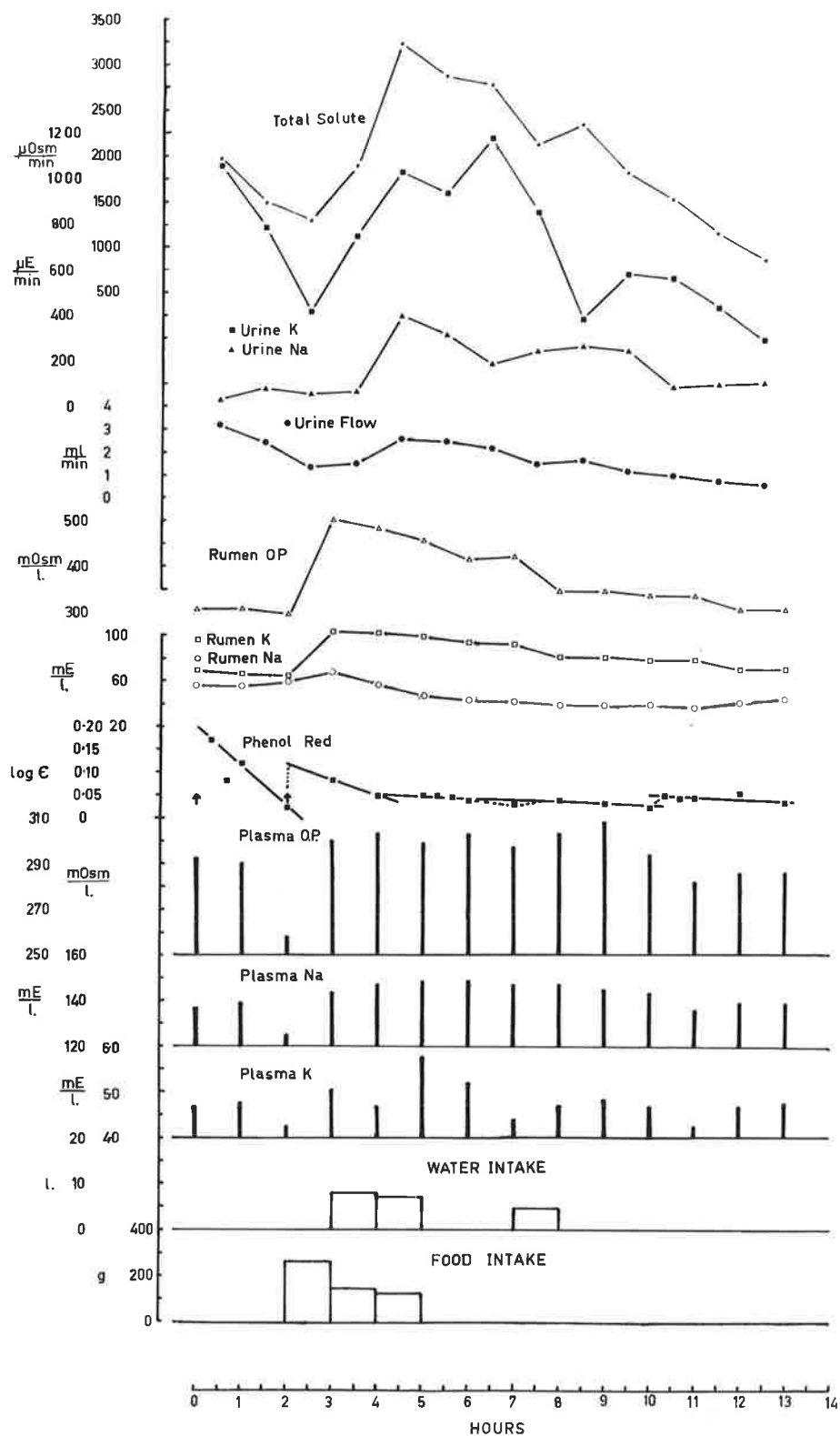
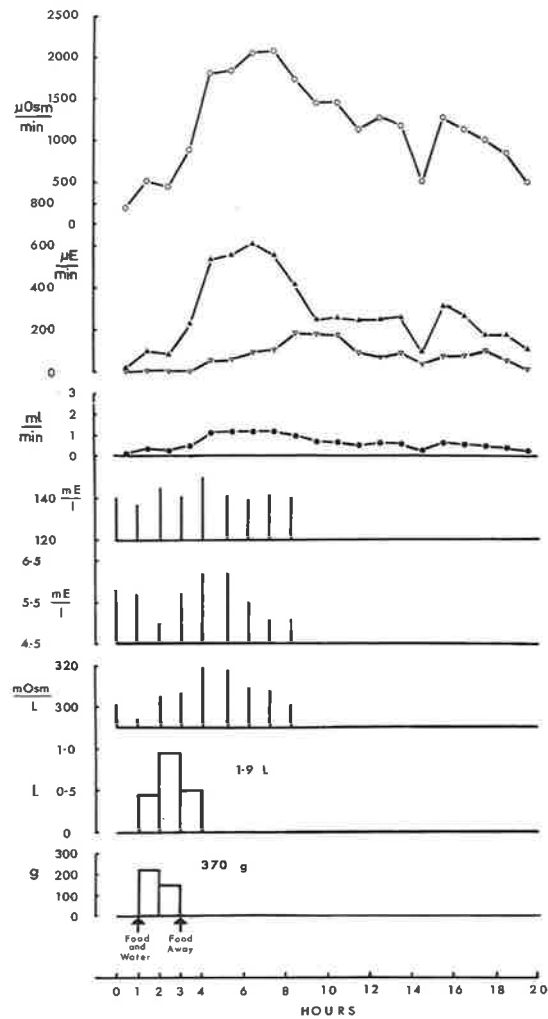


Fig. 16. Comparison of the changes in plasma electrolyte concentrations and in urinary excretion in sheep F following the ingestion of 275 and 995 m-equiv of K in non K supplemented and K supplemented lucerne chaff.



**FREDA**

POTASSIUM INTAKE

← 275 mE

995 mE →

○ URINE OSMOLALITY

▲ URINE POTASSIUM

▼ URINE SODIUM

● URINE FLOW

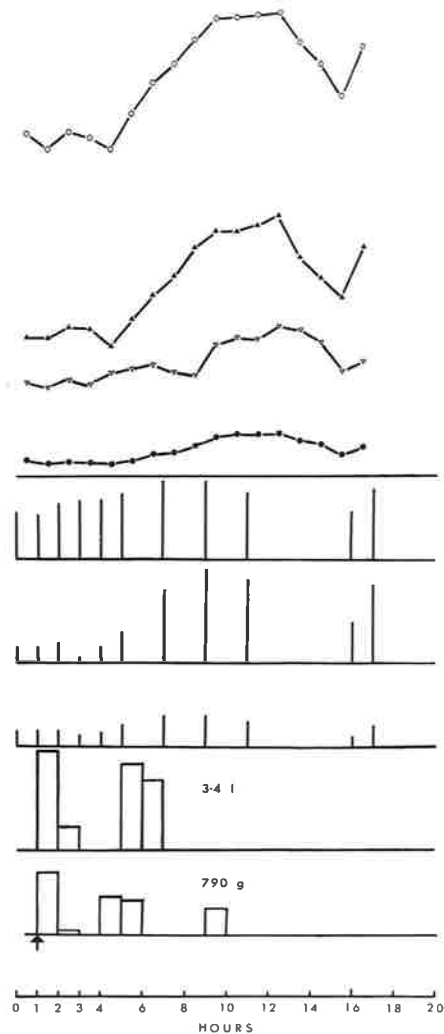
PLASMA SODIUM

PLASMA POTASSIUM

PLASMA OSMOLALITY

WATER INTAKE

FOOD INTAKE



range of 400 to 850 m-equiv/l, while maximum [Na] depended on the K intake, falling as the K intake rose. Rates of Na loss rarely exceeded 400  $\mu$ -equiv/min but from the number of results obtained, it is difficult to assess the effect K intake had on Na output.

Changes in urinary K excretion tended to parallel alterations in plasma [K]. These changes were rarely coincidental but were slightly delayed. Sodium gave a different result since the plasma [Na] rose during the hr after feeding began but the urinary Na output did not change. Once the increase in the rate of urinary Na excretion began, the changes which followed were often parallel with those in plasma [Na]. In a few instances (Fig. 15) the rate of Na excretion remained elevated in the face of a fall in plasma [Na].

Urinary osmolality also varied between individual sheep and depended on water intake. Just as the urinary [Na] and [K] were not necessarily at a maximum when the rates of K and Na excretion were at a maximum so the maximum urine OP did not necessarily coincide with the maximum rate of solute loss. However, the highest urine OP's found were in the first 3 to 8 hr following eating, and ranged from 1500 to 2250 m-osmol/l. The maximum rate of solute loss increased with increasing K intake as would be expected from the rates of K excretion. Maximum rates of excretion of osmotically active solutes

ranged between 1800 and 3300  $\mu$ -osmol/min.

(iv) Changes in plasma and rumen electrolyte concentrations and urinary electrolyte and water excretion following the addition of K salts to the rumen

Most of the results reported in this section were associated with measurements of the rate of water shift from the rumen following the addition of K salts to the rumen and hence only the most pertinent findings will be given.

The addition of 400 m-equiv of K as either Cl or  $\text{HCO}_3$  to the rumen was remarkable for the small and erratic effects it had on plasma electrolytes (Fig. 17, 18). A rise in plasma [K] was anticipated in view of the findings in section iii(c) but this did not always occur. Furthermore, no regular increase in plasma [Na] such as occurred after eating was seen after the addition of 400 m-equiv of K to the rumen.

The pattern of change in rumen electrolytes following the addition of 400 m-equiv of  $\text{KHCO}_3$  or KCl was similar to that found following normal eating. The [K] of the rumen rose to between 90 and 145 m-equiv/l. while after some delay the [Na] either fell slightly or remained constant. This constancy or decline in [Na] continued for between 5 and 12 hr after the addition of the K supplement before it began to rise.



The rumen OP rose to 350 to 450 m-osmol/l., levels similar to those found after eating. Neither the [K] or OP of the rumen fluid was maintained, but began to fall immediately, attaining presupplement levels between 6 and 14 hr after the addition of the K supplement to the rumen.

In sheep receiving no rumen K supplement, the rumen [K] usually tended to fall with time while the rumen [Na] slowly rose. If the initial concentration of K was low (20 to 30 m-equiv/l.) and [Na] was high (100 - 120 m-equiv/l.) changes in the [K] or [Na] of rumen fluid were small.

The urinary excretion of water and electrolytes following the addition of 400 m-equiv of K to the rumen was most irregular. Urinary losses were measured 4 times following the addition of  $\text{KHCO}_3$  but only once with the addition of KCl. With either  $\text{KHCO}_3$  or KCl (Fig. 17,18) there was no significant or regular alteration of urine flow rate. Thus there was no particular difference in the total 24 hr water loss between sheep with and without K added to the rumen (Fig. 19).

Comparison of the excretion rates of K in the same sheep with and without K supplements showed that there was an increased loss following the supplement but there was no definite pattern to this loss. For example, sheep N (Fig. 17) increased

Fig. 17. Comparison of alterations in plasma and rumen electrolyte concentrations and in urinary excretion with and without the addition of 400 m-mole  $\text{KHCO}_3$  to the rumen.

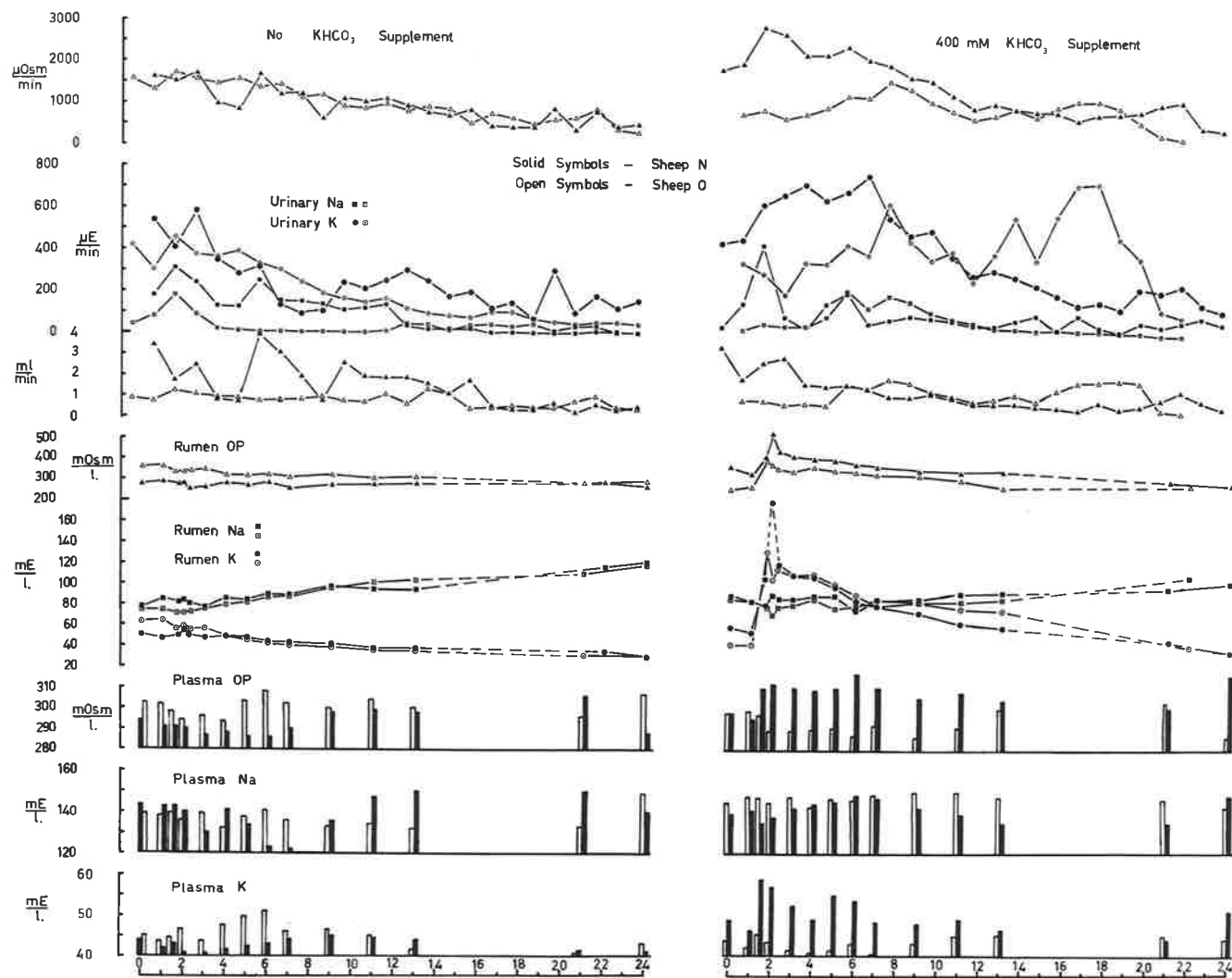
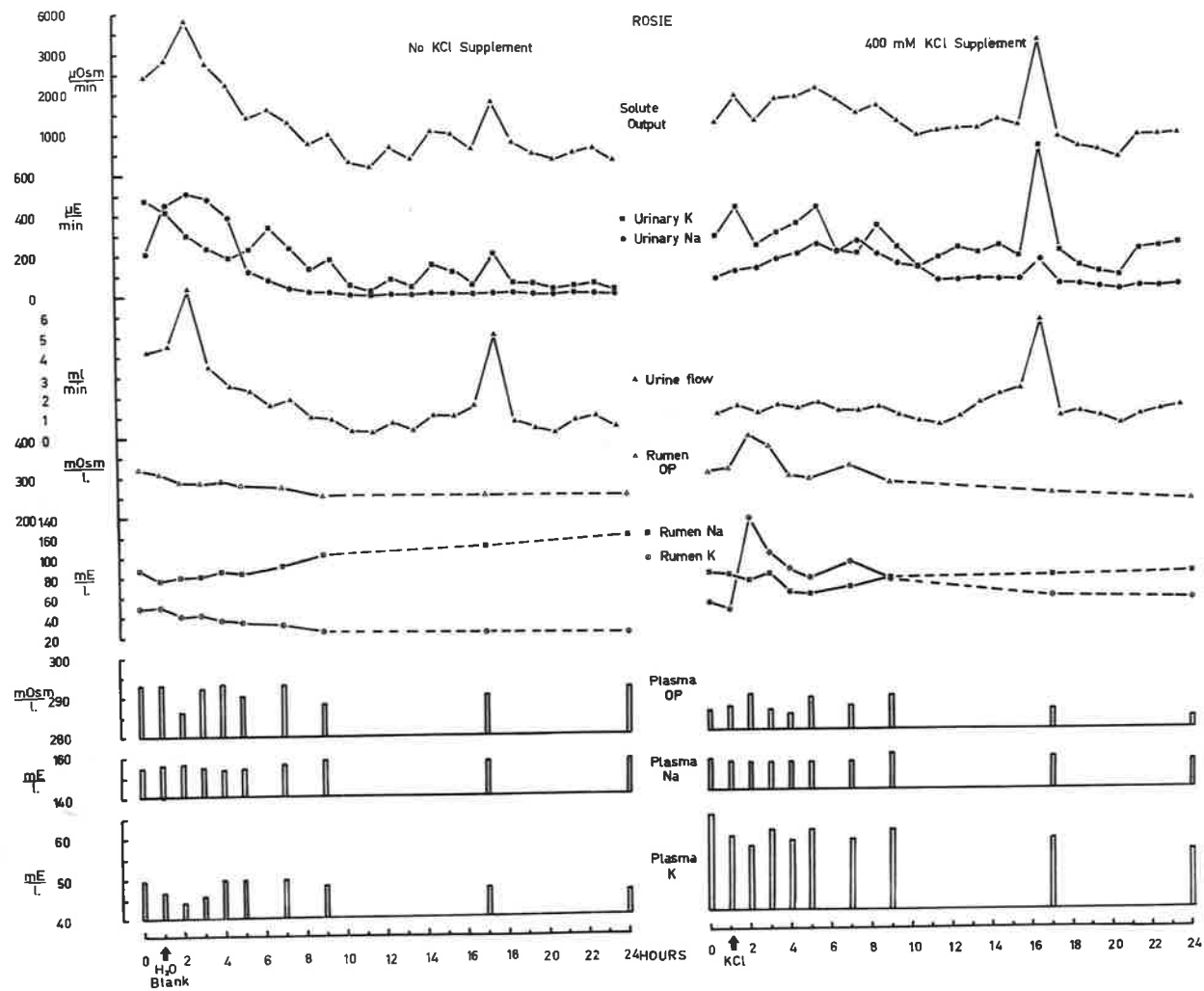


Fig. 18. Comparison of alterations in plasma and rumen electrolyte concentrations and in urinary excretion with and without the addition of 400 m-mole KCl to the rumen.

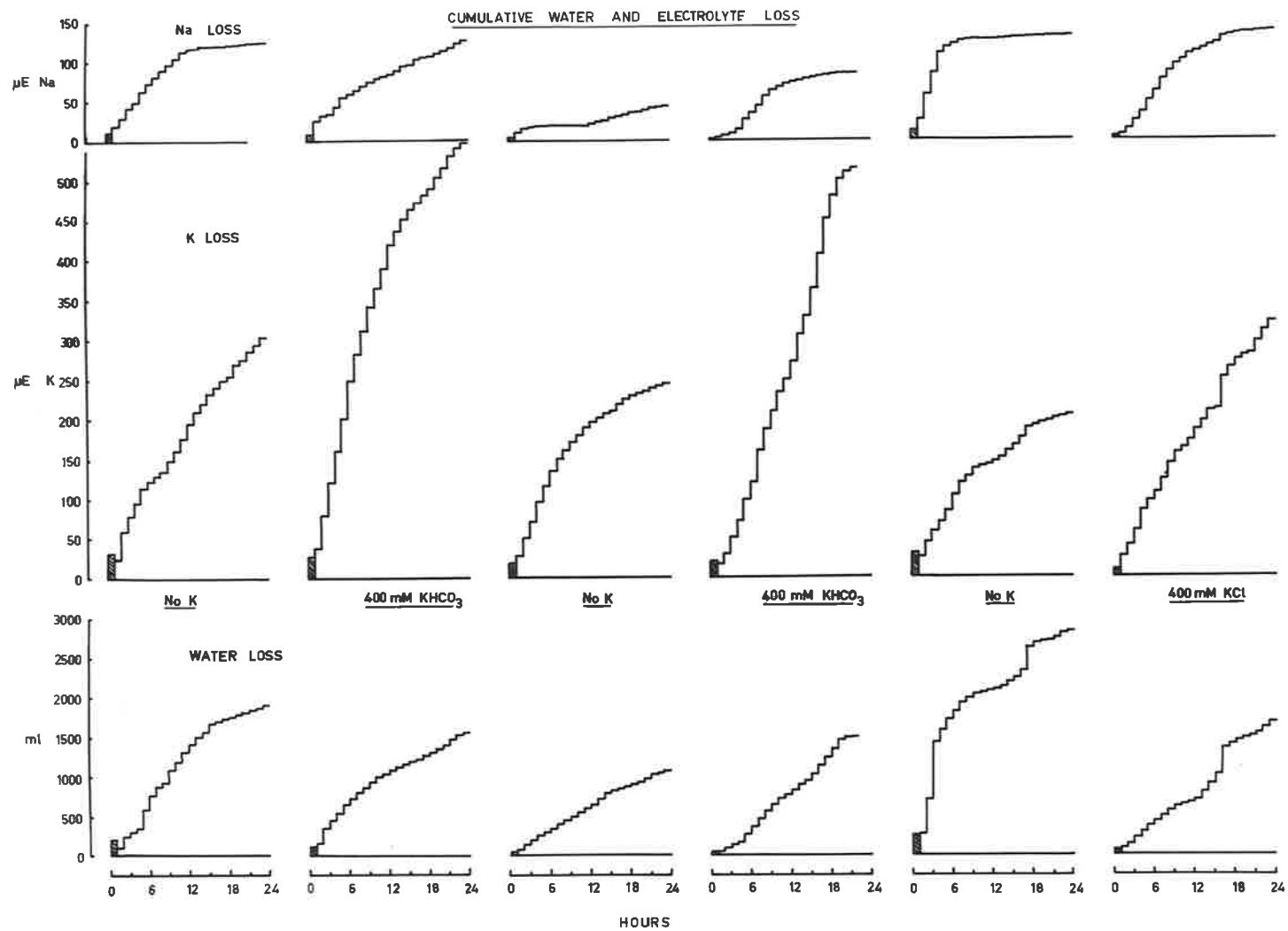


the rate of K excretion immediately the  $\text{KHCO}_3$  was added to the rumen and the rate increased up till 6 hr, after which it began to decline. However, sheep O did not exceed the initial rate of K excretion for over 4 hr and the maximum rate of K loss of 730  $\mu$ -equiv/min was not achieved until the 17th hr after the addition of the K to the rumen. The Na excretion was not altered by the addition of K to the rumen.

From the 24 hr cumulative losses of water, K and Na in 6 sheep with and without either  $\text{KHCO}_3$  or KCl added to the rumen, it appears that the total loss of K in the sheep which received  $\text{KHCO}_3$  was greater than in those with no K supplement or the sheep with KCl added to the rumen. Between 11 and 17 hr were required for the excretion of 400 m-equiv of K, the amount equivalent to that added to the rumen. Even at 24 hr the total loss of K did not equal the sum of the mean loss with no K supplement plus the 400 m-equiv added to the rumen.

The addition of K to the rumen had no effect on the total 24 hr loss of Na in the urine. It is interesting to observe that the water loss tended to follow the Na excretion with no apparent effect of K output on water loss. This was reflected in the [K] of the urine which reached levels as high as 320 m-equiv/l, compared with the maximum in a non rumen supplemented sheep of 480 m-equiv/l.

Fig. 19. Cumulative losses of K, Na and water in the urine without the addition of any K to the rumen or following the addition of 400 m-mole  $\text{KHCO}_3$  or  $\text{KCl}$  to the rumen. (Corresponds to Fig. 17 and 18).





It is difficult to explain the urinary loss of K in the sheep which received 400 m-mole of KCl as the K apparently moved out of the rumen (rumen [K] fell) yet there was no significant alteration in the [K] of the plasma or rise in K excretion in the urine. One point should be noted and that was the high [K] of the plasma initially and the maintenance of the concentration at greater than normal levels for much of the 24 hr.

(v) The uptake of water, potassium and sodium from the rumen and lower gut

This section of the experimental work was supplementary to section (ii) and (iv), to determine what effect, if any, additions of K to the rumen had on water and electrolyte movement from the rumen and whether other segments of the gut provided a restraint on water and electrolyte movement similar to that of the rumen. In several instances an insufficient number of experiments was carried out to allow a statistical analysis of rates of movement. The curves of uptake to the blood and for the rumen, rumen disappearance curves allow certain conclusions to be drawn, however.

Fig. 20 illustrates typical water (TOH) and  $^{42}\text{K}$ , rumen disappearance and plasma uptake curves after the administration

Fig. 20. Plasma uptake and rumen disappearance curves  
of TOH and  $^{42}\text{K}$  after the addition of TOH and  $^{42}\text{K}$ ,  
with and without 400 m-equiv of K to the rumen.

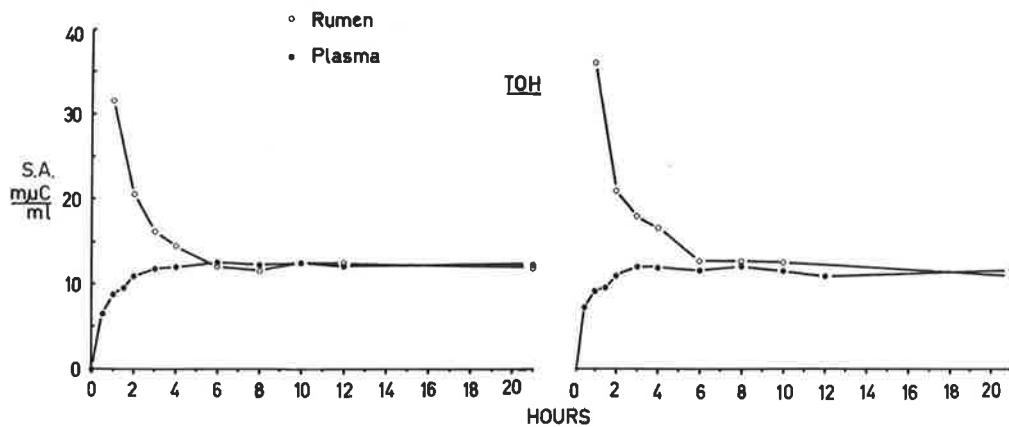
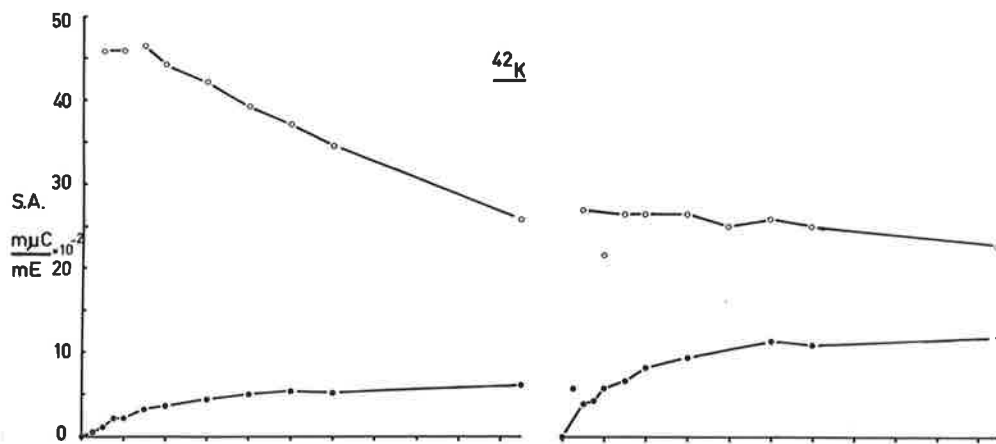
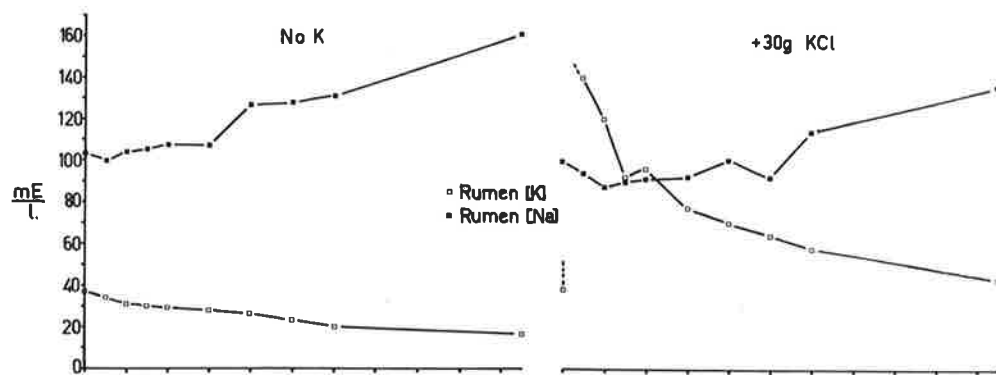
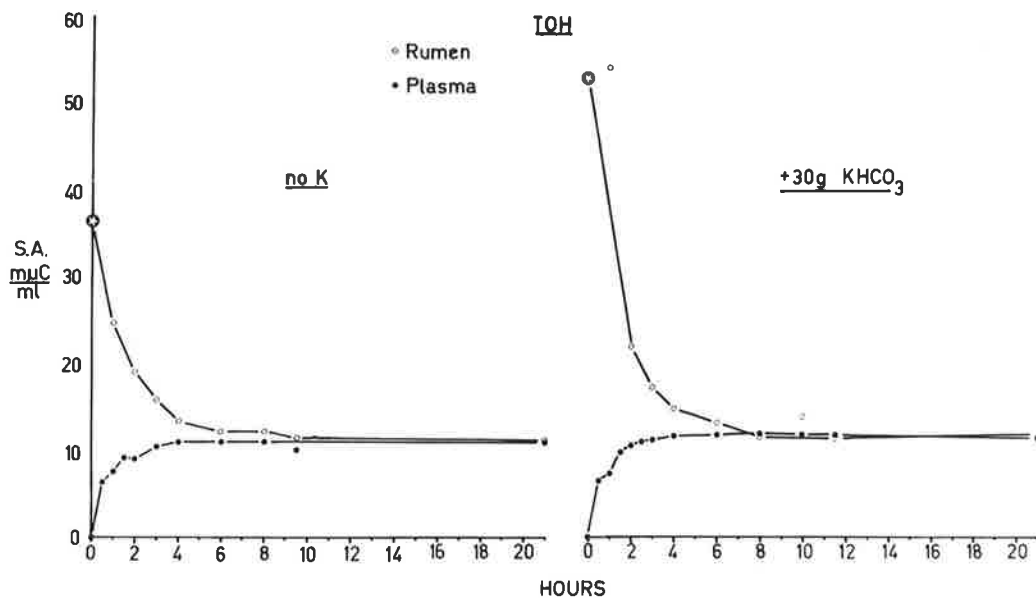
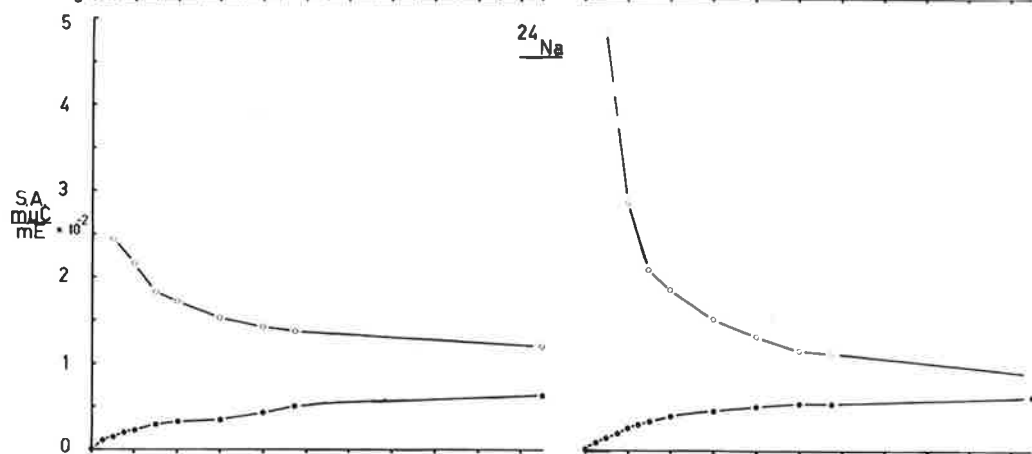
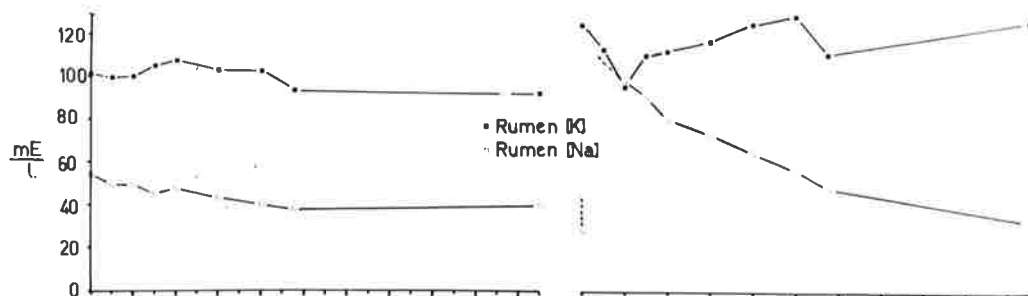


Fig. 21. Blood uptake and rumen disappearance curves of TON and  $^{24}\text{Na}$  after the addition of TON and  $^{24}\text{Na}$ , with and without 400 m-equiv of K to the rumen.

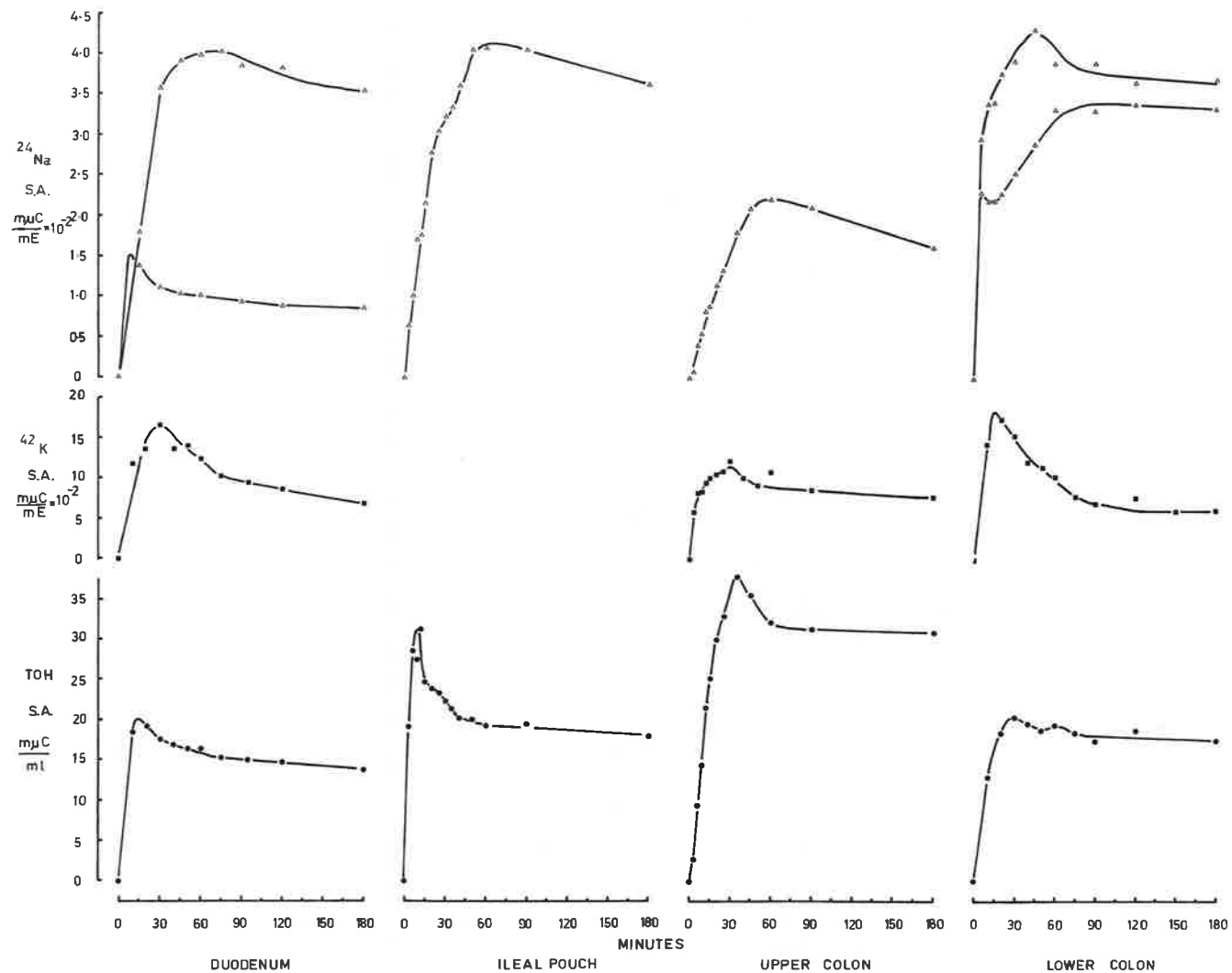


of 300  $\mu\text{C}$  of TOH and 50  $\mu\text{C}$   $^{42}\text{K}$  into the rumen, with and without the addition of 400 m-equiv of K. Because there were difficulties associated with the separation of  $^{42}\text{K}$  and  $^{24}\text{Na}$  during counting only one of these isotopes was added to the rumen at any time. Therefore, the rumen disappearance and blood uptake curves of  $^{24}\text{Na}$  and TOH are shown separately (Fig. 21).

Equilibration of TOH between blood and rumen in the examples illustrated, took place in approximately 7 hr while  $^{42}\text{K}$  and  $^{24}\text{Na}$  were still not equilibrated at 12 hr or even 24 hr (not shown in Figs) - i.e. the plasma or whole blood S.A. was still increasing at 12 and 24 hr.  $^{24}\text{Na}$  was much closer to being equilibrated than  $^{42}\text{K}$ .

When TOH and  $^{42}\text{K}$  or  $^{24}\text{Na}$  were placed in the duodenum (via a duodenal fistula), ileum (Pavlov pouch) upper colon (via a colonic fistula) or lower colon or rectum (by a catheter) the peaks of radioactivity in the blood or plasma were reached within 5 to 75 min (Fig. 22). These times illustrate a very rapid uptake of water, K and Na from the various gut segments, into the blood, compared with their uptake from the rumen. It may be assumed that there was movement from blood to lumen as well as from lumen to blood, since both  $^{42}\text{K}$  and  $^{24}\text{Na}$  were placed in the gut segments with electrolyte concentrations similar to plasma. Thus the solutions added to the gut were isotonic with plasma and no concentration gradient existed.

Fig. 22. Plasma uptake curves of TOH,  $^{42}\text{K}$  and  $^{24}\text{Na}$  following their addition, in solutions isotonic with plasma, to the duodenum, ileal pouch, upper colon and lower colon.





Although considerable differences between the rumen and other gut segments in allowing the passage of water, K and Na to plasma were demonstrated (Fig. 20, 21,22) no information on uptake from the omasum and abomasum was obtained. In one experiment the movement of TOH from blood into the abomasum was measured and it was found that there was a very rapid rise in abomasal S.A. This high S.A. was not maintained as the abomasal S.A. decreased to well below plasma levels due to dilution with water of lower S.A. from the rumen.

Excluding the omasum and abomasum only the rumen wall appeared to provide an effective barrier to the movement of water, K and Na from the gut to the blood.

(a) TOH uptake from the rumen

On the basis of the slope of the rumen disappearance curves and the above results, it appeared that a simple two compartment system could be postulated for the body in regard to water movement. A similar system was employed by Merrell, Gellhorn and Flexner (1944) in studies of the exchange of Na between the blood and extravascular fluid of the guinea pig. By considering the rumen as one compartment and the rest of the body as the second, the rate of water shift between the rumen and plasma could be described by a single exponential function. This function is described by the equation  $y = c + ae^{-kt}$  where

$y$  = rumen S.A.,  $a$  = the intercept of the disappearance curve with the  $y$  axis,  $c$  = the S.A. at equilibration,  $t$  = time and  $k$  the slope of the line. The slope,  $k$ , is a function of TEW, rumen volume and the fraction  $R$ , of the rumen volume which is transferred in unit time.

$$\text{i.e. } k = \frac{R}{q} \text{ where } q = \frac{\text{TBW} - \text{Rumen Volume (V)}}{\text{TBW}} = \frac{T-V}{T}$$

In nearly all instances the graph of  $\log (y-c)$  and time was a straight line which supports this proposal (Fig. 23). If this situation was correct the plasma uptake curves should also be described by the function proposed but such is not always the case. Therefore, the model is too simple and a further compartment(s) exists. Because the rumen disappearance curves are described satisfactorily by the equation given it appears that an extra compartment(s) is associated with the plasma. Although shifts of water between the plasma and this compartment(s) should alter the rate of disappearance of TOH from the rumen these movements must be so rapid as to have an insignificant effect on the rumen disappearance curves. Making this assumption all the curves of TOH disappearance from the rumen were analysed by the simple function proposed. It was also assumed that the rumen volume remained constant or was changing in a regular manner.

TABLE 7

	Volume by TOH	Volume by Phenol Red
	1.	1.
1.	3.96	4.15
2.	4.58	4.61
3.	5.45	5.63
4.	6.74	5.81 *
5.	6.75	6.56
6.	3.81	3.65
7.	7.04	4.51 *

\* Rejected

TABLE 8

## ESTIMATES OF CONSTANTS OF TOH DISAPPEARANCE

## CURVES FROM THE RUMEN

Sheep	a $\pm$ S.E.		c $\pm$ S.E.		TBW (1.)	Rumen Vol (1.)
A	7.80	0.60	3.45	0.53	28.97	8.88*
	16.91	1.85	3.05	1.40	32.76	5.01
G	13.67	0.54	3.80	0.14	26.33	5.72
	6.96	0.20	3.52	0.20	25.54	8.58*
J	34.92	2.58	8.12	0.97	24.62	4.65
M	18.41	0.75	3.43	0.10	29.26	4.58
	14.36	0.84	4.00	0.98	25.02	5.45
	12.73	0.21	3.87	0.11	25.82	6.02
	31.13	1.59	13.31	0.25	22.54	6.75
P	10.39	0.81	3.83	0.11	26.13	7.04*
	21.03	0.48	4.22	0.24	23.69	3.96
	13.78	0.43	3.93	0.28	25.43	5.65
	15.11	1.13	13.85	0.32	21.66	10.36*
R	44.22	5.34	8.30	0.93	24.09	3.81
	29.14	2.77	8.81	0.49	22.69	5.27
T	22.90	5.16	6.78	1.79	29.48	6.74*
	40.25	1.50	12.67	0.10	23.68	5.67
	14.21	0.99	7.37	0.32	27.13	9.26
	24.67	0.69	7.13	0.22	28.04	6.29
	14.09	3.35	10.63	1.64	28.21	12.13*
	24.70	1.20	11.57	0.23	25.93	8.27*

\* Rejected

TABLE 9

KINETICS DISAPPEARANCE OF TOHFROM THE RUMEN

Sheep	Initial Rumen K conc. ( $\frac{mM}{l.}$ )	Rumen Vol. (l.)	$k \times 10^{-3}$	$\pm$ SE	$R \times 10^{-3}$ $\text{min}^{-1}$	RY ml/hr
J	29	4.65	9.85	1.39	7.99	2228
T	29	9.26	8.89	1.09	6.51	3616
T	29	6.29	10.68	0.52	8.29	3127
P	38	5.65	10.74	0.91	8.53	2826
T	50	6.74	10.43	4.30	8.05	3255*
M	51	5.45	4.78	0.96	3.74	1224
T	54	8.27	10.00	0.67	6.43	3186*
P	64	7.04	14.95	1.43	10.92	4614*
G	84	8.58	6.75	0.65	11.48	2310*
T	92	12.13	6.30	3.34	3.59	2614*
R	102	5.27	13.09	1.63	10.05	3177
P	103	10.36	6.30	0.74	3.29	2034*
M	105	6.75	12.45	0.80	8.73	3534
G	112	5.72	10.18	0.63	7.97	2736
T	116	5.67	11.93	0.35	9.08	3089
M	121	6.02	10.00	0.39	7.67	2772
A	121	8.88	6.61	1.48	4.58	2436 *
P	135	3.96	10.46	0.57	8.71	2070
M	136	4.58	9.77	0.46	8.23	2262
R	145	3.81	12.83	1.99	10.80	2466
A	190	5.01	6.50	2.07	5.97	1794

Initially rumen volumes were estimated by the phenol red dilution technique. Later the volume was deduced from the analysis of the rumen TOH disappearance curve. This was performed as follows. By extrapolating the line of slope  $k$  to the  $y$  axis, the value " $a$ " was obtained. Adding this value to the equilibration S.A. ( $c$ ) gave the S.A. of the rumen at zero time. Hence knowing the quantity of TOH (total activity) added to the rumen the rumen volume could be calculated  $-V = \frac{\text{Total Activity}}{a + c}$  (Fig. 23). In 5 out of the 7 experiments good agreement between the rumen volume estimated by this technique and the phenol red dilution method was found (Table 7).

Rather than fit the line of the rumen disappearance curve described by the exponential function visually a computer analysis was performed using a non-linear least squares technique. Estimates of the slope,  $a$  and  $c$  along with measured values of rumen S.A. and time were given and holding  $c$  constant, the fit of  $y = a + ce^{-kt}$  was determined for a maximum of 10 iterations. Then allowing all parameters to vary the fit of  $y = a + ce^{-kt}$  was redetermined. The values of  $k$ ,  $a$  and  $c$  derived by this process were then used to calculate TBW, rumen volume,  $q$  and  $R$ . Multiplying  $V$  by  $R$  gave an estimate of the total water shift to and from the rumen in unit time (Table 8, 9).

Fig. 23. Graphic illustration of the method of analysis of rumen TOX disappearance curves which are described by the equation  $y = c + ae^{-kt}$ .  $\log (y-c)$  is plotted against time to derive (1) the initial rumen S.A.  $(a + c)$  for the calculation of rumen volume and (2) the slope of the line  $(k)$  for calculation of the fractional transfer rate  $(R)$ .

The assumption of a two-compartment system is verified if the plasma uptake curve, plotted as  $\log (c - y)$  against time, yields a straight line which extrapolates to the value  $y = c$  when  $t = 0$  and has the same slope  $(k)$  as that shown by the rumen disappearance curve.

Three such pairs of curves are illustrated where  $\log (y - c)$  has been plotted in terms of cpm/ml water and the star symbol represents initial S.A. as derived from the graphical analysis.

$y$  = rumen (or plasma) S.A.  
 $c$  = body water S.A. at equilibrium  
 $a$  = the  $Y$  intercept of the line  $\log (y-c)$  against time.

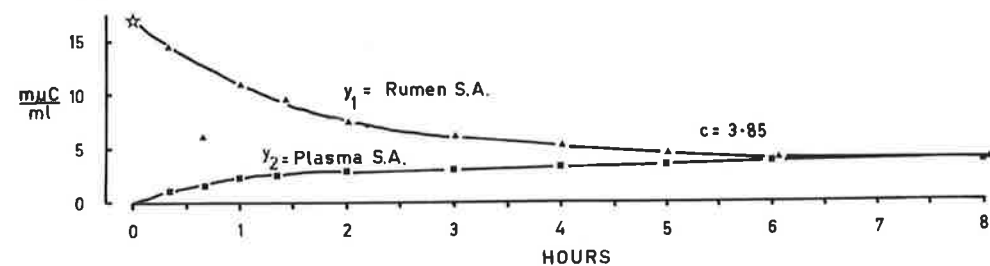
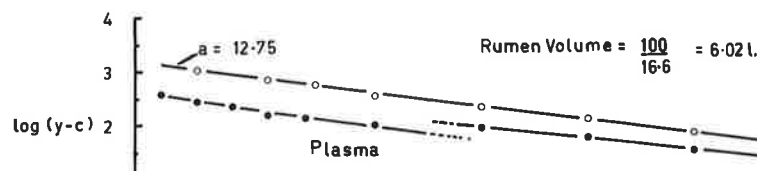
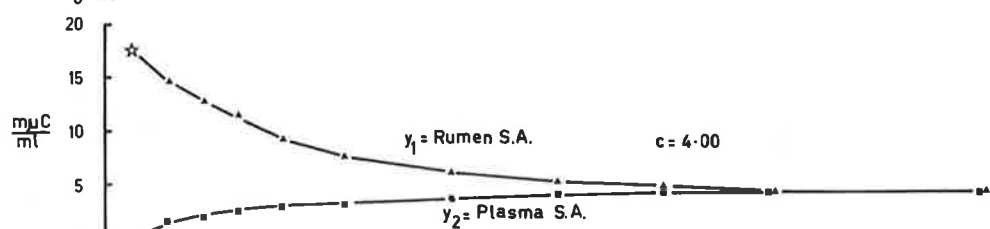
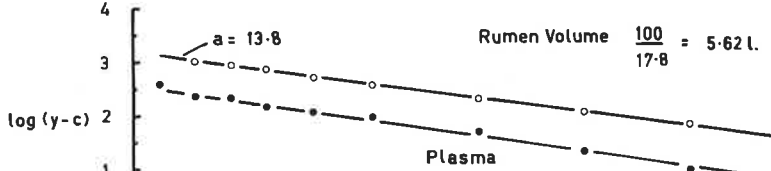
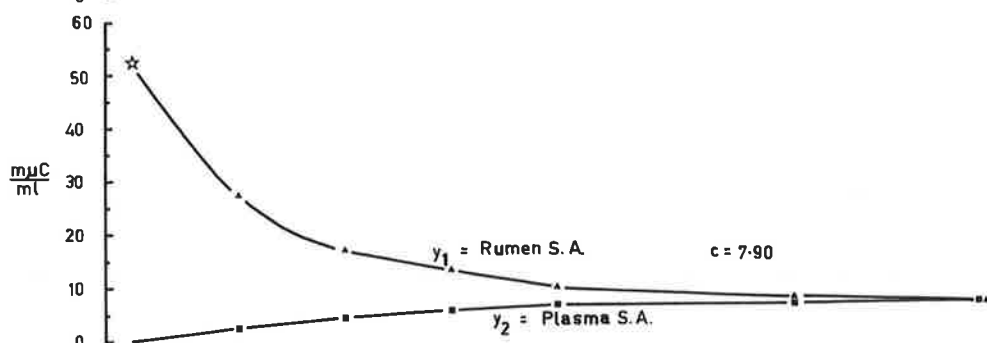
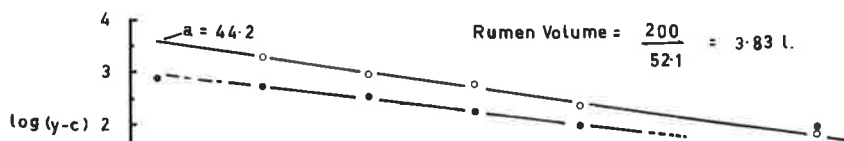
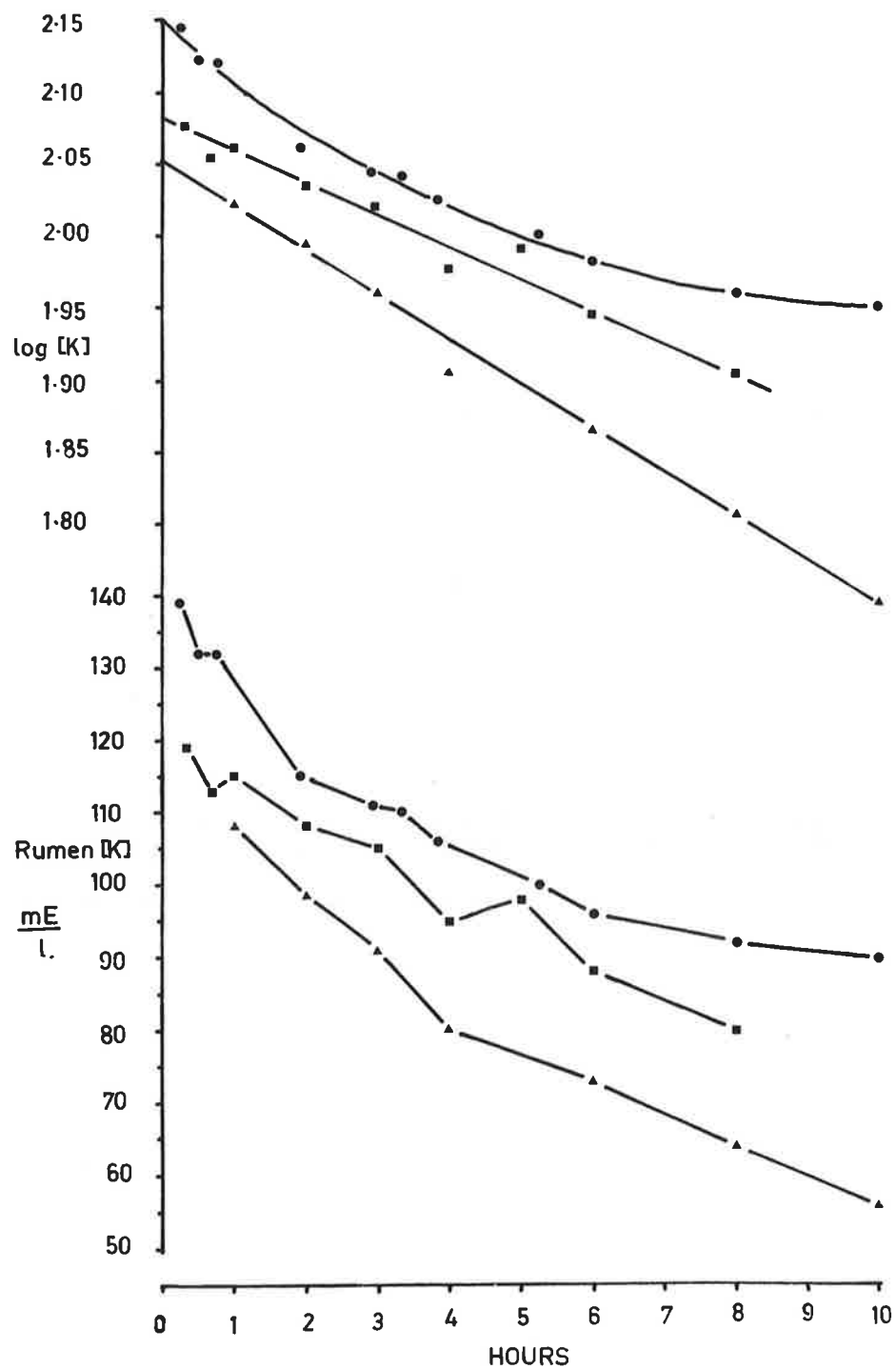




Fig. 24. Illustration of the method of determining the initial rumen  $[K]$  after the addition of  $K$  to the rumen. The plot of log rumen  $[K]$  against time is extrapolated to zero to obtain log  $[K]$  at  $t = 0$ .



Using this method comparisons between  $k$ ,  $R$ ,  $BV$  and rumen volume or rumen  $[K]$  at zero time were made (Fig. 25, 26). Regression between these parameters were calculated.

To determine whether the  $[K]$  of the rumen altered the total water transfer in the rumen. 400 m-mole of  $KCl$  or  $KHCO_3$  was added to the rumen simultaneously with the TOH. This was carried out in 13 of the 21 experiments and the rumen  $[K]$  after the addition of the  $K$  salt was used as the reference point of  $[K]$ . This value was estimated by extrapolating the curve of either the  $[K]$  or  $\log [K]$  and time to zero time. The  $\log [K]$  plot often approached a straight line making extrapolation quite simple (Fig. 24).

A positive relationship was determined between rumen volume and total water movement as is indicated by the regression line of Fig. 25. Seven experiments were discarded from these results owing to inaccurate estimates of rumen volume. The criterion for rejection was based on B.Wt., TSW, SE of estimates of  $a$ ,  $c$ , and  $k$ , and phenol red estimates of rumen volume.

No other significant relationships could be found between rumen volume and other parameters nor between the initial rumen  $[K]$  and the different parameter (Fig. 26). It is of interest to observe that the slope,  $k$  of the rumen TOH disappearance curve, does have quite a range of values especially

Fig. 25. Comparison of TBW, the slope,  $k$ , of the rumen TOH, disappearance curve, the fraction,  $R$ , of the rumen water volume shifting in unit time, the total volume of water,  $RV$  transferred to and from the rumen per hr and the estimated rumen volume,  $V$ .

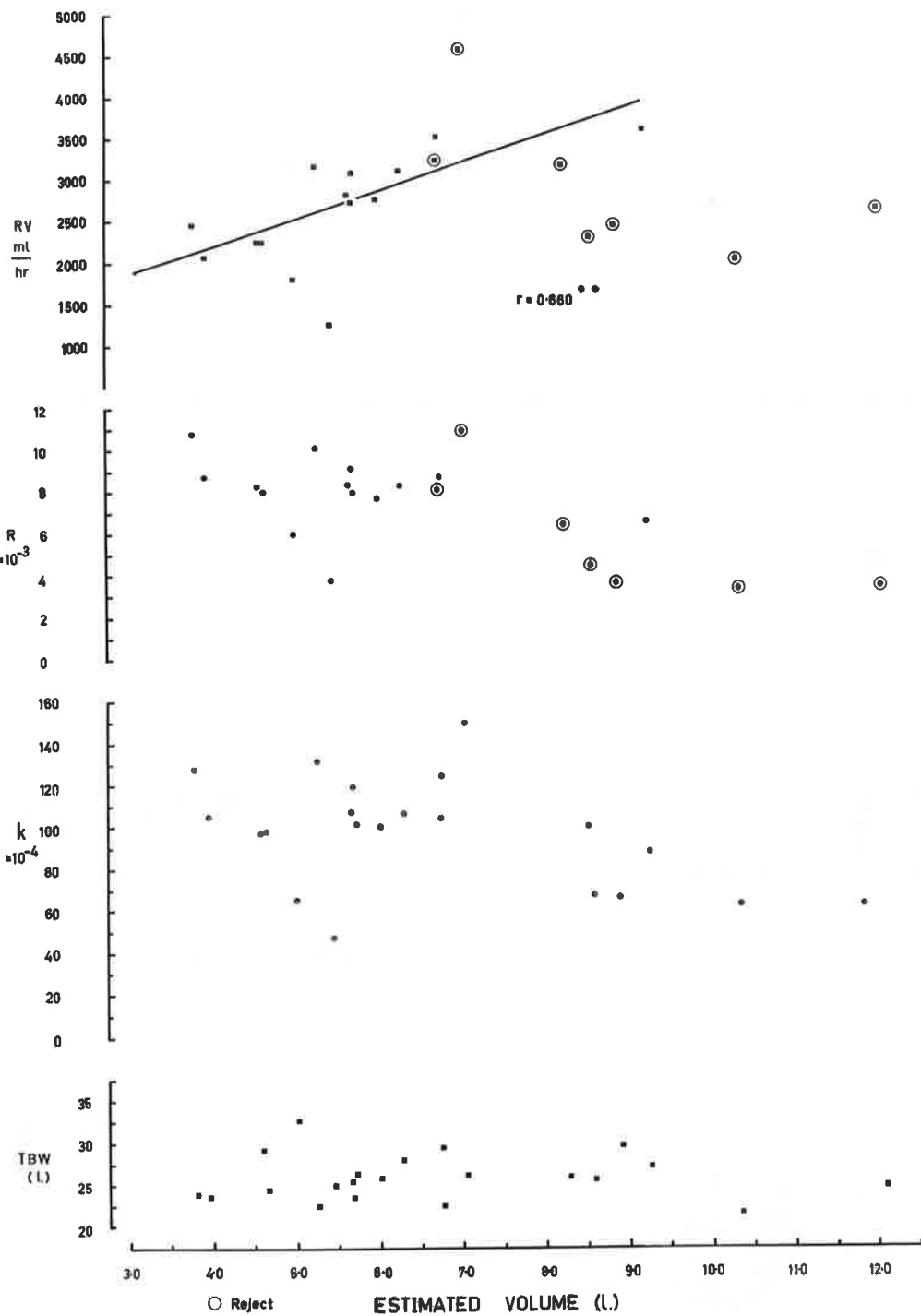
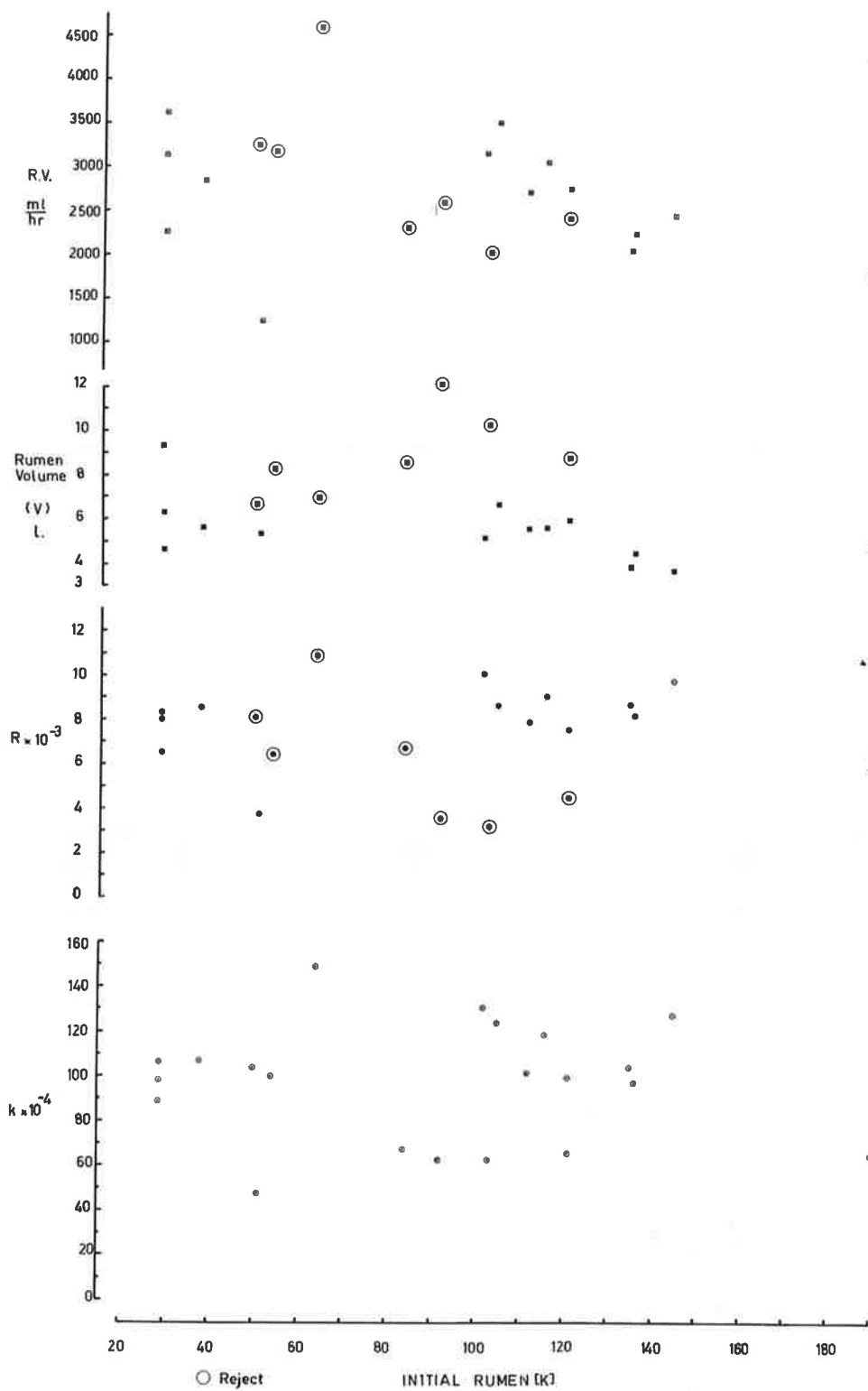


Fig. 26. Comparison of the slope,  $k$ , of the rumen TOH disappearance curve, the fraction,  $R$ , of the rumen volume moving in unit time, the estimated rumen volume,  $V$ , the total volume of water,  $RV$ , transferred to and from the rumen per hr and the measured or estimated initial rumen  $[K]$ .



if the values of  $k$  for those experiments disregarded due to unacceptable rumen volumes are included. There is no reason for doubting the validity of several of these values of  $k$  as the fit of many of these estimated slopes is excellent. It is possible that  $k$  may depend upon rumen volume, although it was not shown in these results, yet it is independent of the estimate of rumen volume. Hence all the values of  $k$  cannot be disregarded like those of  $R$  and  $RV$  which depend upon the estimated ~~of~~ rumen volume for their calculation.

Following the estimation that a rumen of mean vol. 5.58 l. had a total water transfer of 2640 ml/hr attempts were made to assess what contributions saliva flow and transepithelial water flow made to the equilibration of TOH between rumen fluid and blood. The amount of TOH returned to the rumen in the saliva was measured by measuring saliva flow and salivary S.A. (Fig. 27). Only a relatively small quantity of TOH was returned to the rumen by the saliva, and although rumen S.A. should be lower as the result of the salivary dilution (initial salivary S.A. low) this was quite insufficient to account for the rates of TOH equilibration observed. It was therefore proposed that much of the equilibration of TOH took place across the rumen wall. This proposal was supported by the fact that where artificial saliva (McDougall, 1948) was used to replace the natural saliva



Fig. 27 Plasma uptake and rumen disappearance curves of TOH following the addition of TOH to the rumen. Saliva flow, saliva TOH S.A., and amount returned to the rumen in the saliva are shown.

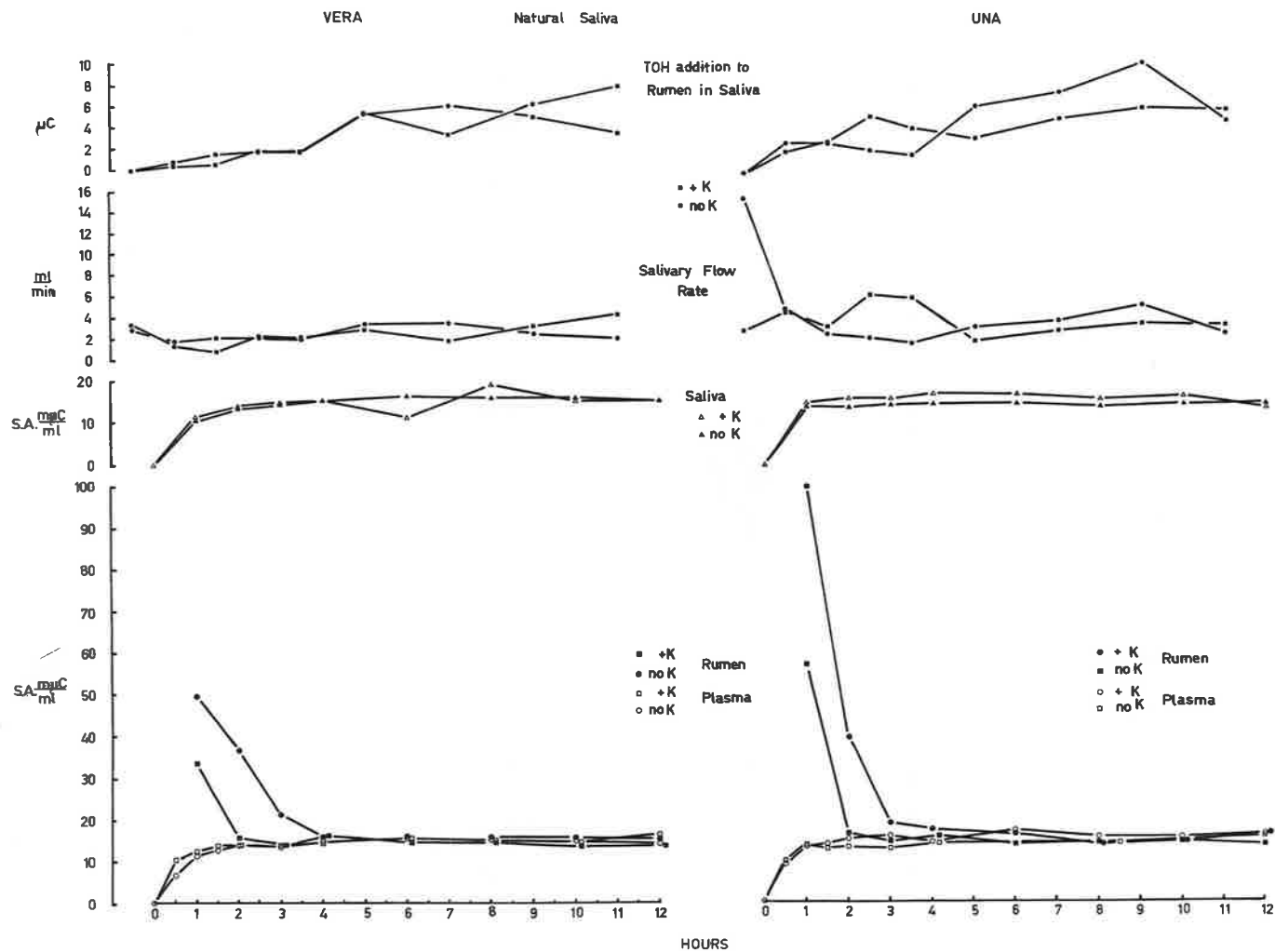


Fig. 28 Plasma uptake and rumen disappearance curves of TOH following the addition of TOH to the rumen and replacement of natural saliva with artificial saliva. Saliva flow, salivary TOH S.A. and amount of TOH contained in the saliva are shown.

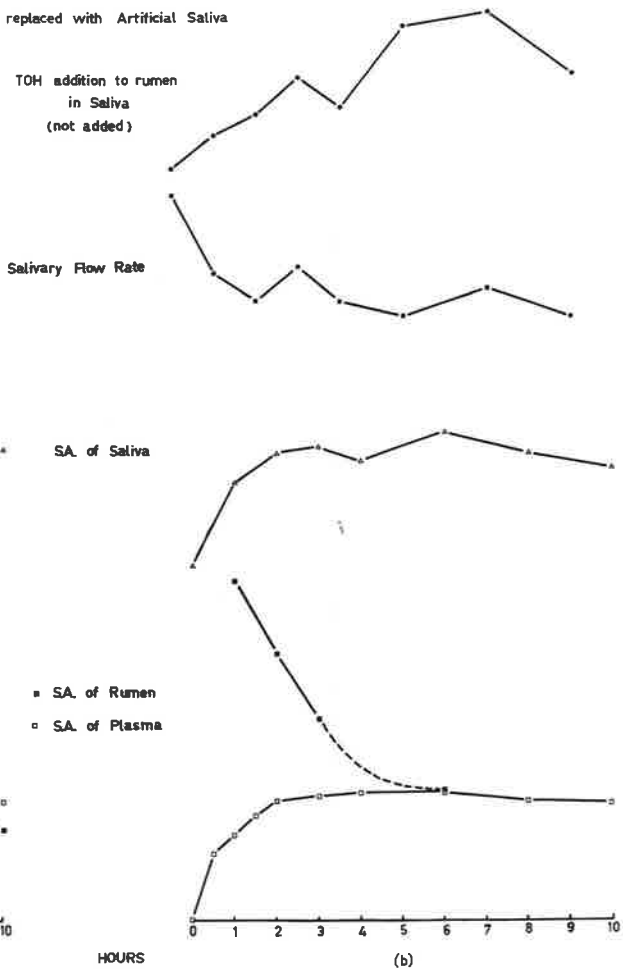
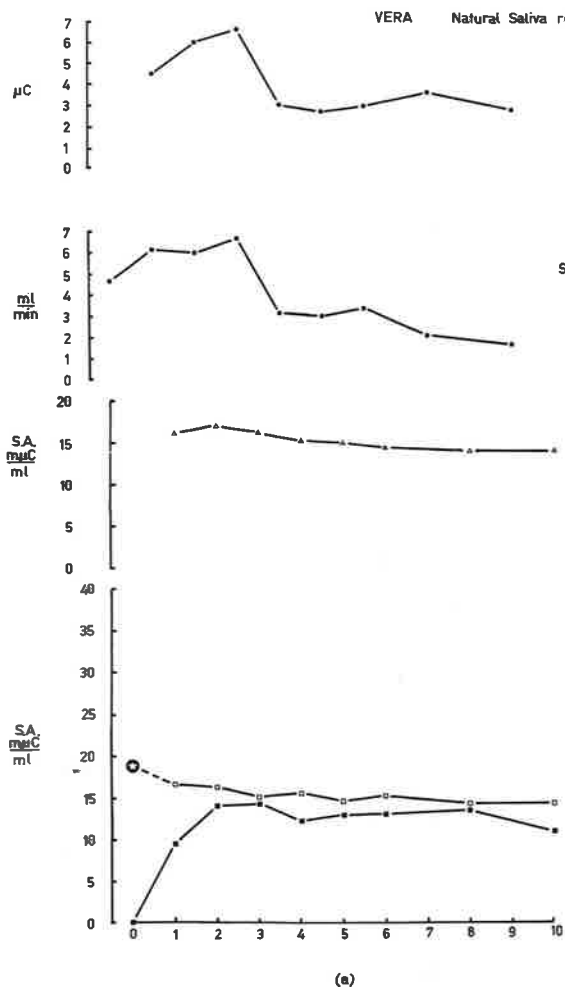


Fig. 29. Plasma uptake and rumen disappearance curves of  $^{42}\text{K}$  after the addition of  $^{42}\text{K}$  with and without 400 m-equiv of K, to the rumen. Salivary flow rates, salivary  $^{42}\text{K}$  S.A., and quantity of K,  $^{42}\text{K}$  and Na returned to the rumen in the saliva are compared.

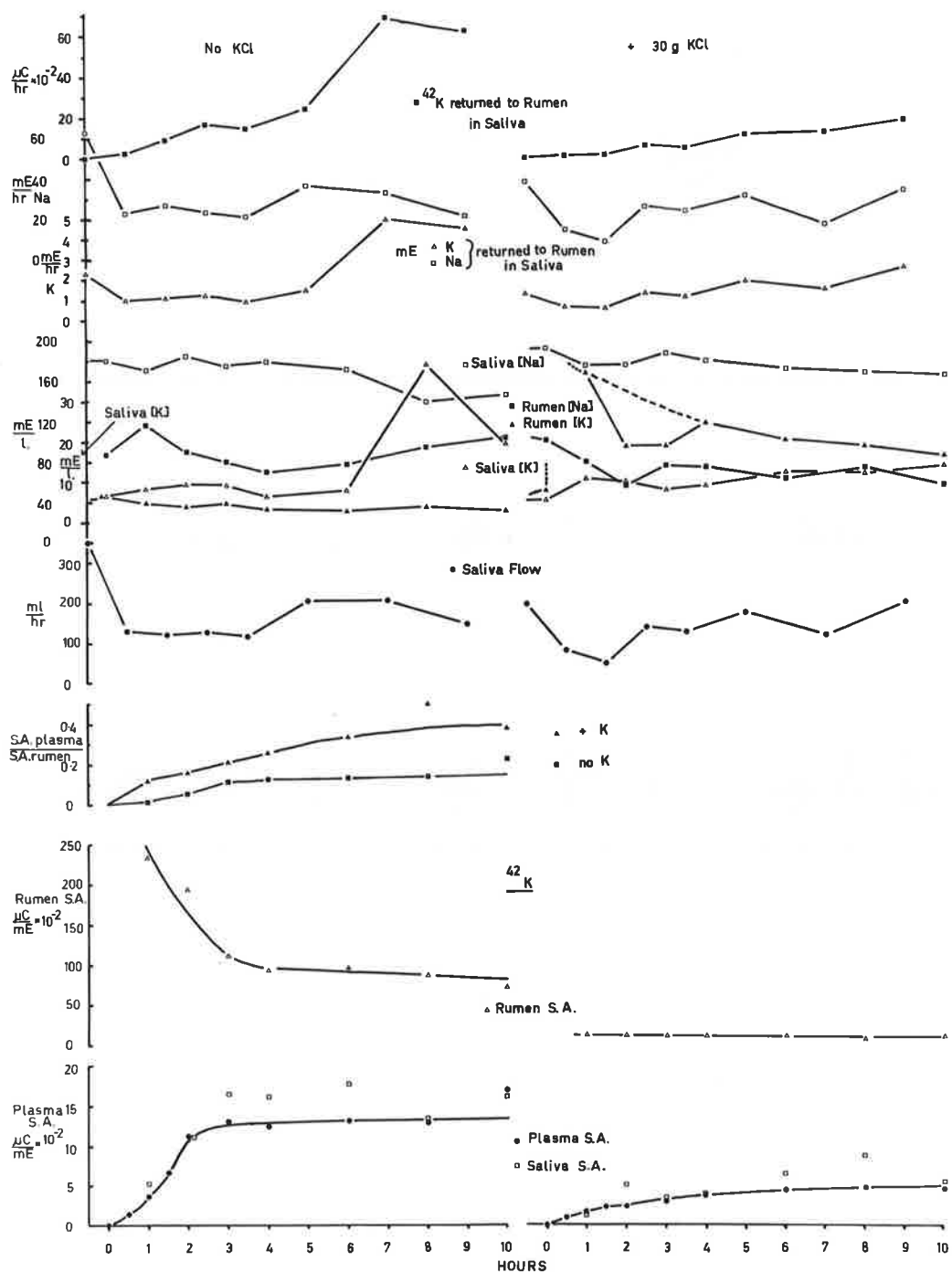
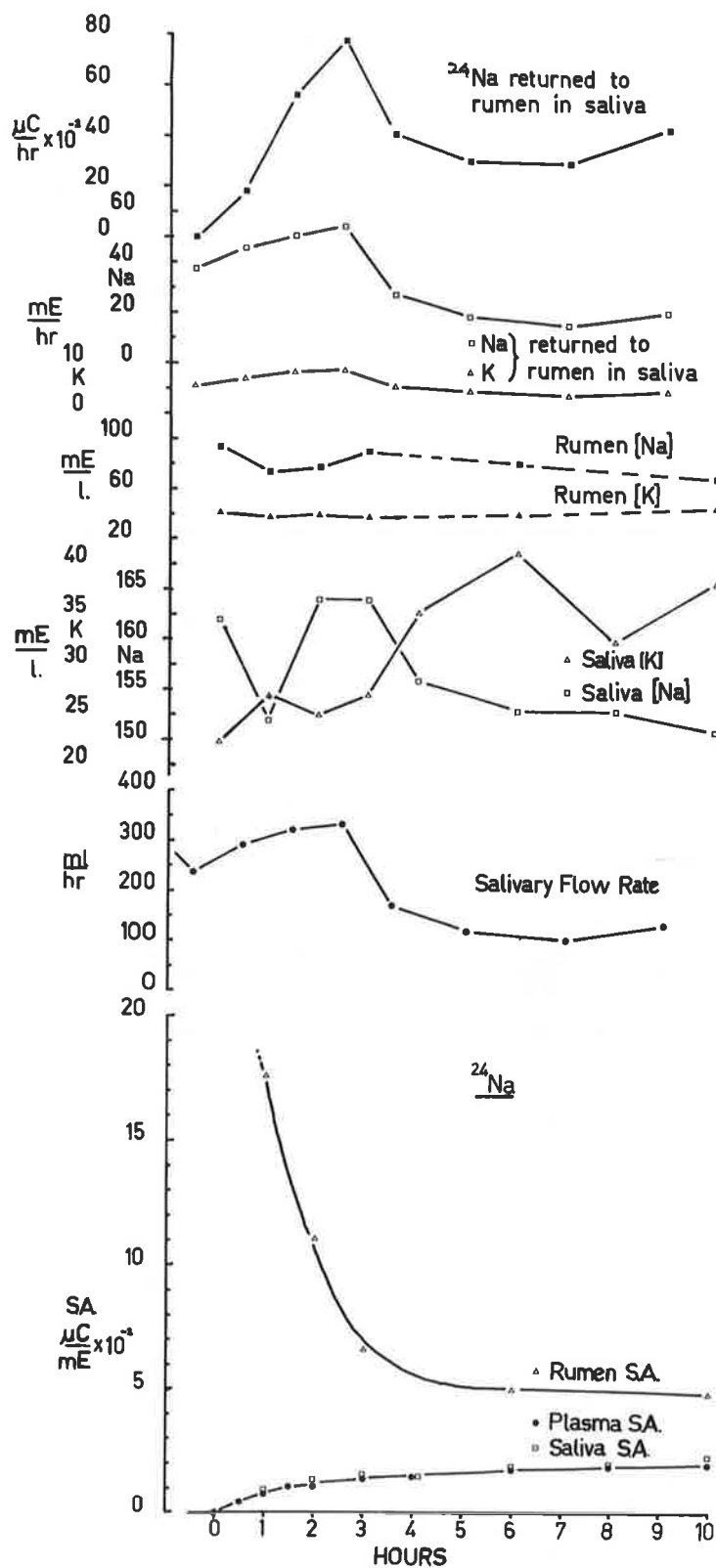


Fig. 30. Illustration of alterations in saliva flow, and quantities of Na,  $^{24}\text{Na}$  and K returned to the rumen in the saliva after addition of  $^{24}\text{Na}$  to the rumen. Plasma and saliva uptake and rumen disappearance curves for  $^{24}\text{Na}$  are illustrated.





returned to the rumen there was no alteration in the equilibration time (Fig. 28).

These results were inconclusive since the TOH may not have been moving into the blood via the rumen wall but from more highly permeable sites in the lower gut to which rumen fluid had been displaced. However, during the first hour of measurement of saliva flow when no fluid was entering the rumen through the oesophagus and thus much less rumen fluid than normal should be leaving the rumen, the rise in blood TOH, S.A. was unaffected.

(b)  $^{42}\text{K}$  and  $^{24}\text{Na}$  uptakes from the rumen

These studies were undertaken to determine what effects, if any, the addition of 400 m-equiv of K to the rumen had on the rates of uptake of  $^{42}\text{K}$  and  $^{24}\text{Na}$  from the rumen.

Fig. 20, 21, 29, 30 and 31 illustrate typical plasma uptake and rumen disappearance curves, with and without supplements of K and in K depleted sheep. These figures show the difficulty of visual comparison of uptakes of K since when K was added to the rumen the rumen  $^{42}\text{K}$  S.A. was reduced. There were too few experiments to make a statistical analysis of the curves. A simple and reasonably effective method of comparison was therefore employed. The ratios of rumen and plasma S.A.

Fig. 31. Plasma and rumen disappearance curves for  
TOH and  $^{42}\text{K}$  following the addition of TOH and  
and  $^{42}\text{K}$  to the rumen of K depleted sheep.

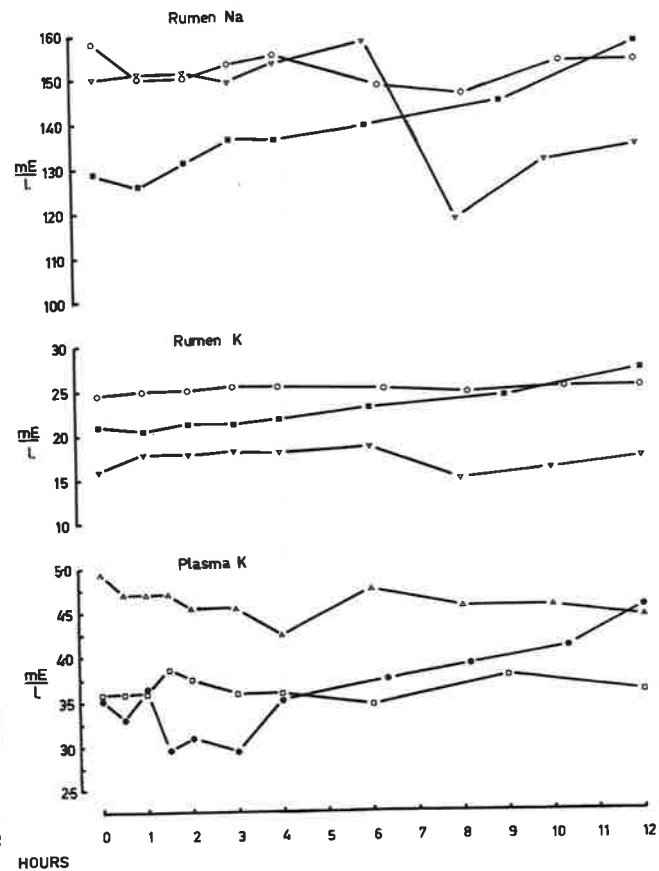
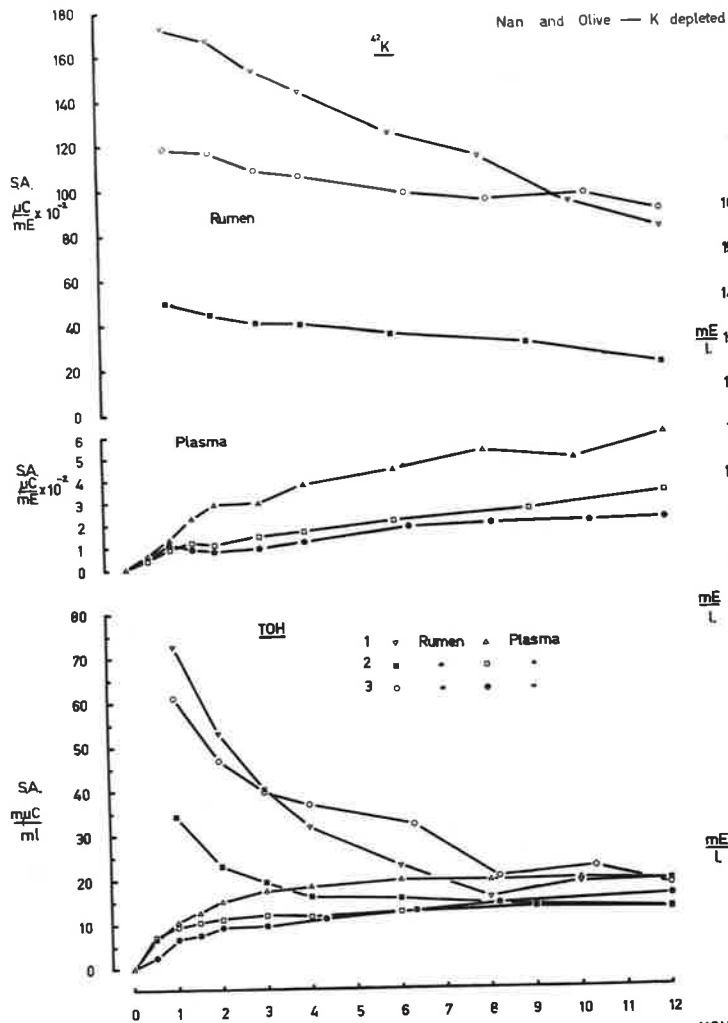
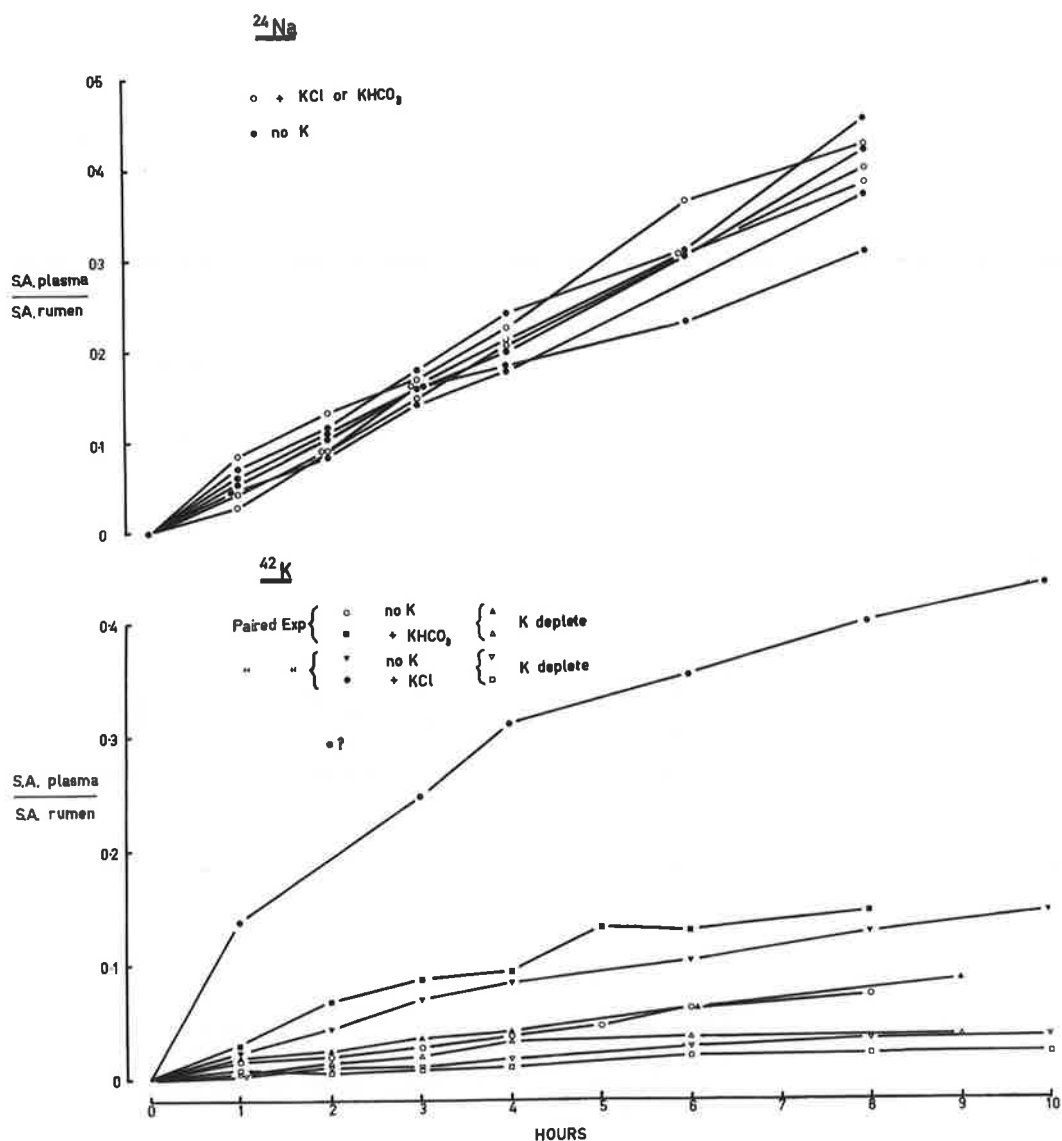


Fig. 32. Comparison of the changes in rumen : plasma ratios of S.A. of  $^{42}\text{K}$  or  $^{24}\text{Na}$  with and without the addition of 400 m-equiv to the rumen of normal sheep or in K depleted sheep.



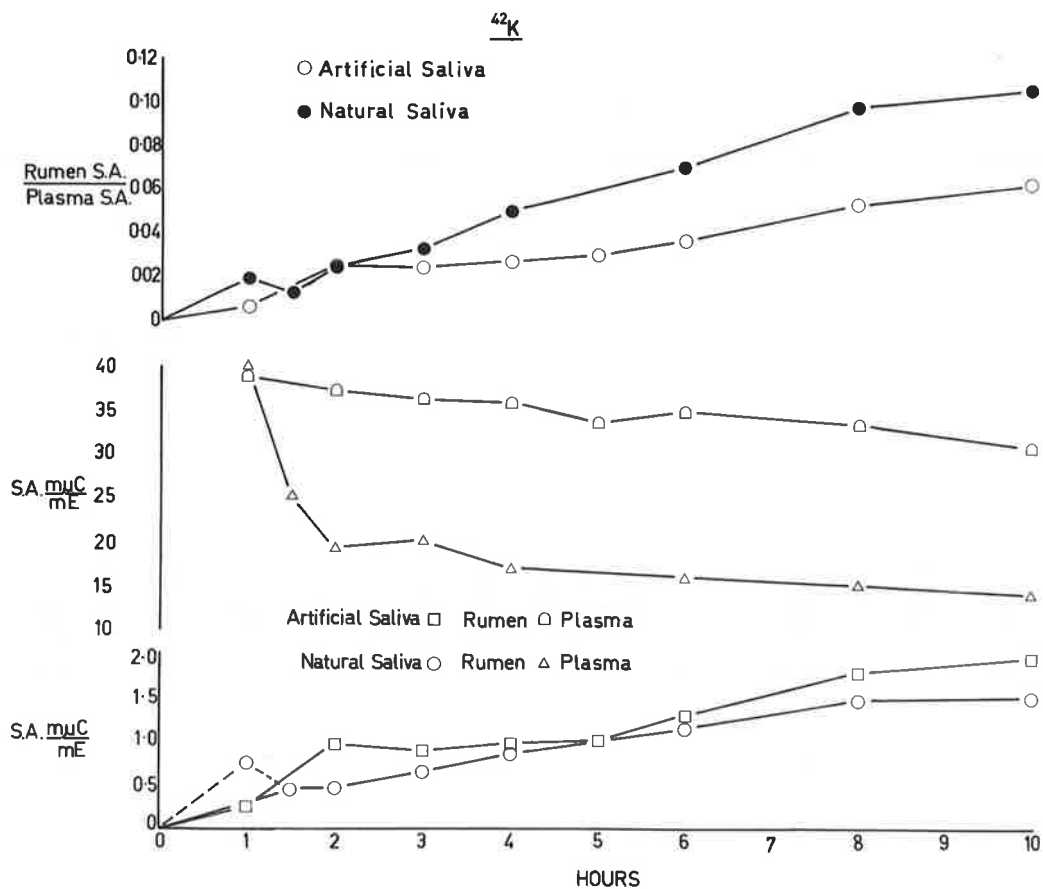
were plotted against time to give an indication of the equilibration rate.

Potassium uptake was increased by the addition of K to the rumen though if the paired animals are ignored the rate was significantly faster in only 1 of the 4 experiments (Fig. 32). In K depleted animals (Fig. 31) the "equilibration rates" were usually reduced below normal indicating a slower rate of shift of  $^{42}\text{K}$  between rumen and plasma (Fig. 32). No particular difference in the equilibration rate of  $^{24}\text{Na}$  could be observed regardless of the rumen [K] (Fig. 32).

The alterations in the "equilibration rate" of  $^{42}\text{K}$  produced by the addition of K to the rumen were not due to alterations in either the  $^{42}\text{K}$  S.A. of the saliva or the salivary flow rate (Fig. 29). Following the addition of K to the rumen the contribution made by the saliva to the total amount of K in the rumen was very small. However, the quantity of Na returned to the rumen in the saliva was quite significant though again in terms of the amount of  $^{24}\text{Na}$  quite small initially, but rising to quite substantial quantities later (Fig. 30).

Because the relationship between log rumen [K] and time often tended to be linear, it seemed that some idea of the rate of K movement from blood to rumen through the rumen wall would be useful. As with the TOH experiments, the natural saliva was

Fig. 33. Entry of  $^{42}\text{K}$  into the rumen from the blood during natural salivary input or replacement of saliva with artificial saliva.





replaced with artificial saliva. The "equilibration rate" of  $^{42}\text{K}$  in these experiments was approximately half as fast as during normal salivary flow so there was a small but significant quantity of K entering the rumen across the rumen wall or by splashback from the omasum (Fig. 33). Apparently both saliva and transepithelial transport account for shifts of K into the rumen from the blood though the "rates of equilibration" indicate that uptake from the rumen is much faster.

(vi) Effect of hormones and pharmacological agents on renal handling of electrolytes

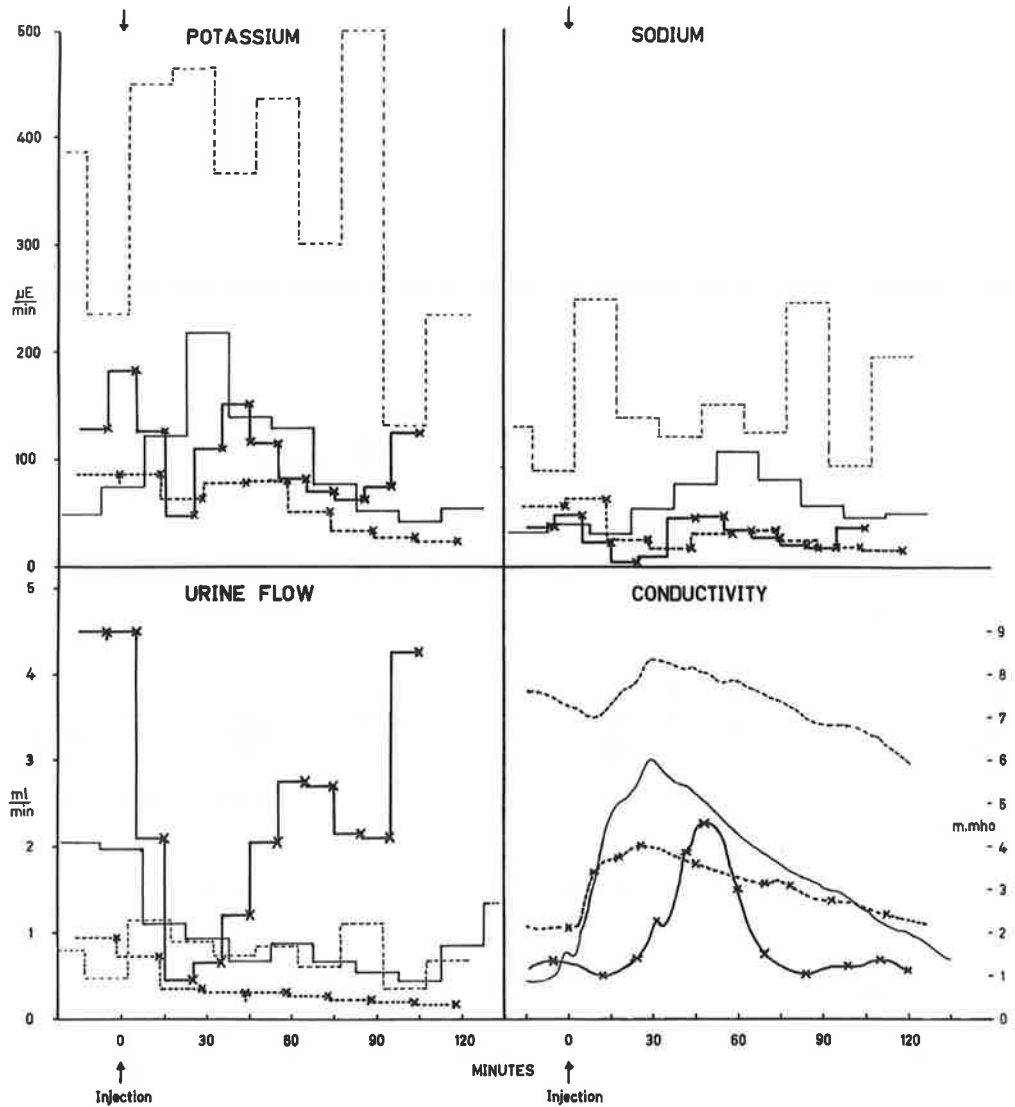
(a) Hormones

(1) Vasopressin (Pitressin)

Fig. 34 and 35 illustrate the effects of an infusion and injections of varying doses of vasopressin on the renal function of normal sheep fed 900 g of a mixture of oats and lucerne chaff/day (K intake of approx. 300 m-equiv/day). In all instances there were increases in the rate of water loss when the urine flow was below 1 to 1.5 ml/min the increase ranging from 50 to 300% of the initial rate while at rates greater than 1.5 ml/min vasopressin caused a reduction in flow rate.

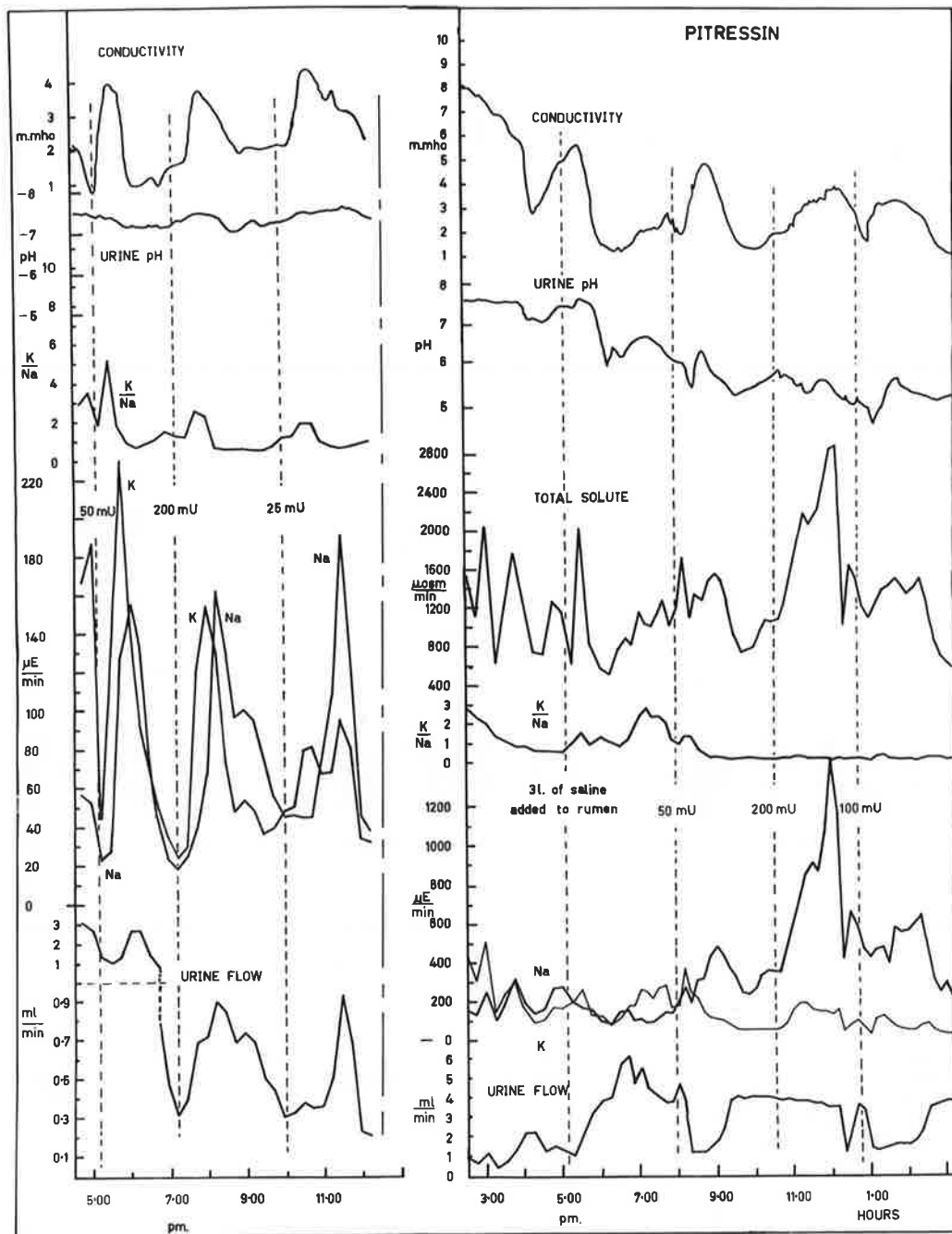
Fig. 34. Range of effects of Pitressin on renal excretion of water and electrolytes in sheep fed a mixture of oaten and lucerne chaff.

# EFFECTS OF PITRESSIN



A -x- 50 mU  
 B — 200 mU  
 C ... 100 mU  
 D -.- 50 mU

Fig. 35. Effects of Pitressin on renal excretion of water and electrolytes during normal flow and a saline diuresis. (Sheep fed a mixture of oaten and lucerne chaff). When urine flow exceeded 1.5 ml/min Pitressin reduced the flow rate but with a urine flow less than 1.0 ml/min it increased the rate. Pitressin increased the excretion of K and Na regardless of its effect on flow rate. During a saline diuresis the rate of Na excretion was increased more than that of K. In one instance shown the increase in Na excretion was such that for some time it prevented the normal reduction of a high rate of urine flow.

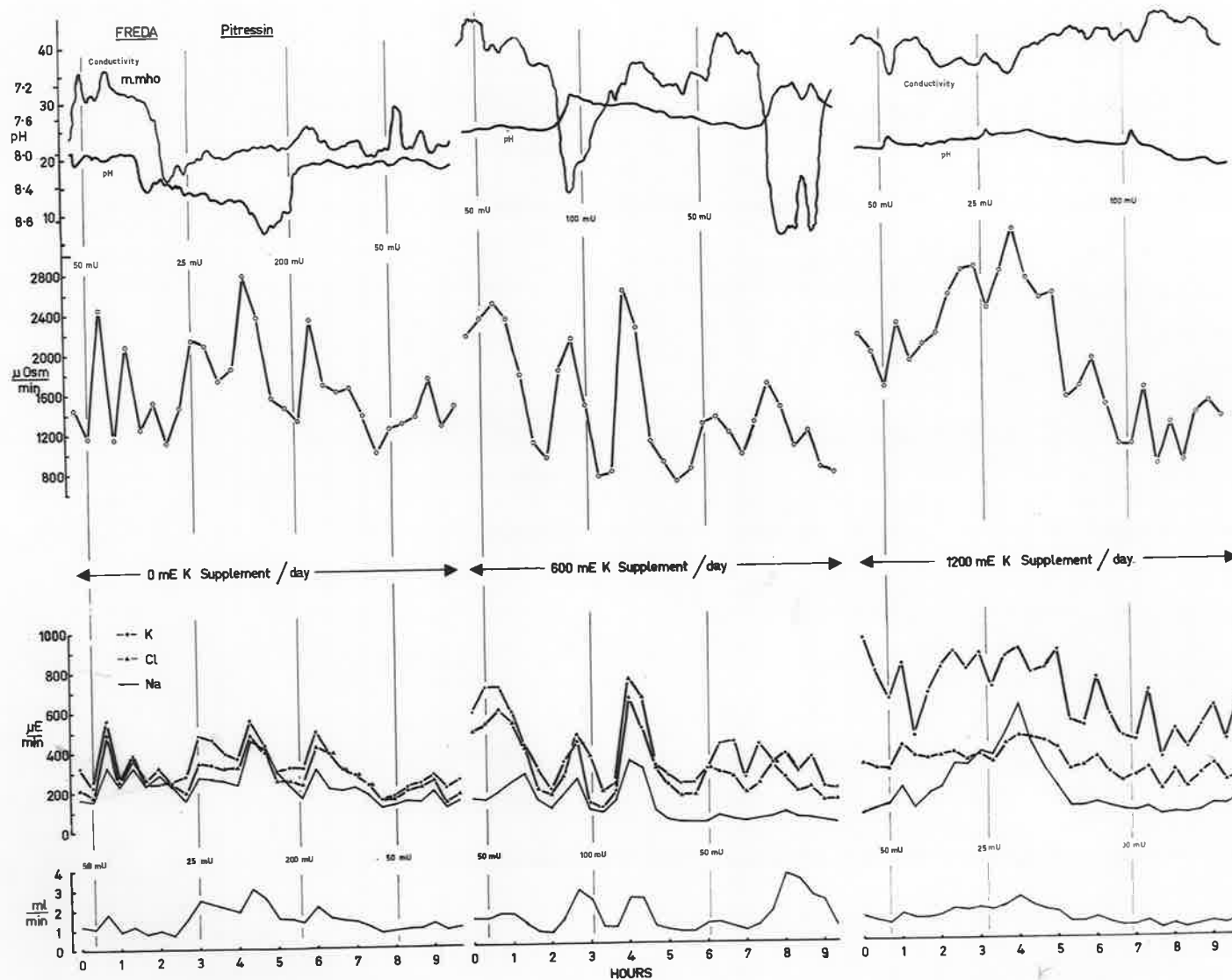


Regardless of the effect of Pitressin on flow rate, there were obvious increases in the rate of K excretion, ranging from 10 to 170  $\mu$ -equiv/min. The smallest dose of Pitressin used with normal sheep was 25 mU or 120 mU/hr and at this level the findings described above occurred (Table 10).

Potassium supplementation Sheep F responded to Pitressin in the normal manner when eating chaff containing no additional K (Fig. 36). When receiving a K supplement of 600 or 1200 m-equiv/day random fluctuations in water, K and Na output made it difficult to determine the effects of Pitressin. One regular response to Pitressin by sheep F with the K supplement of 1200 m-equiv/day, was a small rise in  $H^+$  excretion. This increase in  $H^+$  excretion (decrease in pH) was more pronounced in sheep S and W (Fig. 37,38) with both the daily K supplements of 600 and 1200 m-equiv.

Sheep S inherently drank more than sheep F and W and hence her urine output and flow rate was always higher. Thus Pitressin with a dose as low as 2 mU caused a reduction in flow, though it still at times increased the rates of excretion of K and Na. The urine flow rate of sheep W on a K supplement of 600 m-equiv/day was commonly below 1.0 ml/min and as in the example illustrated (Fig. 38) even when the flow rate was in

Fig. 36. Effects of Pitressin on the renal function of Sheep F, receiving a daily ration of 900 g of lucerne chaff supplemented with 0, 600 or 1200 m-equiv of K. While sheep F was not receiving any K supplement Pitressin produced noticeable increase in K, Na and Cl excretion. However, with K supplements of 600 and 1200 m-equiv/day random fluctuations in water, K, Na and Cl excretion made it difficult to determine if Pitressin had any effect. With a K supplement of 1200 m-equiv/day Pitressin did produce a short, small but detectable reduction of urine pH.





**Fig. 37.** Effects of Pitressin on the renal function of Sheep S, receiving a daily ration of 900 g of lucerne chaff supplemented with 600 or 1200 m-equiv of K.

Pitressin markedly depressed the rate of urine flow at both levels of K supplementations. The rate of K excretion was often increased despite the reduction in urine flow. This increase was more noticeable when the rate of K loss prior to an injection of Pitressin fell towards the end of the experiment. Urine pH was significantly reduced by Pitressin at both levels of K supplementation.

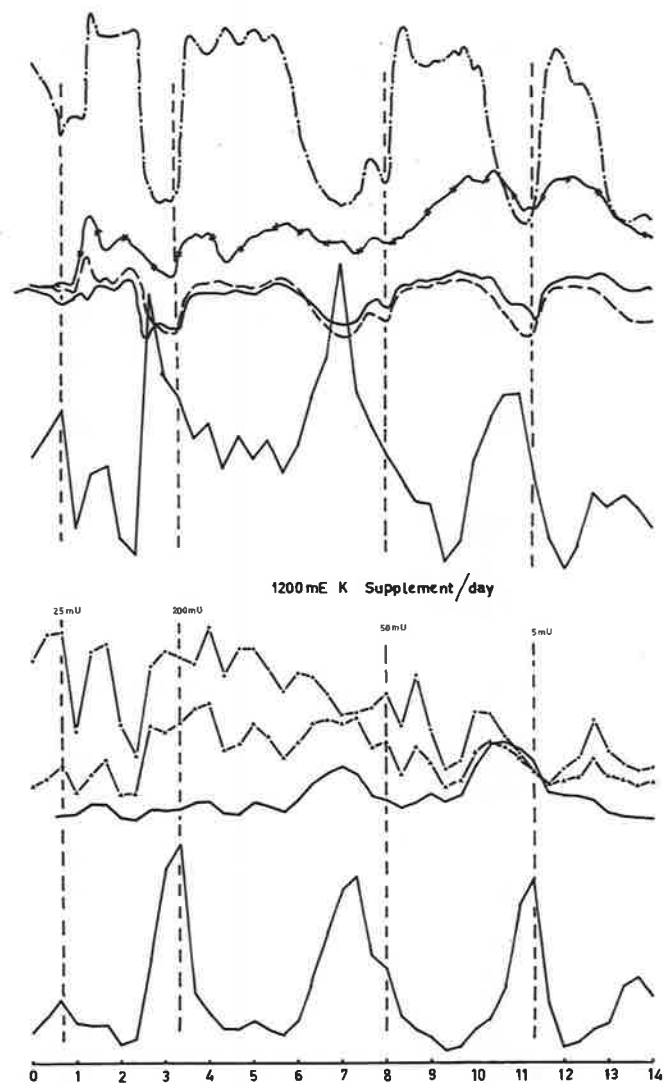
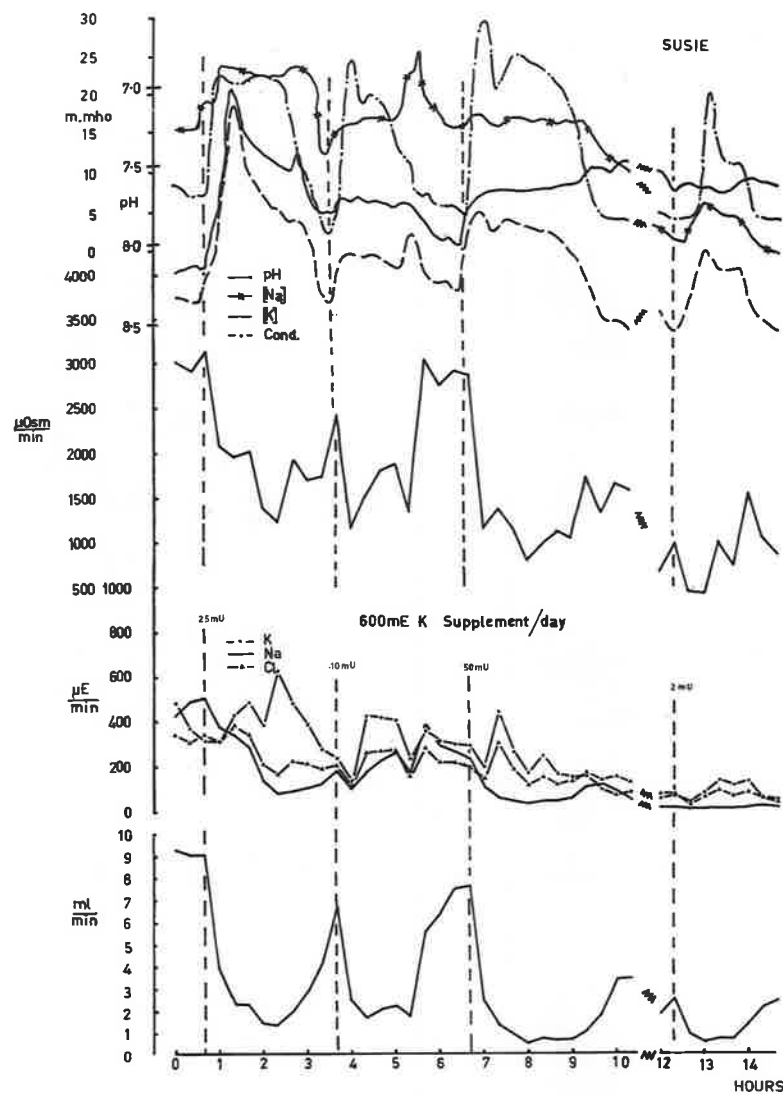


Fig. 30. Effects of Pitressin on the renal function of sheep W, receiving a daily ration of 900 g of lucerne chaff supplemented with 600 or 1200 m-equiv of K. With both K supplements Pitressin produced small but detectable increases in the rate of K, Cl and at times Na excretion. The effect on urine flow was irregular and was independent of the initial flow rate. A small reduction in urine pH occurred with a K supplement of 1200 m-equiv/day. (This fig. also illustrates the difficulty of standardising experiments. During the study of the effects of Pitressin or other hormones sheep were always fed 14-16 hr prior to the commencement of urine collection. It was hoped that by this time the rates of water and electrolyte loss would be stable and reflect the K intake. In this instance both the urine flow and rate of K excretion were less with a 1200 m-equiv K supplement than with a 600 m-equiv supplement cf. K excretion of sheep F and W (Fig. 36, 37).

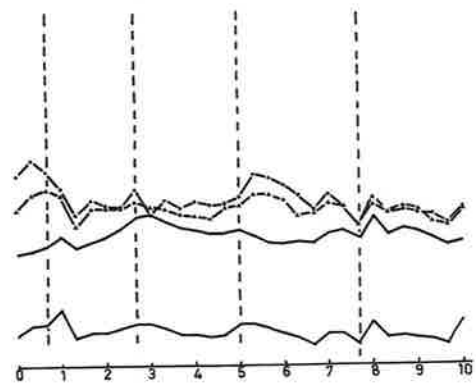
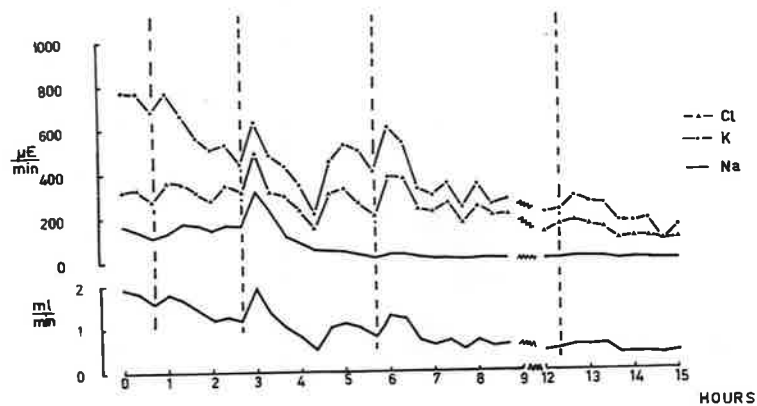
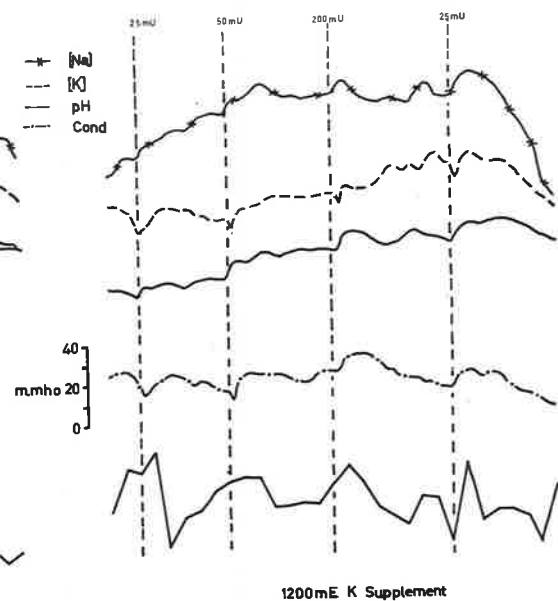
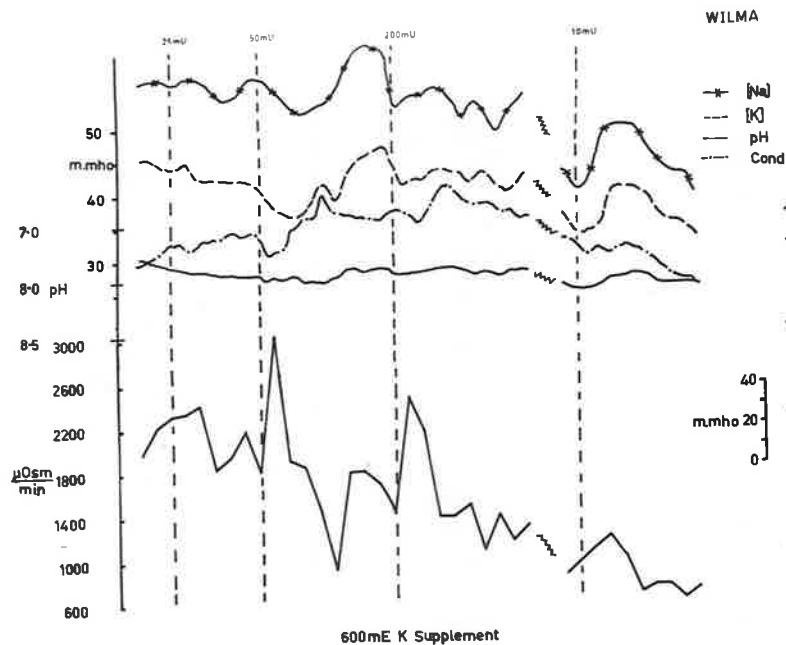


TABLE 10

RESPONSES TO VASOPRESSIN INJECTIONS (2-200 mU)

	Intake m-equiv K/day			
	8-53	250-700	1000-1250	1750-1950
Urine flow rate > 1.5 ml/min	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Urine flow rate < 1.5 ml/min	↓↓→	↑↑↑	↑	↑→
K excretory rate	↑→	↑↑↑	↑↑→	↑→
Na excretory rate	↑→	↑↑	↑→	↑→
Urine pH	↓→	→	↓→	↓↓
Minimum dose tested (mU)	5	25	5	2

↓ Decrease

↑ Increase

→ No change

excess of 1.0 ml/min Pitressin usually caused an increase in flow. With a K supplement of 1200 m-equiv/day the flow rate normally exceeded 1.0 ml/min though at 1.0 ml/min or less Pitressin caused a variable alteration in flow as well as slightly increasing the rates of excretion of K and Na (Fig. 38, Table 10).

(Fig. 39 is a photograph of the actual chart recording of changes of urine pH, conductivity,  $[K]$  and  $[Na]$  of sheep S following injections of Pitressin during K supplementation of 600 m-equiv/day).

Potassium depletion The effects of Pitressin on sheep depleted of K by feeding a diet low in K were also studied and typical responses are illustrated in Fig. 40. Pitressin had a small but variable effect on water output, which was independent of the initial flow rate. All injections of Pitressin caused a rise in urine  $[K]$  and  $[Na]$  and at times increased the rates of output of those electrolytes. There was a simultaneous fall in urine pH in some instances. Doses as low as 5 mU were effective in increasing K excretion though these rates were very low (Table 10).

In one sheep which showed no decrease in plasma  $[K]$  but whose muscle K content was lower than normal (5.1 m-equiv/100 g wet tissue cf. 6.9 m-equiv/100 g wet tissue) and which had a very low K excretion (below

Fig. 39. Photograph of chart recording of urine pH, conductivity, [K] and [Na] during the administration of Pitressin to sheep S. (+ 800 m-equiv K supplement).

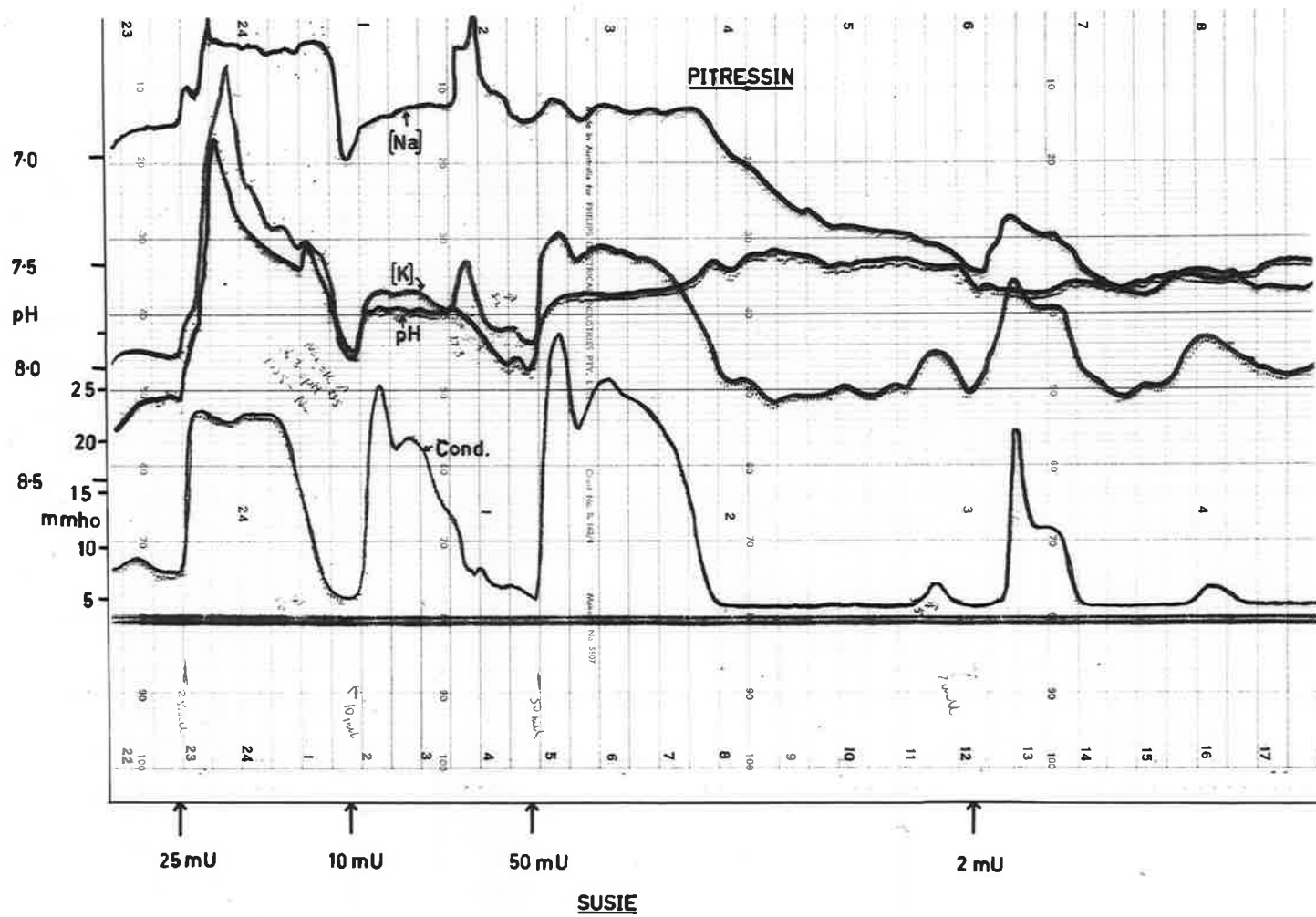
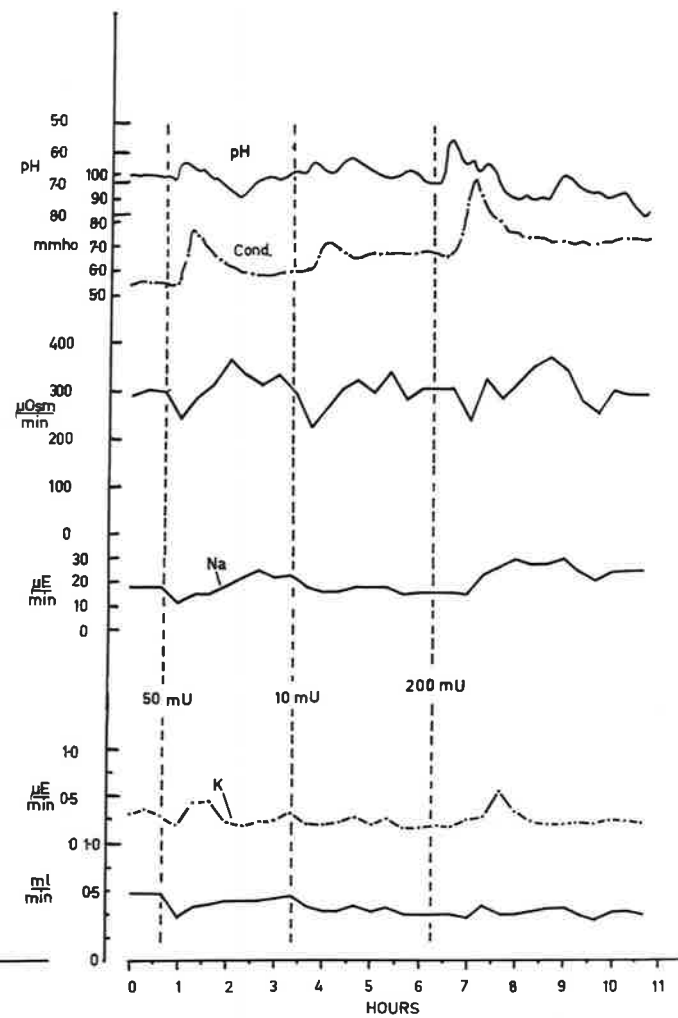
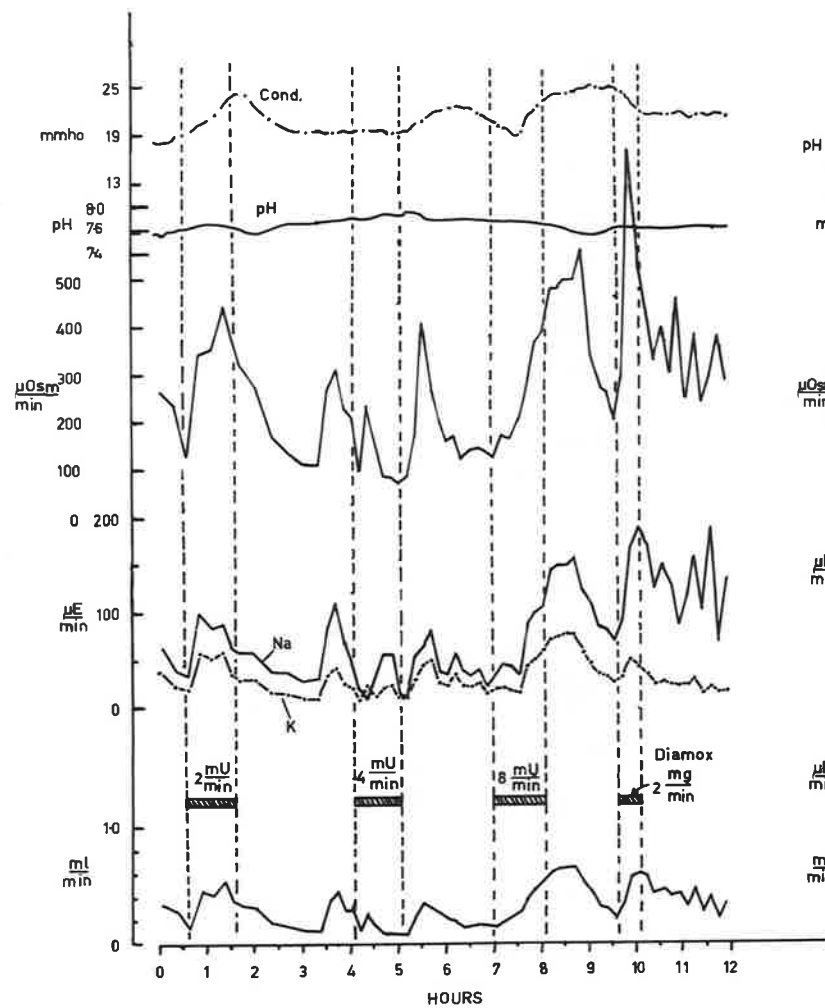




Fig. 40. Effects of I.V. administration or infusions of Pitressin on the renal excretion of water and electrolytes of sheep receiving a low K diet. The effects of an infusion of acetazolamide is also illustrated.



50  $\mu$ -equiv/min) infusions of Pitressin at 2 and 8 mU/min caused increases in urine flow of 0.35 and 0.50 ml/min respectively. At the same time K excretion increased by 40 and 63  $\mu$ -equiv/min while Na excretion increased by 65 and 115  $\mu$ -equiv/min respectively. The RPF and GFR of this sheep were also exceedingly low ranging from 85 to 115 ml/min for RPF and 13.5 to 26 ml/min for GFR over a 3 hr period.

After a long period of K depletion urinary [K] fell to below 0.75 m-equiv/l. and rates of excretion were less than 1.0  $\mu$ -equiv/min. Pitressin, as stated, did at times produce increases in these excretory rates but when compared in magnitude with the increases in normal sheep it is seen how insignificant they really are (Fig. 40).

## (2) Cortisol

Cortisol was infused intravenously into 4 sheep receiving varying supplements of K/day. Infusion rates ranged from 60 to 1000  $\mu$ g/hr.

No definite response of the kidney to any dose or at any particular K intake was observed. There was some indication that cortisol may have increased the rate of urine flow in some instances but increases were normally towards the end of a 2 to 3 hr infusion period and extended for 1 to 2 hr beyond the completion of infusions. This suggested a pure ECF expansion

diuresis as the cortisol was infused with normal saline at a rate of 1 ml/min. As the responses to cortisol were so indefinite no figure of cortisol infusions has been included.

### (3) Aldosterone

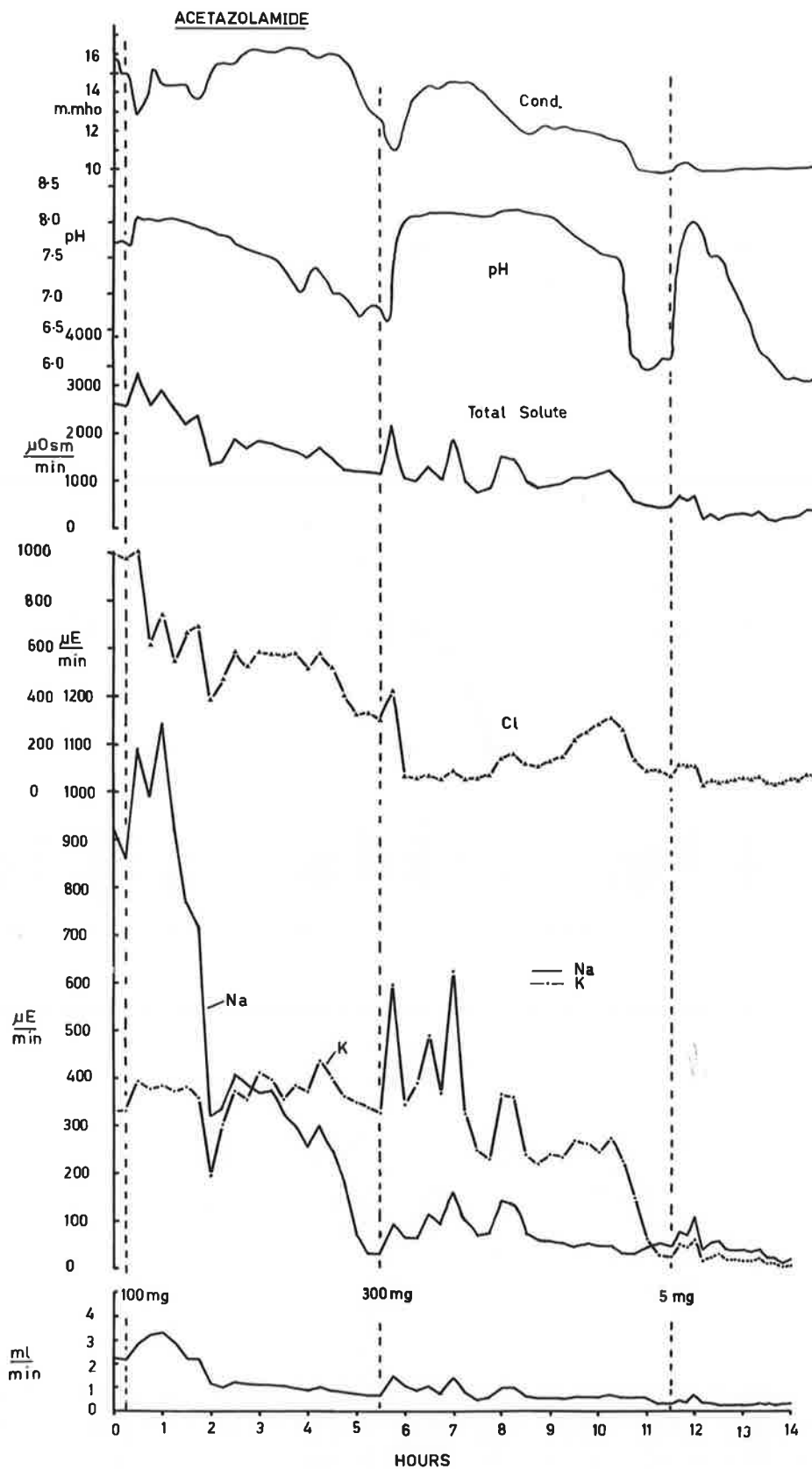
Doses of d-1 aldosterone (Aldocorten) ranging from 50 to 1000 µg/hr were infused intravenously into sheep receiving no K supplement or 1200 m-equiv of K/day. Apart from a clear decrease in urinary [Na] and Na excretion, and a small decrease in flow, aldosterone had no effect on other urinary parameters. There was definitely no alteration in the rate of K excretion.

As it was thought that the endogenous secretion of aldosterone may have been high in all sheep an attempt was made to suppress aldosterone secretion using Dexamethasone. Unfortunately a 2 hr infusion of Dexamethasone (2 mg/hr following a priming dose of 0.5 mg) had very similar effects to aldosterone itself causing a marked reduction in the rate of Na excretion and some reduction in the rate of urine flow. Therefore the plan of following a sustained infusion of Dexamethasone with aldosterone was abandoned.

### (b) Pharmacological agents

In the search for the mechanisms and control of the excretion of K by the kidney, various pharmacological substances

Fig. 41. Effects of the carbonic anhydrase inhibitor, acetazolamide, on the renal excretion of water and electrolytes in the normal sheep.



were injected or infused intravenously. These substances included 6-amino-n-hexoic acid (Epsilon-amino-n-caproic acid or EACA), 5-acetamide-1,3,4-thiadiazole-2-sulphonamide (acetazolamide - Diamox), 6-chloro-3,4-dihydro-3-(5-norbomen-2-yl)-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide (cyclothiazide), 2,3-dichlor-4-( $\alpha$ -ethylacryloyl)phenoxy acetic acid, (ethacrynic acid) and 4-chloro-N-(2-furylmethyl)-5-sulphamoyl anthranilic acid (frusemide - Lasix). Lysine the natural amino acid of which EACA is a structural analogue was also injected and infused.

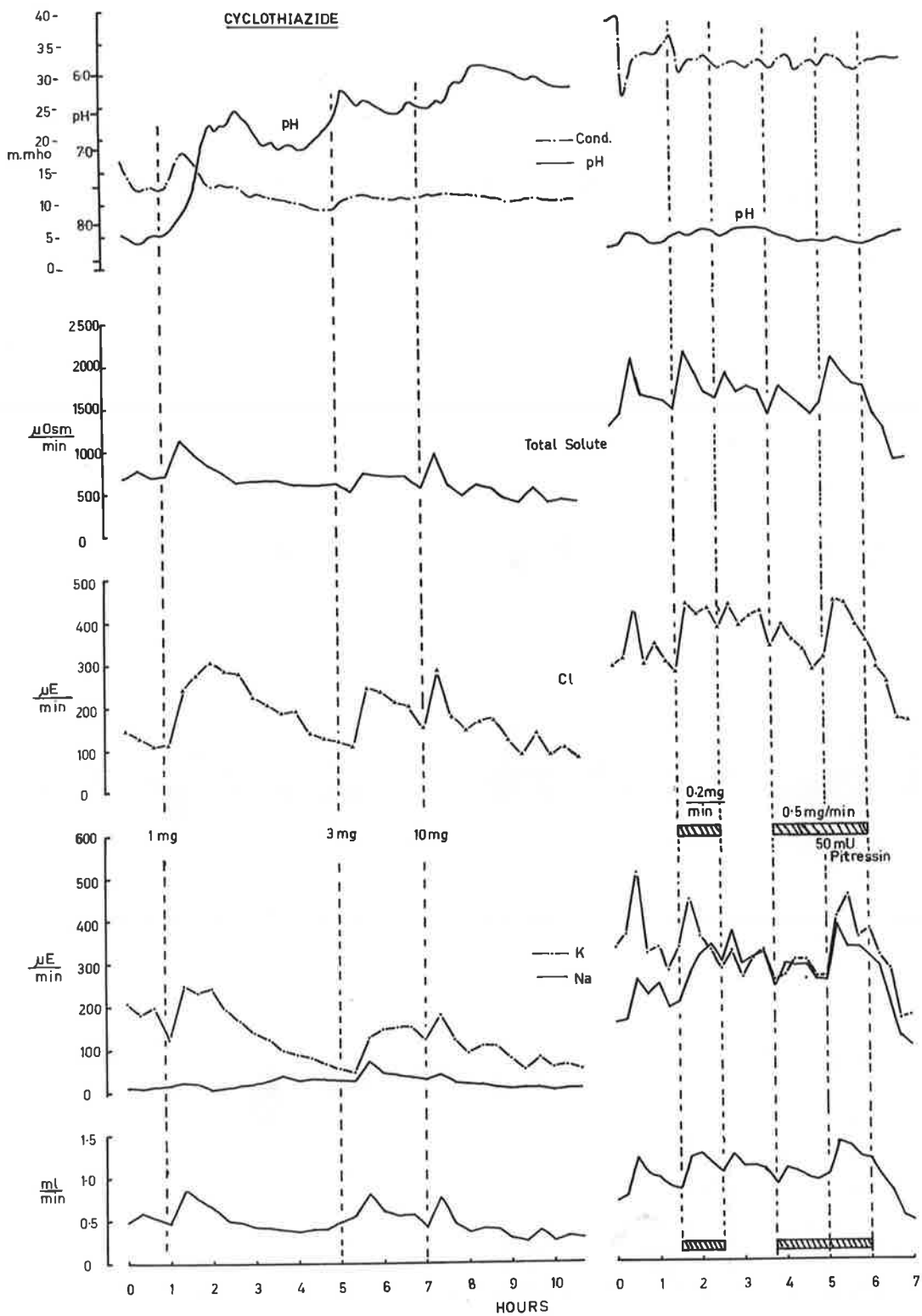
(1) Acetazolamide (Diamox)

Acetazolamide, a carbonic anhydrase inhibitor, administered in varying doses by I.V. injection produced the anticipated rise in urine pH to values of 8.0 to 8.4 and increases in the urinary excretion of water, K and Na. Urinary [Cl] and excretion was markedly reduced (Fig. 41).

The effects of acetazolamide on water and electrolyte output were to some extent independent of dose. Large doses often extended the duration of increased output more than the magnitude and greatly increased the period of high  $K^+$  excretion (Fig. 41).

Fig. 42. Alterations in the renal output of water and electrolytes produced by I.V. injections and infusions of cyclothiazide into the normal sheep. The effects of a single injection of vasopressin during an infusion of cyclothiazide is also shown.





In a "low K" sheep (Fig. 40) the rise in Na excretion produced by an infusion of acetazolamide at 2 mg/min exceeded that of K though the rate of K excretion still rose. Normally rises in the K excretion rate were greater than those of Na though this depended to some extent on the initial pattern of K, Na and H excretion.

### (2) Cyclothiazide

Cyclothiazide has been reported to be an effective diuretic in man, dog and rat causing substantial increases in the rate of water, K and Na excretion with the Na loss exceeding that of K. In sheep this compound produced only minor changes in urinary excretion (Fig. 42). There were small increases in the urinary losses of water, K, Na and Cl which were partly independent of the size of doses used, the larger doses only extending the period of increased loss. Urinary pH was usually unaffected by cyclothiazide.

### (3) Ethacrynic acid

Ethacrynic acid whether given by infusion or injection produced an exceedingly large urinary diuresis in all sheep. This diuresis was predominantly due to a large increase in Na excretion though there were significant increases in the rate of K excretion. Urine flow rates reached levels as high as

Fig. 43. Effect of I.V. injections or an infusion of  
ethacrynic acid on the renal function of normal  
sheep.

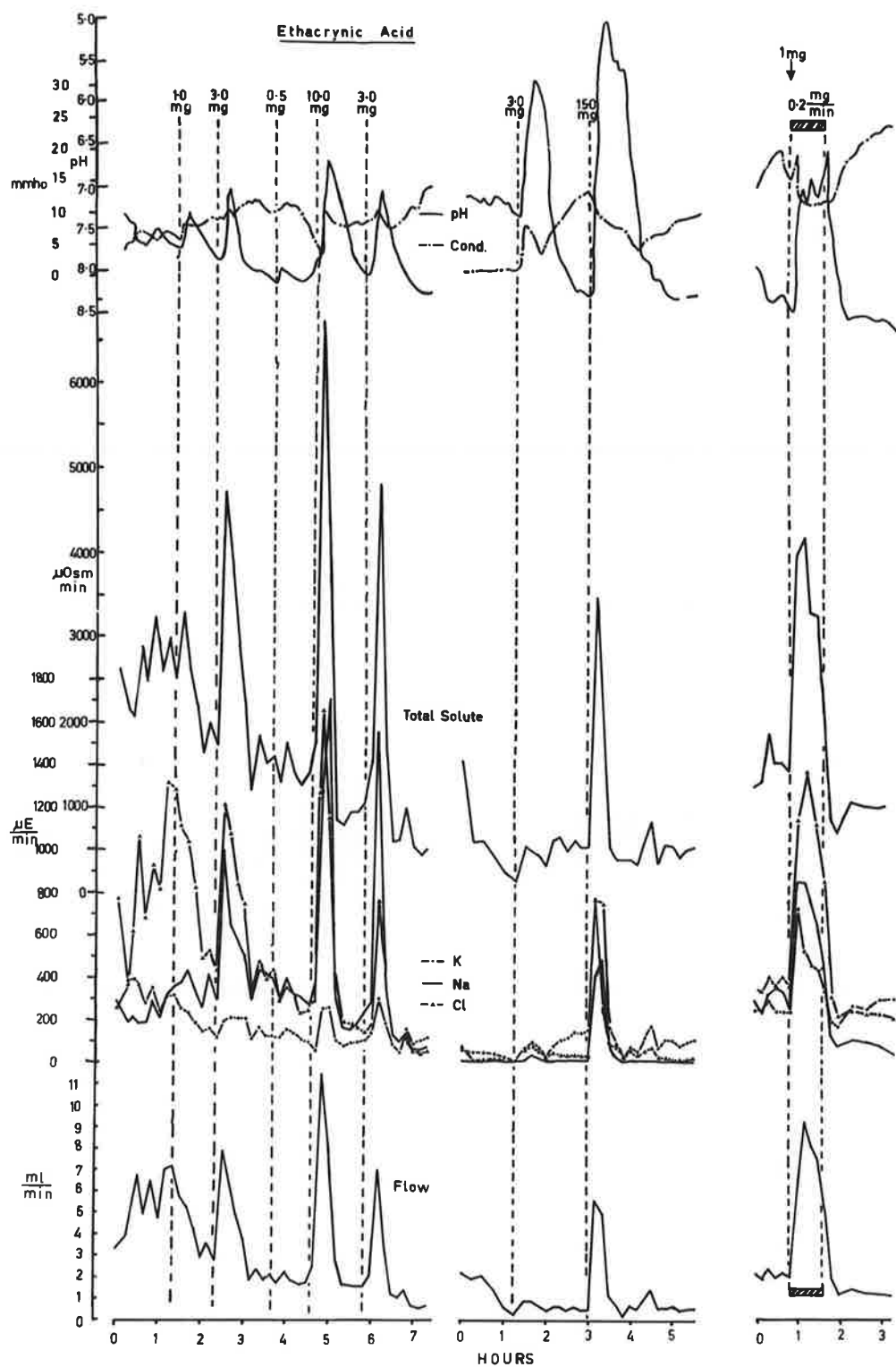
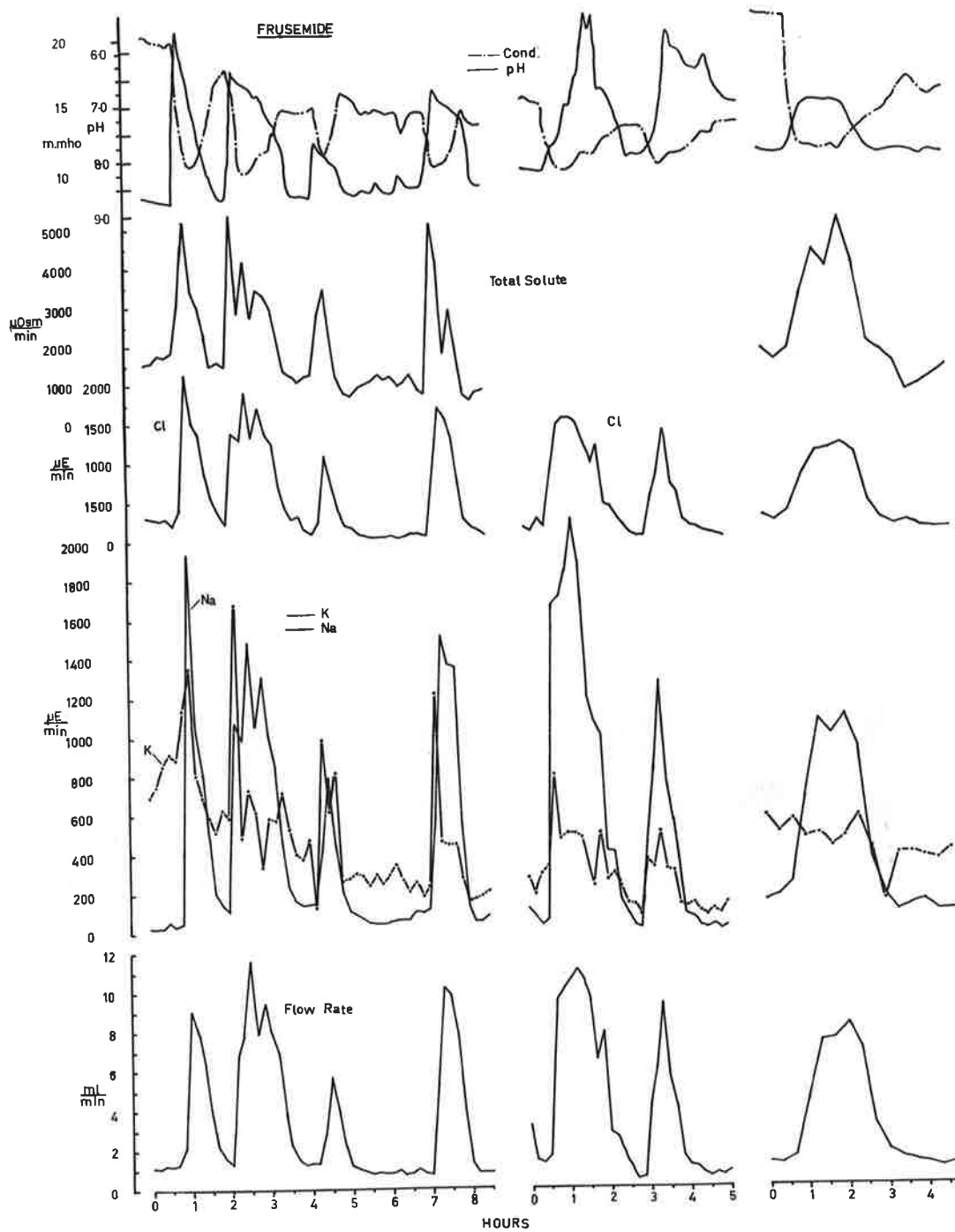


Fig. 44. Effect of I.V. injections or an infusion of frusemide on the renal excretion of water and electrolyte of normal sheep.




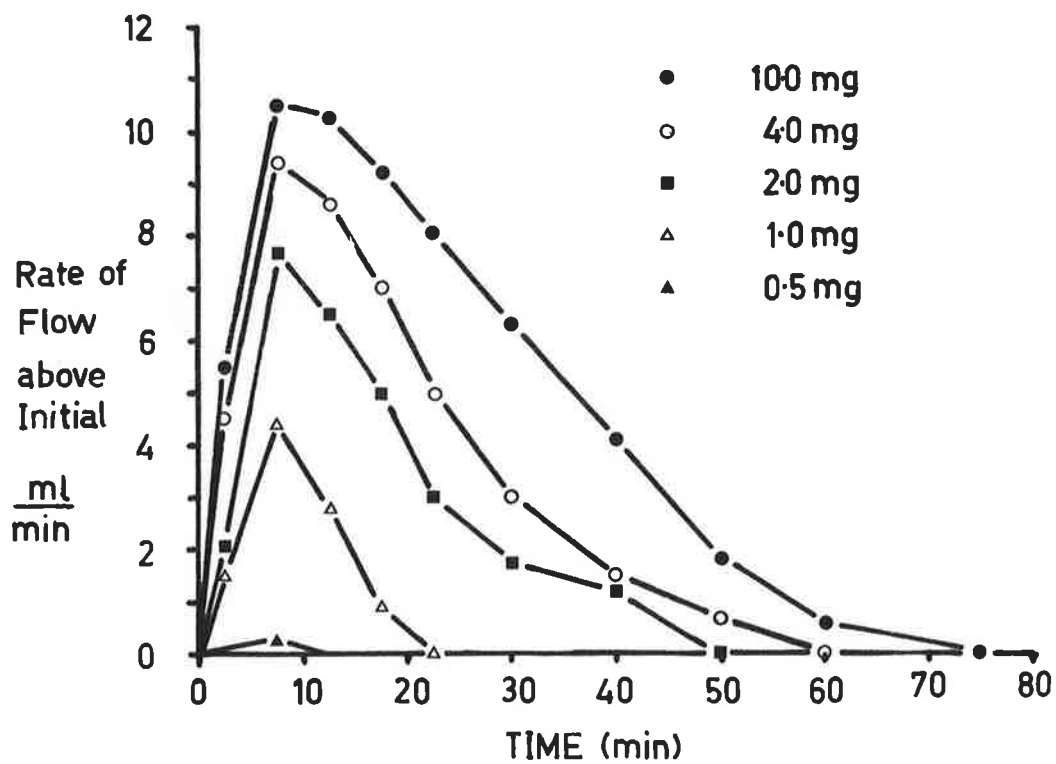
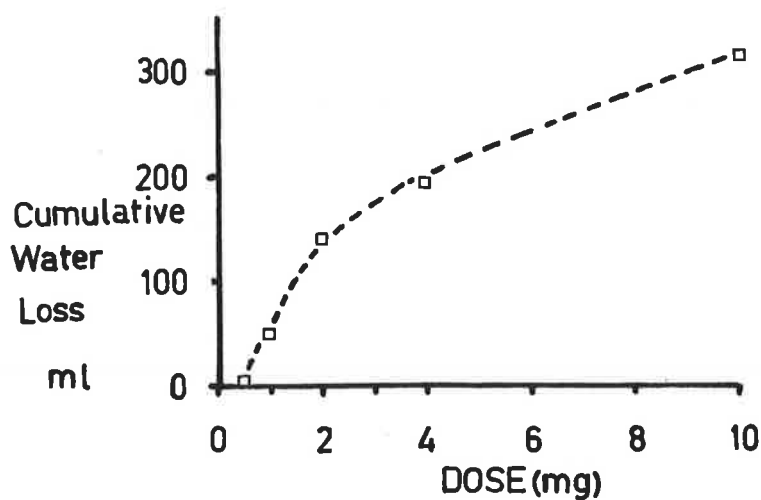
↑ 2mg    ↑ 10mg    ↑ 1mg    ↑ 0.5mg    ↑ 4mg    ↑ 40mg    ↑ 20mg     0.1mg/min

Fig. 45. Dose-response curves relating renal water loss to size of dose of frusemide.





10 ml/min with rates of excretion of Na in the order of 1000 to 1400  $\mu$ -equiv/min (Fig. 43). These rates of excretion were attained within 20 to 40 min of an injection and 20 to 60 min from the beginning of an infusion. However, during infusions the high rates attained initially were not maintained and fell slowly throughout (Fig. 43). This decrease in the natriuresis was presumably associated with a decline in RBF and GFR following an initial rise. There was a marked fall in urinary pH to values as low as 4.9 units following the administration of ethacrynic acid.

The dose response to ethacrynic acid was not determined though as with acetazolamide and cyclothiazide, increasing the dose beyond 10 mg did not increase the magnitude of the natriuresis but only extended its duration.

#### (4) Frusemide

Frusemide produced in sheep a large natriuresis very similar to that found with ethacrynic acid. Other effects were also very similar to those of ethacrynic acid though the maximum diuresis attained was slightly larger with measured water output reaching rates of 12 ml/min and measured maximum rates of Na and K excretion in the order of 2250 and 1400  $\mu$ -equiv/min respectively (Fig. 44).

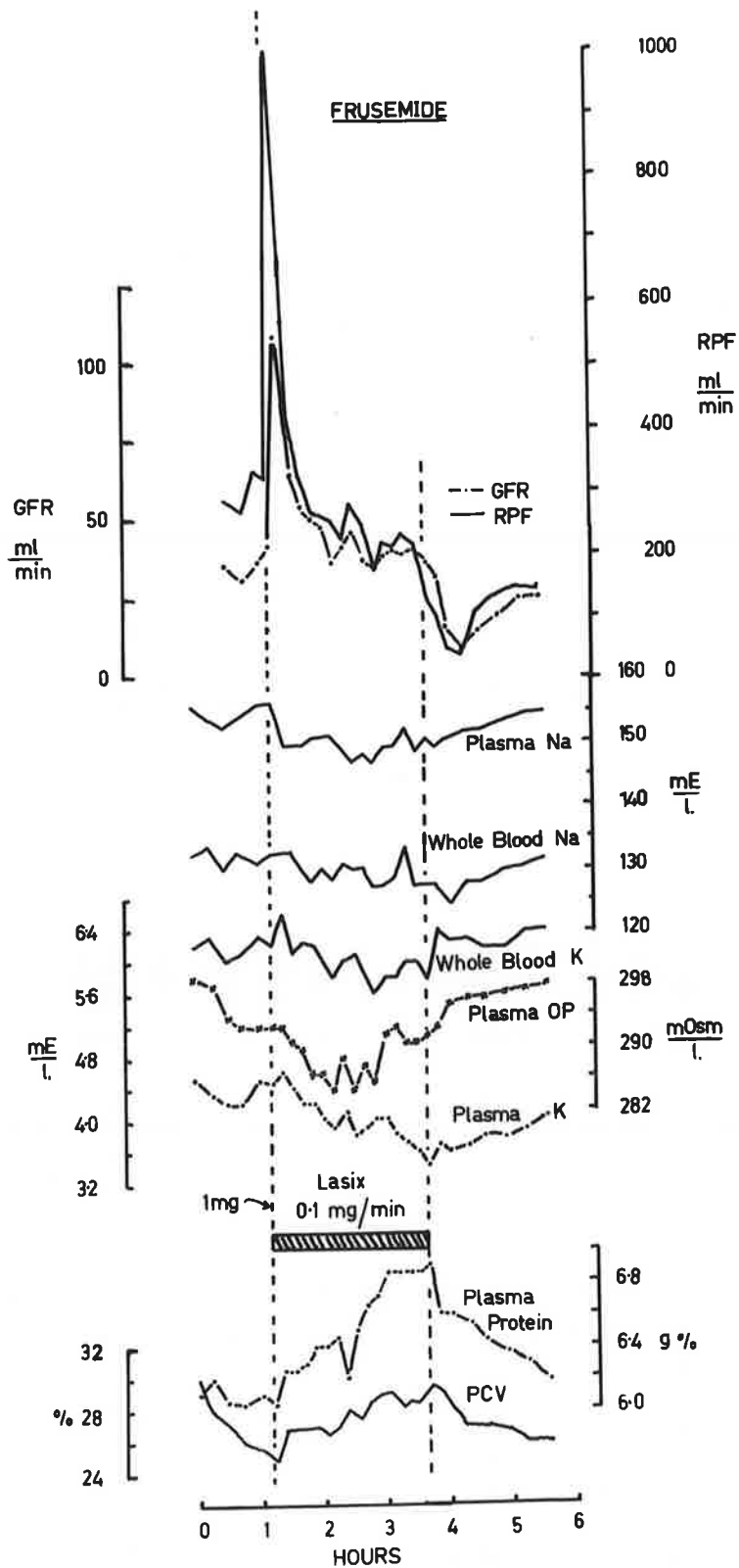
Doses of frusemide above 5 mg I.V. did not increase the magnitude of the diuresis but extended the period of natriuresis. Fig. 45 illustrates a rough dose-response pattern and it can be seen that the kidney is responsive to smaller doses of frusemide than ethacrynic acid.

Like ethacrynic acid, frusemide produced a large increase in  $H^+$  secretion, urinary acidity falling to levels as low as pH 5.5. This fall in pH was associated with a rise in Cl excretion, the rates of excretion of Cl being the same or slightly greater than those of Na.

RpF and GFR both showed small increases in the first 40 to 60 min of an infusion but then fell to preinfusion levels (Fig. 46).

By its alterations of renal function, frusemide produced several changes in whole blood and plasma electrolyte concentrations and volumes. It caused a rise in PCV, Hb, and plasma protein, with a decline in plasma and whole blood [K] and [Na] (Fig. 46). Infusions of 0.1 mg/min for 2 hr reduced plasma [K] and [Na] by as much as 1.5 m-equiv/l. and 20 m-equiv/l. respectively. At the end of an infusion it took from 2 to 5 hr for plasma concentrations to return to normal.

Fig. 46. Alterations in RPF, GFR, plasma and whole blood electrolyte concentrations, PCV, Hb and plasma protein concentrations produced by an infusion of frusemide.



(5) Epsilon Amino-Caproic Acid and L-lysine

Both these substances have been reported (see discussion) to produce a large kaliuresis and decrease in plasma [K] in man and dog. They were infused into sheep in an attempt to produce similar results. It was also possible that they would lower the cellular [K] (of the kidney in particular) and thus allow the study of the effects of the hormones, vasopressin, aldosterone and cortisol in such a situation.

Fig. 47 illustrates a typical response pattern to infusion of EACA on the blood and plasma electrolytes and the renal function of sheep D. Contrary to the findings in dog and man there was no large urinary kaliuresis. The Na excretion rate rose sharply during the first hr of infusion before rapidly declining to preinfusion levels. There was, however, a very large water diuresis which reached a peak of 10 ml/min at between 1 and 2 hr before declining slowly until 1½ hr after the infusion ceased. The peak of the diuresis did not correspond with the peak of Na excretion which furthermore did not correspond with a large initial rise in RPF and GFR. RPF attained a rate of 1200 ml/min and GFR reached 175 ml/min before they both fell sharply to below preinfusion rates. The RPF continued to decline up to 3 hr after the end of the infusion to a minimum of 120 ml/min. Urine pH and

conductivity fell during the infusion as did the  $[K]$  and  $[Na]$ .

At the end of the infusion there was a sharp rise in Na excretion followed by a rise in K excretion rate a short time later.

Some remarkable changes in the plasma and whole blood were produced by infusions of EACA. PCV rose from 25 - 30% to a maximum of 44%, plasma protein rose from 5 - 6% to 6 - 7g% and blood Hb rose from 8 - 11% to 13 - 18 g%. Associated with these changes there was a decline in plasma  $[K]$  from 4 - 5 m-equiv/l. to as low as 2.8 m-equiv/l. The plasma  $[Na]$  rose from 135 - 145 m-equiv/l. to 145 - 155 m-equiv/l. and was still at these levels up to  $3\frac{1}{2}$  hr after the end of an infusion. Whole blood  $[K]$  rose by as much as 1.0 to 1.5 m-equiv/l. and these levels were maintained until 30 min after an infusion ended. At this time it began to decline, like the PCV, Hb and plasma protein values to preinfusion levels. While the whole blood  $[K]$  rose the whole blood  $[Na]$  fell from 140 - 150 m-equiv/l. to 130 - 136.5 m-equiv/l.

During an infusion of EACA there was a rise in pulse rate, from 60 - 70 beats/min to 115 - 120 beats/min at the end of the infusion. This continued to rise to 150 - 160/min for up to  $1\frac{1}{2}$  hr after the end of an infusion. Pulse rate then

fell rapidly to reattain the preinfusion values 3 to 4 hr from the completion of infusion. Simultaneously with the rise in pulse rate there was a rise in respiration rate from 45 - 55/min to a maximum of 120 .. 125/min.

It seemed possible that increased heart and respiration rates could have been due to fever caused by pyrogens in the infusion mixture, but rectal temperatures showed little change. Solutions for infusions were prepared using sterile water and flasks and filtering the final solution through a sterilised Zeitz filter. Pyrogen tests on these solutions using rabbits showed no pyrogenic contaminants.

As with EACA, administration of the natural amino acid L-lysine, did not produce similar effects in sheep to those found in dog and rat, nor did it produce similar effects to EACA.

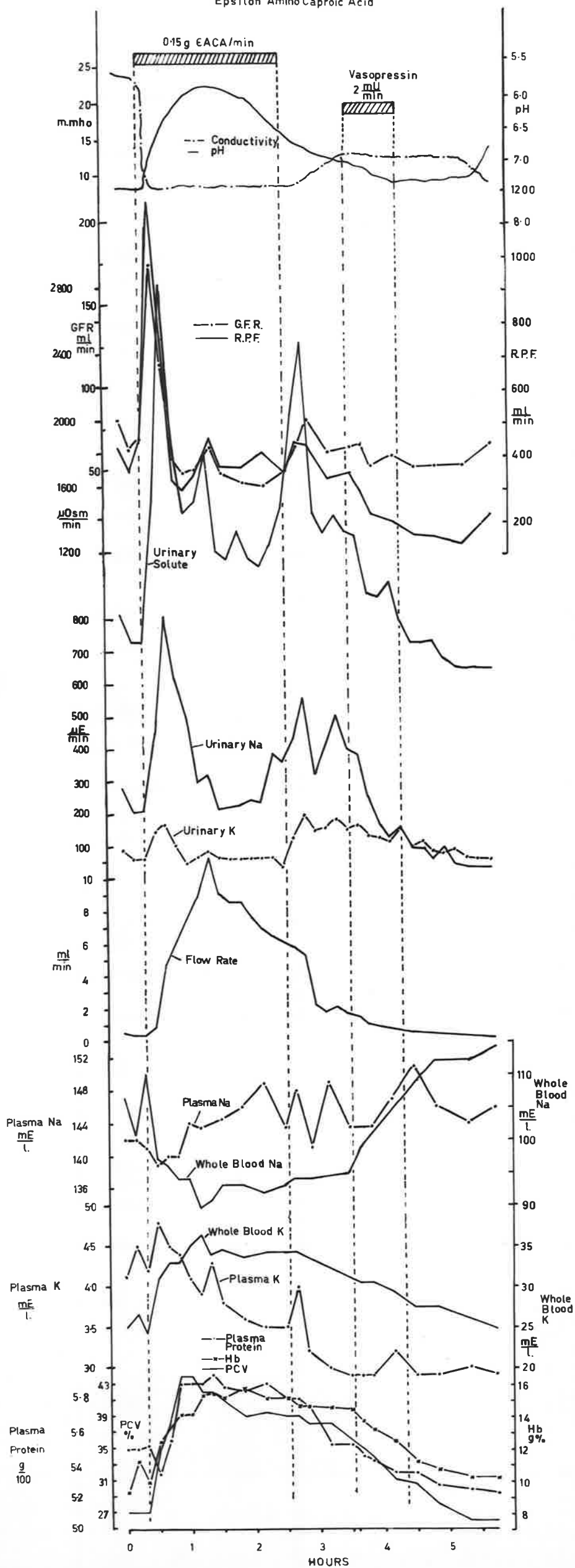
Lysine administered intravenously by a constant infusion of 4 g/hr or by successive hourly injections of 4g did not cause any diuresis but slightly reduced urine flow and K and Na excretion. Unlike EACA it had no effect on urine pH.

The effects of either an infusion or successive injections of L-lysine on plasma electrolyte concentration was variable with in one instance the [K] increasing and in another decreasing. No alterations of PCV or plasma protein concentration were observed. There were, however, small rises in

Fig. 47. Response of the normal sheep to infusions of epsilon-amino caproic acid - alterations in renal function and plasma and whole blood parameters.



# Epsilon AminoCaproic Acid



pulse and respiration rate but they were not of the order of those produced by EACA infusions.

(v) Combined effect of pharmacological agents and vasopressin on renal function

Attempts were made to discover whether the effects of Pitressin on electrolyte excretion were independent of its effects on flow and where the possible sites of action on electrolyte movement in the nephron might be. To do this, vasopressin was administered either by injection or infusion to sheep already receiving infusions of the various pharmacological agents described.

No experiments were performed on a background of acetazolamide. Two experiments were made with cyclothiazide, the results of one being shown in Fig. 42 where Pitressin produced a further increase in the rates of water  $K$ , Na and Cl. excretion. The increases were greater than those produced with the cyclothiazide alone. These effects were not due to alterations in RPF or GFR which were measured but are not shown in Fig. 42.

Fig. 48 illustrates the effect of injections of Pitressin during frusemide and ethacrynic acid infusions into different sheep. Comparing this figure with Fig. 35 or 37 (Section 6 (i)) the first obvious feature is the absence of a

sharp reduction in the relatively high rate of urine flow caused by an injection of Pitressin. Fig. 48-b shows that there was a slight increase in urine flow rate during the first 40 min after an injection of 200 mU of Pitressin, while in Fig. 48-a, c urine flow continued to decline in a similar manner to that found during any prolonged infusion of frusemide or ethacrynic acid. The second pertinent feature of Fig. 48 is the small but significant increase in the rate of K output and in two instances (Fig. 48-b,c) in Na, Cl and total solute excretion after an injection of Pitressin.

The urine conductivity shows a typical rise in Fig. 48-a, following the injection of Pitressin (cf. Fig. 35,37). This is not obvious in Fig. 13-b,c.

Pitressin was either infused or injected 6 times during frusemide and 4 times during ethacrynic acid infusions and in no instance was there any sharp decrease in urine flow, and on occasions the flow increased. Following all 10 injections or infusions there was an increased rate of K and at times Na excretion. In only 1 of 4 experiments during which RPF and GFR were measured did Pitressin cause an increase in RPF and GFR.

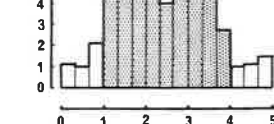
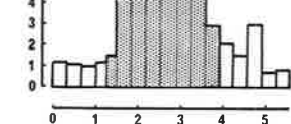
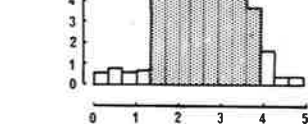
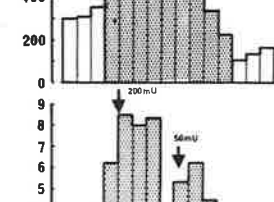
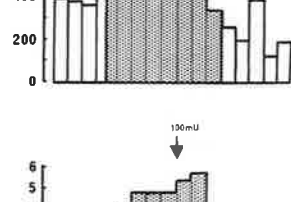
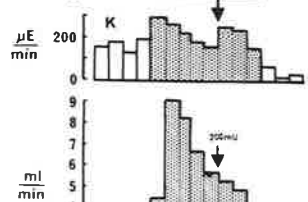
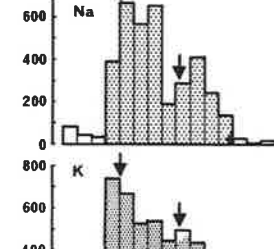
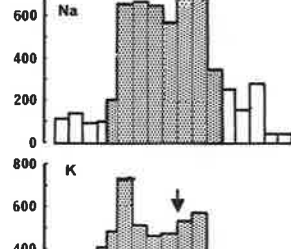
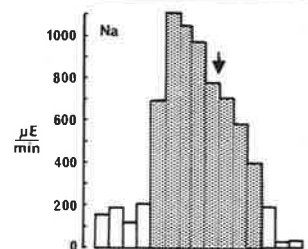
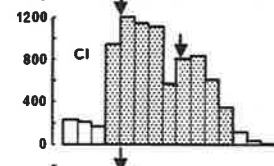
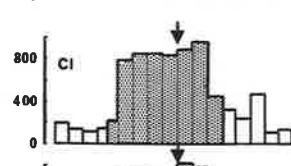
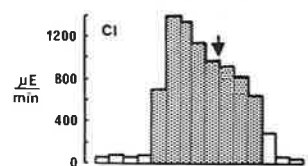
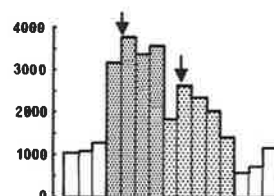
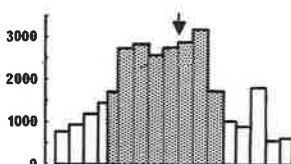
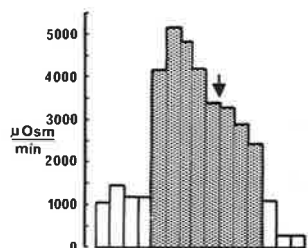
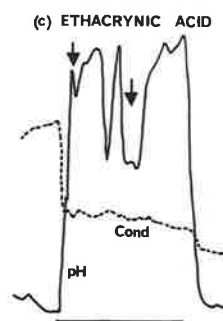
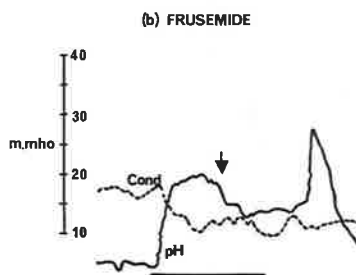
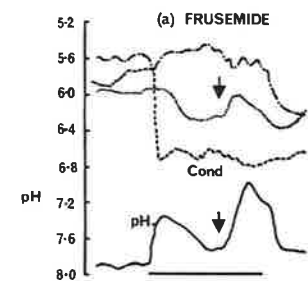
During an EACA infusion an injection of 50 mU of Pitressin (Fig. 49) caused a sharp transitory rise in urine

flow and the rate of K loss. However, this infusion of EACA was somewhat abnormal as regards the effects on renal function for the urine flow increased above preinfusion levels for only 1 hr though there was the usual reduction in K excretion. RPF and GFR were as in other experiments, reduced by the EACA infusion and the rate of Na excretion was also increased. Plasma and whole blood were altered in the expected fashion as described previously.

There were sharp transitory rises in the RPF and GFR following the injection of Pitressin. RPF rose by 300 ml/min and GFR by 35 ml/min. These rises corresponded with the changes in flow rate and K excretion though Na excretion was scarcely altered.

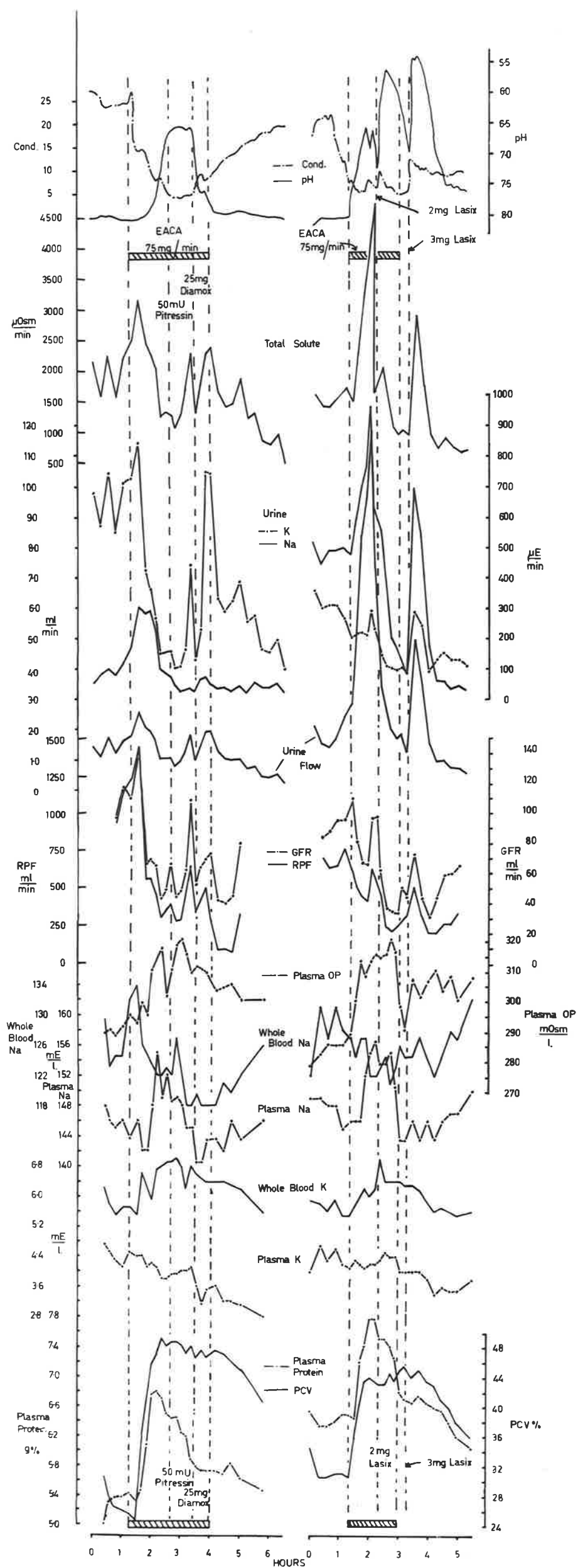
An injection of 25 mg of acetazolamide an hr after the injection of Pitressin produced a larger rise in urine flow and K excretion than did the Pitressin alone and there was also a small but significant rise in Na excretion. The acetazolamide was injected to examine changes in urine pH. There was a rapid rise in pH towards normal from the low value induced by the EACA. Like Pitressin, acetazolamide produced a rise in RPF and GFR.

Fig. 48. Effects of injections of vasopressin during infusions of frusemide or ethacrynic acid, on the renal excretion of water and electrolytes of normal sheep.



HOURS

Fig. 49. Effects of injections of Pitressin or Lasix during infusions of epsilon-amino caproic acid into the normal sheep - alterations in renal function.





An injection of 2 mg of frusemide during an EACA infusion caused no increase in urine flow beyond the high flow rate already present, nor was there any increase in Na and K excretion (Fig. 49). But there was a substantial rise in  $H^+$  excretion or a fall in urine pH down to pH 5.7. Thirty min after the end of the EACA infusion an injection of 3 mg of frusemide produced a normal response though not of the usual magnitude.

### 3. Summary of Results

Mechanisms which allow sheep to tolerate the ingestion of large quantities of K were examined over a range of K intakes. Body fluid volumes and electrolyte metabolism, as well as the control of K excretion by the kidney were investigated.

#### (1) Effects of alterations in K intake

##### (a) K supplementation

##### (i) Body fluids and electrolytes

A number of significant findings came from raising the K intake of Merino sheep from 450 to 2000 m-equiv/day by adding varying quantities of a mixture of K salts to a basic lucerne chaff diet. There was a linear relationship between the water intake, water turnover as measured using TOH, and the quantity of K ingested each day. The increase in water turnover varied between animals. Those with the highest water turnover on a basic lucerne ration increased water turnover proportionately more as the K intake was increased (the lowest water turnover increased 50% while the highest increased by 97%).

An increase in TBW and ECV resulted from the increased water turnover which occurred as the K intake was raised. Total body water reached values as high as 84% of the body weight. The body weight also rose as the K intake increased but much of this rise in weight was due to the water retention and rise in TBW. As a percentage of total body water, the extracellular volume was unchanged, showing that a large proportion of the water retained was in the extracellular fluid. Plasma volume did not alter as a result of increasing the K intake though the plasma protein concentration rose by 1g%.

When the results from all sheep were grouped there was no significant change in the plasma [K]. The plasma [Na] and OP decreased significantly when the level of K in the diet was raised. However, the total amount of Na in the extracellular fluid was unchanged since the extracellular volume rose.

(ii) Rumen and salivary electrolyte concentrations

Measurements of the concentrations of electrolytes in the rumen at three intervals during the day (before feeding, 4-6 and 8-10 hr after feeding) showed that there was a substantial rise in the [K] and OP and a fall in the [Na]

after eating. These changes still existed 8-10 hr later. Similar changes occurred after feeding during K supplementation though the concentrations were significantly different from those in normal sheep. Raising the K intake produced a significant rise in the rumen [K] at all three periods of the day while the [Na] was depressed. The resting rumen [K] rose as high as 118 m-equiv/l. while [Na] fell to as low as 24 m-equiv/l. This interchange of [K] and [Na] resulted in only a small rise in rumen osmotic pressure at any of the three sampling periods.

Salivary composition did not change for any of the three collection periods in the sheep on a basic lucerne chaff ration. As the K intake was raised, the [K] of the saliva rose while the [Na] fell. The composition of the saliva in the supplemented sheep, varied during the day. The Na:K ratio of the saliva in K supplemented sheep suggested that aldosterone secretion was increased by the addition of extra K to the diet.

#### (111) Urine and faecal concentrations and outputs

As expected the additional water intake which resulted from increasing the K intake produced a proportional increase in urine volume. Despite the rise in urine volume, there were rises in the concentration of urinary K at the lower

levels of K supplementation and the urinary output of K rose. At maximum levels of K supplementation the urinary [K] again fell but remained above presupplementation levels. Excretion of K in the urine was never less than 97% of the urinary plus faecal K loss with the result that there ~~were~~ insignificant changes in the quantity of K excreted in the faeces. As the K intake was raised and urine volume rose, the urinary [Na] tended to decrease though the total urinary excretion was unaltered. Sodium balances were often negative on the maximum K supplements or during water restriction. If Na intake in the water was included, Na balance as expressed in Table 3 was raised, but then Na loss in suint was not measured and hence this would have reduced the Na balance.

The increase in water turnover and urinary volume varied with individual animals. These differences could be related to the individual sheep's ability to concentrate the urine, particularly in respect to K. However, water restriction of one sheep showed that the ability of the kidney to increase the urinary [K] was not limited. The final volume of water drunk and urine voided was obviously resolved by the animal as a balance between drinking and concentrating the urine.

Faecal excretion was altered by increasing the K intake so that the wet faeces weight increased as the level of K supplementation rose. A large proportion of the rise in weight was due to an increase in free water though in some

instances the dry matter content also fell. The dry matter digestibility of the food was variable throughout, though in some instances digestibility fell slightly as the K intake rose.

(b) K depletion

Feeding a diet of low K content resulted in a slow loss in body weight. This was associated with slow decreases in total body water and extracellular fluid.

Only scanty information on the levels of rumen electrolytes was obtained though the [Na] was never found to be below 100 m-equiv/l, while the [K] fell to as low as 15 m-equiv/l. No measurements of salivary flow and composition were made.

Plasma electrolyte concentrations were altered by the low K diet but not as much as anticipated. The plasma [K] decreased to as low as 3.5 m-equiv/l, while the [Na] tended to rise, reaching values of 189 m-equiv/l. These values were not maintained with the plasma [K] varying from 3.5 to 5.3 m-equiv/l, and [Na] from 147 to 189 m-equiv/l.

The reason for the ability of the sheep to exist for much longer than expected while on the low K diet became apparent when urinary and faecal electrolyte excretion was measured. Despite maintenance of an average daily urine excretion of 450 to 750 ml, the urinary excretion of K was in

the order of 0.50 to 1.75 m-equiv/day. This was achieved by reduction in the urine [K] to levels as low as 1.15 m-equiv/l. The urinary Na loss of approximately 15 m-equiv/day was also low, despite the ready access of animals to a salt lick.

The faecal loss of K exceeded the urinary loss. Faecal K content ranged from 80 to 130 m-equiv/kg DM with a resultant faecal K loss in the range 8 to 13 m-equiv/day. These values are similar to those of sheep on a maximum K supplement. By achieving such low total K losses, both sheep were able to sustain small daily negative K balances of 4 to 6 m-equiv.

(1) Effects of eating or intraruminal loading of K.

(a) Rumen

After eating normal or K supplemented lucerne chaff there were several distinct patterns of change in the rumen electrolyte concentrations. The rumen [K] rose as high as 160 m-equiv/l. while the [Na] despite the addition of Na in the diet, fell. The fall in the [Na] was dependent on the pre-feeding concentration which in turn, as already described, depended on the level of K intake. A rise in rumen OP also occurred with feeding and on the maximum supplement the rumen OP was often in excess of the plasma OP at any time of day.

Once eating ceased, the rumen [K] began to fall rapidly (it also fell during drinking) though the total time required to reach prefeeding levels ranged from 12 to 18 hr. Increases in the rumen [Na] did not occur for some time after eating ceased though the prefeeding concentration was achieved at about the same time as that of K.

Alterations in the rumen concentrations of electrolytes in sheep with 400 m-equiv of  $\text{KHCO}_3$  or KCl added directly to the rumen were somewhat similar to those following feeding. Naturally there was a sharp rise in the rumen [K] which began to decline immediately. The rumen [Na] was slightly depressed by the addition of K though the magnitude of this depression depended on the initial [Na] - the higher the initial concentration the larger the depression. Rumen osmotic pressure rose after the addition of K and declined in a similar manner to the [K].

(b) Plasma

There were increases in the plasma [K], [Na] and osmotic pressure following eating. Plasma [K] rose by up to 24% while the [Na] rose by up to 7%. Maximum concentrations were achieved within 4-10 hr of the commencement of eating after which they slowly declined to prefeeding levels. All



the changes in plasma concentrations were independent of the level of K in the diet and in fact the largest changes occurred with no K supplement.

Surprisingly, the alterations in the concentration of plasma electrolyte following the addition of 400 m-equiv of K salts to the rumen were not as pronounced as those which followed eating. Alterations were irregular despite the fact that changes in rumen concentrations were similar to those which occurred during and following eating. The plasma [K] in several instances rose immediately following the addition of K in to the rumen yet at other times the rise was scarcely detectable or occurred many hours later. In all situations, the decrease in the rumen [K] was similar. There was no significant rise in the plasma [Na] after the addition of K to the rumen but the rumen [Na] did not fall to the same extent as after eating. Presumably therefore, the absorption of Na from the rumen was less after an intraruminal load of K than after normal feeding. Changes in the plasma osmotic pressure normally followed a similar pattern to the [Na] with a small effect from changes in the [K].

Salivary concentrations during and after eating were not measured but when K was added directly to the rumen there

was a tendency for the [K] to rise for several hours.

This occurred if there was a rise in the plasma [K].

(c) Urine

Overall, the pattern of urinary [K], apart from a slight delay, was similar to the pattern of changes in plasma [K]. However, the magnitude of the rate of K excretion was not related to the plasma [K] and depended entirely on the quantity of K ingested.

The rate of K excretion rose within 1-2 hr of the commencement of feeding and reached a peak at times ranging from 4 to 14 hr. Rates with an unsupplemented diet reached a maximum of between 500 and 800  $\mu$ -equiv/min, whereas with the highest K supplement (1200 m-equiv/day) the maximum rate attained was 1400  $\mu$ -equiv/min. Flow rate followed a similar pattern to K excretion.

Sodium excretion was apparently independent of alterations in the plasma [Na] and a natriuresis occurred after the increase in K excretion and at times after the peak of the kaliuresis. The magnitude of the natriuresis was independent of the quantity of K ingested in the food.

The pattern of electrolyte excretion following the addition of salts to the rumen was, like the pattern of plasma electrolytes, irregular. Changes in the rate of K excretion again followed the pattern of plasma [K] with the result that the peak of the kaliuresis ranged from 4 to 17 hr after adding K to the rumen. Sodium excretion was unaffected by the addition of K to the rumen.

Using  $\text{KHCO}_3$  as the salt for intraruminal loading approximately 75% of the additional K was lost in the urine in 24 hr (quantity above unsupplemented excretion). The 24 hr losses of Na and water were unaltered so the additional K loss was the result of an increased concentration of K in the urine.

(3) Uptake of TOH,  $^{42}\text{K}$  and  $^{24}\text{Na}$  from the gut

During measurement of changes in plasma, saliva, rumen and at times, urinary excretion of electrolytes in normal or rumen supplemented sheep, the rates of uptake of TOH,  $^{42}\text{K}$  and  $^{24}\text{Na}$  were estimated.

The disappearance of TOH from the rumen could be described by a single exponential function thus suggesting the simple proposal that in regard to water the sheep may be divided into two compartments, the rumen and the rest of the body. Using this equation, measurements of total water

movement to and from the rumen, with and without the addition of K to the rumen were made.

It was found that the total water movement to and from the rumen was dependent on the rumen volume since the greater the volume the larger the total water movement. The [K] of the rumen had no significant effect on water movement, though there was a trend for a decrease in total water movement at a very high rumen [K].

The mean total water movement regardless of [K] determined in 14 measurements for a mean rumen volume of  $5.58 \pm 0.36$  l. was  $2.64 \pm 0.18$  l./hr or 63.5 l./day.

Following measurements of salivary flow and the TOH S.A. of the rumen fluid, plasma and saliva during the return of natural or artificial saliva to the rumen, it was concluded that saliva played some part in the equilibration of TOH between the rumen fluid and the blood, but that a large part was played by transepithelial water movements.

The uptake of  $^{42}\text{K}$  and  $^{24}\text{Na}$  from the rumen were comparatively slow processes and the time for equilibration between blood and rumen fluid was in excess of 24 hr. An increased rate of  $^{42}\text{K}$  absorption occurred when K salts were added to the rumen though the uptake rate for  $^{24}\text{Na}$  was not

altered. When the saliva was replaced with artificial saliva there was a very slow backflow of  $^{42}\text{K}$  from the blood into the rumen across the rumen wall.

Absorption of  $\text{TOH}$ ,  $^{42}\text{K}$  and  $^{24}\text{Na}$  from the duodenum, ileum and upper and lower colon was a very rapid process. Following the addition of each isotope in isotonic fluid, to the various segments of gut lumen, peaks in the S.A. of the plasma for all 3 isotopes were achieved in between 5 and 60 min. The S.A. of  $^{24}\text{Na}$  in the blood and rumen almost attained equilibrium at 24 hr when  $^{24}\text{Na}$  was placed in the lower gut.

(4) Effects of pharmacological agents and hormones on renal excretion of electrolytes

Acetazolamide, a carbonic anhydrase inhibitor, raised low urinary pH to values of 8.2 to 8.5 though in many instances the pH was already at these levels. Acetazolamide (10-300 mg) produced increases in urine flow of 10 to 120% depending on the initial flow rate. There were simultaneous rises in K and Na excretion and decreases in Cl excretion. Increases in K excretion ranged from 50 to 300  $\mu\text{-equiv/min}$  and in Na from 50 to 250  $\mu\text{-equiv/min}$ .

The benzothiadiazine diuretic, chlorothiazide, was not very effective in altering the renal excretion of electrolytes. Doses of 1-10 mg/35 kg sheep produced only small increases in urine flow and the excretion of K, Na and Cl.

In contrast to chlorothiazide both ethacrynic acid and furosemide produced a large natriuresis, and chloruresis and a moderate but variable kaliuresis. Maximum effective doses of both these compounds increased urine flow to rates of 10 to 12 ml/min with maximum rates of Na, Cl and K excretion of 2250, 1400 and 2400  $\mu$ -equiv/min. Both compounds reduced urine pH to values of 5.5 to 6.0.

Infusions of epsilon amino caproic acid, a structural analogue of the natural amino acid, L-lysine, usually increased urine flow. After sharp initial rises in electrolyte excretion associated with rises in renal plasma flow and glomerular filtration rate, electrolyte excretion was reduced to below pre-infusion levels. By its alteration of capillary permeability, EACA increased packed cell volume from 25-30% to as high as 48% and increased the concentration of plasma protein by 1.2 to 1.5 g%. This resulted in sharp increases in pulse and respiration rate.

EACA also caused a significant reduction in plasma [K] to levels as low as 2.8 m-equiv/l. while the [Na] rose to as high as 166 m-equiv/l. When EACA infusions were stopped, several hours were required before urinary excretion and plasma parameters returned to normal.

Pitressin whether given by single I.V. injections of 25-200 mU or by I.V. infusions at the rate of 2-12 mU/min produced significant increases in K excretion of sheep fed lucerne chaff. When urine flow was less than 1.0 to 1.5 ml/min these doses of Pitressin increased urine flow by up to 300% whereas at initial flow rates greater than 1.5 ml/min Pitressin reduced the flow. The effect on Na excretion was more variable depending on the initial pattern of electrolyte excretion. In sheep fed K supplemented diets, the effects of Pitressin on K excretion were less pronounced though in several instances K excretion was increased by doses of 5 mU despite reductions in high rates of urine flow. On the highest K supplements (1200 m-equiv/day) Pitressin always produced an increase in urinary  $H^+$  excretion. The magnitude of this effect depended on individual animals.

In sheep receiving a low K diet, Pitressin was able in several instances to evoke small increases in K excretion. Since the initial rates of K excretion were as low as 0.1 to 0.5

$\mu$ -equiv/min, these alterations were functionally insignificant when compared with changes in the normal sheep. Flow rates of less than 1.0 ml/min were further reduced or unaltered by injections of Pitressin in the low K animals. Na excretion was unaltered or increased in these animals though as in the K supplemented sheep there were transitory falls in urine pH.

When Pitressin was infused or injected into sheep infused by any one of the pharmacological agents altering electrolyte excretion, there were further increases in the rate of K excretion and at time in water Na and Cl excretion. Pitressin did not reduce the high rates of flow produced by ethacrynic acid, frusemide and EACA as it did during a normal diuresis though as stated still increased K excretion. Thus the effects of vasopressin on water and electrolyte excretion were dissociated.

dl-Aldosterone infusions into normal or K supplemented sheep always led to a reduction in Na excretion and urine flow but were without significant effect on K excretion. Cortisol infusions had no effect on the urinary excretion of electrolyte or urine flow of normal or K supplemented sheep.



#### 4. Discussion

Sheep tolerate the ingestion of quantities of K greatly in excess of their optimal requirements. In man, the rapid ingestion of 200 m-equiv of K as Kcitrate produces numerous toxic symptoms referable to the nervous system and heart (Bedford, 1954), yet grazing sheep may ingest up to 1250 m-equiv/day (an intake of 1 kg DM containing 5% K).

Excess K from the diet is eliminated mainly in the faeces and urine. Sweating provides a route for K excretion and it has been shown that in man extremely high rates of sweating may result in a slight K deficiency (Toor, Agmon, Zahavi, Wurzel, Rosenfeld, 1968). Nevertheless, at normal rates of sweating the loss of K is small. Estimates of K loss in the suint of sheep are in the order of 1% of the daily K intake (Beal and Budtz-Olsen, 1968, Farnworth, 1956). Therefore, if the plasma [K] is to be maintained at a constant level, K absorbed from the upper digestive tract must be rapidly excreted in the urine or secreted back into the lower gut and eliminated in the faeces with unabsorbed K.

An alternative possibility is that K absorbed following eating may temporarily pass into the cells and be resecreted into the plasma and excreted in the urine when absorption from the gut

declines. It has been demonstrated that rats fed high K diets, develop this mechanism to deal with acute K loads and prevent toxic rises in the plasma [K] (Alexander and Levinsky, 1968).

The proposal that specialised renal and gut mechanisms whereby the excess K could be excreted might exist in the sheep was investigated. Measurements of urinary and faecal electrolyte excretion were made following increases in K intake. Concurrent measurements of body fluid volumes, electrolyte concentrations and water TO were carried out.

Since it was anticipated that the ingestion of K may alter the rates of uptake of K, Na and water from the rumen and their return in the saliva, movements of K, Na and water were followed using radioisotopes.

Finally, the mechanism of K excretion through the kidney and its possible stimulation after eating were investigated using various natural hormones, in particular, vasopressin. Pharmacological agents, alone and in combination with vasopressin were also tested.

(1) Effects of a high K intake on body fluids and electrolytes

(a) Pilot experiment

Although there have been no reports of the effect of K intake on water intake and TO in sheep, St. Omer and Roberts

(1967) noticed a higher water consumption and urine volume in heifers fed a "high" K ration compared with a "low" K ration. However, the highest K intake they considered was approximately 4.3 m-equiv/kg/day or 1.7% of the dietary dry matter intake whereas the lowest used in the present experiments was approximately 19.5 m-equiv/kg/day (2.5% of the DM intake). The maximum K intake was approximately 65 m-equiv/kg/day (8.7% of DM intake). Potassium comprises 1-5% of the dry matter of normal herbage (Spector, 1956; van der Horst, 1960) so the highest K content was well in excess of that normally available to grazing sheep. The total K intake/day of sheep grazing young pasture containing 4% K as dry matter has been calculated as 1500 m-equiv or 30 m-equiv/kg (Howard, personal communication). As mentioned, in Chapter I, the dry matter of the saltbush, Atriplex nummularia, which is grazed by sheep, may contain as much as 8-15% Na and 3-4% K as dry matter (Macfarlane, Howard and Siebert, 1967; Wilson, 1966). Therefore it was decided to investigate the tolerance of sheep for K at equivalent levels to those of Na.

In the pilot experiment K intake did not exceed 5% of the total dry matter intake yet there was a significant rise ( $p < 0.05$ ) in water TO as the K intake was increased. The regression of water TO on K intake showed that 0.9 l. of water was turned over for every 100 m-equiv of K ingested in a temperate environment (15-35°C).

(ii) Main experiment

(a) Body fluids and electrolytes

The tendency for TBW to increase with increasing K intake was confounded in the pilot trial by environmental variation since the sheep were housed in partially exposed pens. This presumably also affected water intake and TO but not enough to prevent significant changes in water TO with alterations in K intake. To remove this environmental effect the main trial was carried out in a controlled indoor environment ( $T = 20 \pm 3^{\circ}\text{C}$ ).

There was no apparent effect of the anion associated with the K in the supplements of the pilot trial. It was, therefore, decided that an equimolar mixture of all 3 anions would be most satisfactory. Adding Ac alone in the maximum supplements of 1200 m-equiv/day would have provided too great a source of energy, Cl alone could have produced acidosis while  $\text{HCO}_3$  alone could have led to alkalosis.

If too sudden a change of K intake takes place, animals tend to scour (personal observation) and in order to prevent this occurrence, stepwise increases in K supplements were used rather than a randomised trial. Furthermore, too long a pre-experimental adjustment period was required if

sudden large changes in K intake were used. There was also a limitation on the amount of chaff which could be mixed with the K salts to provide a uniform diet throughout any experimental period for any sheep. Although an attempt was made to use uniform bags of chaff throughout, considerable variation in the basal K content was found (570-700 m-equiv/kg DM) resulting in variations in the intake of individual sheep at the different supplement levels. Added to this variation was the difficulty of getting sheep to maintain a constant intake of the basic 900 g of chaff.

As in the pilot experiment, there was a significant relationship between water TO and K intake. Three of the 4 sheep showed consistent rises in water TO as the quantity of K ingested rose while the 4th sheep showed a rise in TO only at the higher levels of K intake.

The increase in B.Wt. of all 4 sheep as the K intake rose was significant. There were small increases in body fluids (B.Wt. was corrected for wool growth) which were possibly due to the ~~higher~~ energy intake resulting from the Ac included in the supplements. However, sheep F during a period of no supplementation and a similar ration of 900 g of lucerne chaff also gained weight. Its TBW as a percentage of B.Wt. remained relatively constant throughout this period.

Since the TBW as a percentage of B.Wt. increased significantly as a result of increasing the K intake there was water retention. Part of the water retained during the pre-experimental period of adjustment to a rise in K intake was in several instances lost during the experimental period. The final estimate of TBW did not decrease if supplementation was continued so the increased K intake did produce a rise in TBW.

Much of the increment of TBW came from a rise in the ECV though the ECV as a percentage of TBW did not alter. Because the rise in ECV accounted for most of the rise in TBW, there must have been very little alteration in the amount of water in the alimentary tract.

The PV was unaltered, despite variations in TBW and ECV - instead it fell significantly as a percentage of B.Wt. and TBW. Since plasma protein contributes towards the regulation of PV one would anticipate little or no alteration in this parameter yet plasma protein concentration rose significantly as the quantity of K ingested increased. There is no apparent explanation for this increase.

The fall in plasma [Na] and the rise in ECV produced by increasing the K intake allowed the total quantity of Na in the ECF to remain relatively constant. Since the plasma [K]

did not change, the total quantity of K in the ECF rose. It is difficult to ascertain whether the increased retention of K or the increased water TO produced the rise in ECV and TBW. Macfarlane, et al., (1967a) found that among sheep grazing salt-bush those with the highest water TO/day had the highest ECV. These workers and Macfarlane (1965) also found an increased retention of extracellular water when total TO was increased even though the [Na] of the plasma was reduced.

It is not clear how the increased water retention with a lowered plasma [Na] is produced. The control of plasma volume and [Na] by receptors in the heart, hypothalamic receptors, angiotensin and aldosterone (Bartter et al., 1959) would appear to apply in sheep (Blair West, et al., 1963, 1963a, 1967), but in the present situation the [Na] of the plasma decreased and the ECV rose though the total quantity of Na remained constant. Blair-West et al., (1963) demonstrated that a fall in the plasma [Na] of 5.0 m-equiv/l. could produce an increased secretion of aldosterone by the adrenal of the sheep, which should lead to Na retention by the kidney. Observations on the urinary excretion of Na following a rise in K intake indicate that in terms of concentration, Na was being retained by the kidney, though the total Na output was not reduced.

A further indication of an increased level of circulating aldosterone and presumably increased secretion was the change in the Na:K ratio of the saliva as the K intake rose. Denton, Goding and Wright (1959) proposed that the Na:K ratio of the saliva is a good long term indicator of the circulating level of aldosterone in sheep. The Na:K ratio of saliva in one sheep not given access to salt fell from 24 to 5-10 when the K intake was raised. This sheep had access to salt prior to the week of saliva collection though the 4 sheep, in the main trial did not. When the K intake of the second sheep which had continuous access to salt, was raised the fall in the Na:K ratio was less pronounced. During Na depletion, the salivary Na:K ratio of the same sheep declined to a level of less than 2. Sodium and K balances were not measured in either animal though plasma [Na] showed no indication of a Na depletion. Therefore, there was an indication that raising the K intake increased the plasma concentration of aldosterone or some related hormone.

A high circulating concentration of aldosterone may explain the lack of rise in plasma [K] as the K intake increased since continuous infusions of aldosterone into sheep eating grass cubes supplying 45 m-equiv Na and 720 m-equiv K/day produced a marked reduction in plasma [K] and only a very slight rise in plasma [Na] (Scott, 1962). Similar observations have been



made in the rabbit though the K balance was positive and it was suggested that during the initial stages of administration K moved into the cells (Dawborn and Ross, 1967). In rats, feeding a high K diet makes the animals far more tolerant of acute K loads. This tolerance is related to an increased adrenal activity and the ability of the cells to increase their K uptake and thus prevent large rises in extracellular concentration (Alexander and Levinsky, 1968).

In sheep the normal K intake is 3-6 times greater than that of the carnivores and omnivores and hence the adrenal activity may well be regulated to this K load. The cells may be able intrinsically to increase their K content following feeding under the action of aldosterone or a related mineralocorticoid.

## (2) Rumen electrolytes

Scott (1967) altered the [K] and [Na] of the rumen by constant infusions of solutions of K salts. He found that the [K] rose while the [Na] fell. Somewhat similar changes were observed by Goodall and Kay (1965), following changes in diets of different Na and K composition. In the present experiments increases of K intake also resulted in a rise of [K] and a decrease in the [Na] of the rumen. Prefeeding levels of rumen [K] rose from 44-49 m-equiv/l. to 115-130 m-equiv/l, while [Na] fell from 90-98 m-equiv/l. to 24-35 m-equiv/l. Further

increases in the  $[K]$  and decreases in the  $[Na]$  of the rumen occurred following eating. Post feeding alterations became progressively smaller as the K supplement rose. The rumen fluid was always hypertonic to the plasma 4-6 and 8-10 hr after eating commenced though on the maximum K supplement even the prefeeding rumen OP was often greater than that of the plasma.

The alterations in the Na:K ratio of the rumen during K supplementation could not have been due to the alterations in salivary composition. Scott (1967) did not find any change in composition of parotid saliva during K supplementation and his measurements of rumen outflow suggested that the increased rumen  $[K]$  produced an increased rate of both K and Na absorption. Since the Na intake was constant this resulted in decreased  $[Na]$  in the rumen fluid. A similar conclusion was reached by Warner and Stacy (1965) and Stacy and Warner (1966) regarding the fall in rumen  $[Na]$  following feeding or the addition of K, or mannitol-urea to the rumen. They proposed that the increased absorption was the consequence of an osmotic stimulus.

The addition of 400 m-equiv of K by rumen fistula did not produce as significant a fall in rumen  $[Na]$  as those found by Warner and Stacy yet the decrease after eating in normal

unsupplemented sheep was similar. Changes in the rumen [K] after the direct addition of K salts to the rumen or after eating of lucerne chaff were similar. The rumen [K] remained at high levels for 3 to 12 hr after eating (depending on whether eating ceased or not) before it began to decline. Rumen [Na] was depressed for a similar period before it began to rise. After the direct addition of K to the rumen the [K] began to decline almost immediately while the [Na] remained unaltered or decreased for 3 to 6 hr before commencing to rise.

These changes in [Na] and [K] could be due to dilution of the rumen fluid by saliva or by water moving into the rumen from the plasma through the rumen wall, or else to absorption of K and Na from the rumen.

If salivary flow rate was increased, one would anticipate a rise in rumen [Na] rather than a decline, unless the rate of absorption exceeded the rate of entry. Furthermore, during eating additional Na enters the rumen, yet the [Na] fell. Measurements of salivary flow rate following the addition of either KCl or  $\text{KHCO}_3$  to the rumen showed no alteration in flow rate. Therefore, the rapid decline in the high [K] and the lack of alteration or slow fall in the [Na] of the rumen after the addition of K were not due to alterations in the flow of saliva.

Ternouth (1967) claimed that after eating or the addition of a solute load to the rumen, there was an increased influx of water to the rumen. Warner and Stacy (1968) were highly sceptical of these results and the techniques employed in determining them. In continuing investigations of this problem, Ternouth (1968) showed a reduction of the ECV following feeding and he assumed this decrease was due to trans-epithelial water movement into the rumen. Investigations by Warner and Stacy (1968) showed similar changes in ECV following feeding but they concluded this was due to a stimulation of salivary flow by eating.

In relation to this problem, three points have emerged from the present investigations. As postulated, the ingestion of K does alter the rate of electrolyte and water movement. Potassium absorption is accelerated following the addition of K salts to the rumen as is indicated by the more rapid equilibration of  $^{42}\text{K}$ . This could result from a faster out-flow of K from the rumen following the addition of K, and a rapid absorption of K from the low gut. The rate of uptake of  $^{42}\text{K}$  from the duodenum, ileum and upper and lower colon was shown to be rapid, though unfortunately K uptake from the omasum and abomasum was not measured. If fluid out-

flow from the rumen was increased by the addition of K salts a sharper rise in plasma  $^{42}\text{K}$  S.A. would be expected.

From the measurement of salivary flow there is no indication that outflow from the rumen should be increased by the addition of K (as the work of Stacy and Warner (1966) also showed). Furthermore, preventing salivary inflow for an hour following the addition of  $^{42}\text{K}$  and 400 m-equiv of K to the rumen did not alter the rise in the S.A. of the plasma. In this situation outflow from the rumen should be small and hence the K must have been absorbed directly from the rumen. This is in agreement with the results of Scott (1967) who by measuring outflow rates from the rumen and the total loss of K from the rumen determined that a large fraction of the K lost from the rumen was by direct absorption across the rumen wall.

Potassium also enters the rumen from the blood across the rumen wall. This can be seen by considering the curve of rumen  $^{42}\text{K}$  S.A. when  $^{42}\text{K}$  was added to the blood and natural saliva was replaced with artificial saliva. The rate of entry of  $^{42}\text{K}$  into the rumen in the saliva and across the rumen epithelium is many times slower than the rate of absorption. Approximately 1-10 m-equiv of K were found to enter the rumen in the saliva each hr which coupled with some inflow across the rumen wall would

have meant an hourly addition of no more than 15 m-equiv of K to the rumen. Following the addition of K salts to the rumen the high rumen [K] produced, then fell by 10-20 m-equiv/l./hr. Assuming a constant rumen volume of 5.0 l. some 50-100 m-equiv of K were lost from the rumen each hr. Part of this loss would have been by outflow of rumen fluid to the omasum yet as already discussed, much of the K loss from the rumen was by direct absorption. Thus the rate of loss of K from the rumen after the addition of K salts was approximately 3 to 6 times faster than the rate of inflow.

The second point to emerge from these investigations was that there was no apparent difference in the rates of Na uptake from the rumen in a normal sheep and one with K added to the rumen. This is difficult to reconcile with the observed decline in rumen [Na] or the reports of Stacy and Warner (1966) and Scott (1967) that this decline is due to an accelerated Na absorption. However, the decline in [Na] of the rumen which occurred after the addition of K to the rumen was relatively small or non-existent and the method of measuring Na uptake may not have been sensitive enough to detect alterations in uptake rate.

Although there was some indication that raising the K content of the rumen reduced the estimates of the total water movement to and from the rumen, no significant relationship between the initial [K] and total water movement could be found. However, the relationship of rumen [K] and total water flow was confounded by the finding that the total water flux depended on the rumen volume; the larger the rumen volume the greater the total flux of water. The mean total flux determined in 14 measurements on 5 sheep was  $2640 \pm 180$  ml/hr (63.5 l./day) from a rumen of mean volume of  $5.58 \pm 0.36$  l.

These estimates were based on the method described in the results (section (v) (b)) where it was pointed out that various assumptions are made. Estimates of rumen volume probably provide the biggest source of error though there was good agreement between estimates using the phenol red dilution techniques and the extrapolation of the TOH disappearance curve. If rumen volume changed during the initial period of 1 hr when mixing was taking place, both methods would give incorrect estimates of rumen volume. In particular, if the rumen volume increased, which would occur if there was a net transepithelial inflow of water, initial rumen volume would be overestimated. The technique for measuring the initial rumen [K] appeared to be quite satisfactory. It is possible that the relationship between the total water flux and rumen volume may be explained

in terms of surface area - the greater the rumen volume, the greater the area of rumen wall coming in contact with water and hence a higher water flux. The rates of saliva flow (250 ml/hr) show that to achieve the values of total water flux given, the transepithelial inflow and outflow of water must be large. Assuming that outflow from the rumen is no greater than salivary inflow the total transepithelial water inflow and outflow would in the present experiment amount to at least 2000 ml/hr.

The fact that a large transepithelial water transfer does occur was demonstrated by measuring the equilibration time for TOH between rumen fluid and blood or vice versa, plasma, rumen and salivary TOH S.A. and salivary flow rates. This is most clearly seen using the following example:-

e.g. Assume no transepithelial flow and TOH absorbed only from the lower digestive tract.

Salivary flow rate = 250 ml/hr.

Constant Rumen volume = 5.0 l. which with 300  $\mu$ C of TOH added rumen fluid has an initial S.A. of 60  $\mu$ C/l.

In first hr 250 ml of saliva (assume it contains no TOH) reduces S.A. to  $\frac{300}{5.25} = 57.1 \mu\text{C/l.}$



Assume 250 ml of fluid flows onto the omasum and TOH equilibrates rapidly with the blood. (During the next hr the saliva contains some TOH but allowing for this TOH increases final rumen S.A. rather than reduces it).

In the second hr TOH content of rumen =  $5 \times 57.1 = 285.7 \mu\text{l}$ .  
Further dilution with 250 ml saliva -

$$\text{New rumen S.A.} = \frac{285.7}{5.25} = 54.4 \mu\text{C/l.}$$

Continuing until the 6th hr.

$$\text{Rumen S.A.} = 44.7 \mu\text{C/l.}$$

$$\text{Total Activity} = 224 \mu\text{C.}$$

Continuing until the 8th hr.

$$\text{Rumen S.A.} = 40.6 \mu\text{C/l.}$$

However, equilibration is normally achieved by this time and rumen S.A. is less than  $15 \mu\text{C/l}$ . Therefore, considerable dilution of the rumen S.A. must take place by water inflow through the rumen wall.

This example demonstrates that when TOH is added to the rumen the fall in S.A. cannot be due to saliva alone. However, it is possible that there is a transepithelial inflow of water from the blood with little transepithelial absorption and a large outflow to the omasum.

An indication that this did not occur was the normal rise in plasma TOH S.A. during the first hr after the addition of TOH to the rumen while saliva inflow was stopped. During this time, outflow from the rumen should have been small and hence TOH uptake must have been across the rumen wall.

The conclusive demonstration that transepithelial water transfer was the major determinant of the equilibration time of TOH between plasma and rumen fluid came from the addition of TOH to the blood and replacement of natural saliva with artificial saliva. Equilibration still took place in 6 hr or less indicating that back diffusion of water from the plasma through the rumen wall took place at rates comparable to the rates of loss.

Siebert (1968) suggested that the longer equilibration time of TOH placed in the rumen of cattle and camels undergoing dehydration, was the result of a reduction in salivary flow. From these results it appears more likely that it was a result of changes in permeability of the rumen wall. Equilibration of TOH between rumen and blood, when natural saliva was withheld for hourly intervals before its return to the rumen was at times reduced to as low as  $2\frac{1}{2}$ -4 hr.

This indicated either an alteration in rumen epithelial permeability or changes in reflex movements of the rumen. Insertion of an endotracheal cannula into the oesophagus may have stimulated rumen movements, providing greater mixing action and perhaps an increased outflow from the rumen. By increasing rumen movements a larger surface of rumen wall would come into contact with TOH and transepithelial water movements might be increased. Since the rate of passage of TOH from blood to rumen was also increased during the replacement of natural saliva with artificial saliva, this explanation does not seem feasible. Therefore, alteration of the rumen wall permeability either by lack of regular saliva flow or by stimulation of some process by the experimental technique seems likely.

Engelhardt (1963a) using goats, estimated that the total flow of water across the wall of a rumen of volume  $4.0 \pm 1.1$  l. averaged 40 (7-70) l./24 hr. This value did not include salivary inflow and outflow from the rumen and hence the total flow to and from the rumen was even higher. Thus for the volume of rumen fluid calculated in these experiments the estimate of total water movement seems quite satisfactory. Engelhardt (1963, b) also found that the rumen wall was subject to rapid changes in permeability and that increasing the osmolality of

rumen fluid retarded the "Nettoflusssigkeitzufluss" of water into the rumen. Hence the decrease in total water movement measured after the addition of K to the rumen, although not statistically significant, may have been real.

It may be concluded from the present results that although saliva plays some part in the equilibration of TOH between blood and rumen fluid and vice versa, there is a large transepithelial water movement which may be altered by changes in rumen fluid OP and is controlled by changes in rumen epithelial permeability.

### (3) Urine and faeces

There was a significant rise in urine volume following the increase in water intake and TO of sheep ingesting increasing quantities of K. A range of urinary [K] resulted from the variations in the increase in water TO of individual animals. Over this range of [K] the amount of K excreted in the urine/day was proportional to the K intake. Even when sheep B had its water intake restricted there was a marked rise in urinary [K] such that the total K output was unaltered. Unfortunately, the water restriction was not severe enough and the [K] achieved was still below that of sheep F with water offered ad lib.

Initially the rise in urine volume following an increase in K intake was not sufficient to prevent an increase in urinary [K] but beyond an intake of 1000-1100 m-equiv K/day the urinary [K] declined. This was the result of an increase in urine volume and the total loss of K in the urine was still proportional to the quantity of K ingested.

Although some 70-95% of the K filtered at the glomerulus of dogs and rats is reabsorbed proximally (Windhager and Giebisch, 1965) there has been no demonstration of a similar mechanism in sheep. Net secretion of K does occur in the kidney of sheep and cattle (Denton, McDonald, Munro and Williams, 1952; McDonald and Macfarlane, 1958; Anderson and Pickering, 1962). Potassium secretion has been assumed to occur in the distal tubule in a similar manner to that in dogs (Berliner, Kennedy and Hilton, 1951) and rats (Malnic, et al., 1966 a,b). Since no inulin measurements were made of filtration rate it is not possible to say whether alterations in GFR were responsible for the changes in K excretion which took place as the K intake was raised. Two estimates of the average daily GFR using creatinine clearance did not show any substantial alteration in GFR with increasing K intake, so that it is likely there was a large increase in the net secretion of K by the kidney. Scott (1969a,b) however, found an increase in filtered K when the daily K intake

exceeded 880 m-equiv/day and suggested that when the dietary intake of K is high a fraction of the filtered K load augments the secretion of the distal tubule.

Although the present experiments do not allow any conclusions to be drawn regarding the mechanism of K excretion by the kidney they substantiate the original proposal that increasing the K intake should result in a larger water TO and augmented urine volume to prevent excessive concentration of K in the urine. It appears that the [K] of the urine is resolved by individual animals as a balance between water intake and the concentration of K by the kidney. Some animals prefer to maintain a higher water intake and produce a less concentrated urine in respect to K than others. Individual animals still retain the ability to increase the urinary [K] if the necessity arises.

Several recent reports have shown a remarkable consistency in the ratio of urinary to faecal K loss regardless of K intake (English, 1966; Dewhurst, Harrison and Keynes, 1968; Beal and Budtz-Olsen, 1968). The urinary fraction has been found to be of the order of 89-90% of urine plus faecal K loss though in the present experiments it was never less than 97%. On the other hand, faecal Na output ranged from 1.5 to 13.6% of the urinary plus faecal Na loss.

These values are somewhat similar to the 12% found by Beal and Budtz-Olsen (1968) yet Dewhurst, et al., (1968) found values ranging from 89.3 to 98.3%.

There is no apparent explanation for the unusually high loss of K in the urine in the present experiments and lack of rise in faecal K excretion after increasing the K intake. St. Omer and Roberts (1967) using calves and Scott (1967) and Goodall and Kay (1965) using sheep, found an increased faecal excretion of K as the quantity of ingested K rose. Nevertheless Scott (1969a) found a marked difference between sheep in their ability to increase the fraction of K lost in the faeces in relation to their total urinary plus faecal loss. Dewhurst, et al., (1968) reported that there was no change in the faecal excretion of K up to 48 hr after the addition of 1 l. of 0.25 N KCl (250 m-equiv K) to the rumen prior to feeding. In the present experiments all 4 sheep seem to have been able to absorb K from the lower digestive tract and thus prevent its loss in the faeces.

The absence of an increased faecal K loss following rises in K intake conflicts with the proposal that increasing the K intake produces an increased secretion of aldosterone or related mineralocorticoids. There is an increased faecal

loss of K and decreased loss of Na in man (August, et al., 1958, Duncan, Liddle, and Bartter, 1956) and in rabbits, rats and dogs (Dawborn and Ross, 1967; Davis, et al., 1959) following administration of aldosterone or in hyperaldosteronism. However, aldosterone may not function in sheep as it does in these species. This is indicated by the observation that aldosterone does not promote K loss in the urine of sheep as it does in other species (see Discussion (iv)).

#### (iv) Effects of K depletion

Although the prime object of feeding a low K diet was to observe how the kidney functioned in this situation, some measurements of body fluid volumes and electrolyte concentrations were made.

Body weight fell during the period of low K intake, possibly as a result of a reduction in dry matter intake and hence energy intake. St. Omer and Roberts (1967) and Campbell and Roberts (1965) reported a decrease in appetite and dry matter intake of calves and lambs on a low K intake and similar observations were made in the present experiments. It was not possible to determine whether the loss of appetite was due to K depletion since there were no apparent signs of K deficiency in terms of plasma [K]. Telle, Preston, Kinter



and Pfander (1964) suggested that plasma [K] below 3.0 m-equiv/l. were necessary to indicate K deficiency. There were significant reductions in plasma [K] but only to levels as low as 3.5 m-equiv/l. and such concentrations were not maintained consistently.

Total body water fell slowly throughout the period of low K intake though TBW as a percentage of B.Wt. rose in one sheep but was relatively constant in the second. Plasma volume and ECV also fell but rose as percentages of B.Wt. and TBW during the course of the experiment. These alterations in body fluid volumes could have been the result of the low food intake rather than the effects of K depletion as such.

In view of the findings of Telle, et al., (1964) it was anticipated that a diet of the K content used (30 to 35 m-equiv/kg DM) should have produced a pronounced K deficiency leading to muscular paralysis, rapid weight loss and if continued death. Some muscular weakness did develop but once again this may have been the result of the low energy intake.

Despite reports that dogs, rats and man (Relman and Schwartz, 1962) do not have the ability to conserve K during periods of abnormally low K intake, due to the

inability of the kidney to conserve K beyond certain limits, the sheep were able to reduce their daily loss of K in the urine to as low as 0.5 to 1.75 m-equiv. This was achieved by reducing the [K] to values of 1.15 to 2.50 m-equiv. The urinary loss became far less than the faecal loss which exceeded 10 m-equiv/day and was in fact similar to that of normal and K supplemented sheep. Overall, there was only a very small negative K balance of 4 to 6 m-equiv/day, which explains the absence of any obvious signs of K deficiency.

Measurements of GFR for a 3 hr period using inulin clearance, in a sheep fed a paper, maize and molasses diet of low K content, gave values as low as 13 ml/min and a maximum of 26 ml/min. This sheep had a plasma [K] in the order of 4.0 m-equiv/l., with a slight reduction in muscle K content. Urinary K losses in this animal were higher than those observed in the main K depletion experiment but the K intake was also considerably greater. Whether similar reductions in GFR occurred in the sheep on the very low K diet is unknown. However, assuming 95% reabsorption of filtered K in the proximal tubule, no distal secretion and a plasma [K] of 3.5 m-equiv/l. GFR would have to be reduced to rates of 20-25 ml/hr to account for the lowest levels of K excretion found. Such reductions in GFR appear unlikely and thus the

the sheep kidney either possesses. the ability to reabsorb K proximally in excess of 95% or there is a further site of K reabsorption in the collecting duct as found in the rat (Malnic, et al., 1966 a). Whatever the mechanism, the adult sheep kidney has an ability to retain K in excess of that of other species.

(iii) Changes in plasma and urinary electrolytes following feeding or the addition of K salts to the rumen.

Regardless of the K intake of the sheep there was a consistent rise in the plasma [K], [Na] and the plasma OP following feeding. Warner and Stacy (1965), Stacy and Warner, (1966) and Ternouth (1967, 1968) reported rises in plasma [Na] and OP after eating though their periods of feeding varied. Small changes in plasma [K] in the first hr after the initiation of eating were recorded by Ternouth (1968) but he did not state the magnitude of these changes. These 2 groups of workers were at a variance as to the origin of the rises in plasma [Na] and OP but both concluded there was a loss of ECF to the rumen. Stacy and Warner (1966) and Warner and Stacy (1968) determined that the decrease in ECV following eating was due to an increased salivary flow and that the rising OP of the rumen fluid stimulated Na absorption

from the rumen. This in turn raised the plasma [Na] and OP. Ternouth (1967) estimated that there was an increased trans-epithelial flow of water into the rumen after eating which resulted in a decrease in the ECV and an increase in the plasma [Na] and OP. In the present investigations use of plasma protein concentration as a guide to changes in ECV showed there was no substantial decrease in ECV following feeding.

Neither Warner and Stacy's nor Ternouth's proposals account for the rise in plasma [K] of 1-3 m-equiv/l. measured in the present experiments. In particular, an increased salivary flow should reduce the plasma [K] rather than increase it. Measurements of  $^{42}\text{K}$  uptake after the addition of 400 m-equiv of K, to the rumen showed that there was an increased absorption of K from the rumen when the rumen [K] was raised. This agrees with the results of Scott (1967) and it was presumably this increased K absorption which resulted in the observed elevation of plasma [K] following feeding.

Despite consistent rises in plasma [K], [Na] and OP after eating there was no consistent pattern of changes following the addition of 400 m-equiv of K to the rumen. Plasma [K] began to rise in the first hr after the initiation of eating and reached a maximum at between 4 and 10 hr.

After the addition of K to the rumen, the plasma [K] reached a maximum from 1 to 17 hr later. Similarly the plasma [Na] began to rise in the first hour after eating began, but there was often no consistent change after rumen supplementation. The alterations of plasma [Na] appeared to be related to changes in the rumen [Na]. After eating there was always a substantial depression of rumen [Na] whereas there was often only a small decrease or no alteration following the addition of K to the rumen. When there was a pronounced decrease in rumen [Na], plasma [Na] was observed to rise. This suggests that the decrease in rumen [Na], was as proposed by Stacy and Warner (1966) and Scott (1967) the result of an increased Na absorption, and in turn resulted in a rise in plasma [Na].

The absence of any measurable rise in plasma [Na] after rumen supplementation may therefore be anticipated since the rumen [Na] was not markedly reduced and hence there may not have been a significant rise in Na absorption. This point was substantiated by the inability to detect any alteration in <sup>24</sup>Na uptake from the rumen after the addition of K. However, Stacy and Warner (1966) reported that the addition of solutions of K salts, or manitol-urea to the rumen resulted in a decrease in rumen [Na] and an increase in plasma

[Na] and an increase in plasma [Na]. Therefore the lack of any change in these parameters in the present experiment, though they were found after feeding seems unusual. There is the possibility that the initial rumen [Na] was important and since Stacy and Warner fed a Na supplemented diet, their initial rumen [Na] were considerably higher than in the present experiments. Because Na is actively absorbed from the rumen against an electrochemical gradient (Dobson, 1959; Scott, 1966; Ferreira, Harrison and Keynes, 1966; Ferreira, Harrison, Keynes and Nauss, 1966) a high rumen [Na] should reduce this gradient with the result that any subsequent reduction of the [Na] by absorption would occur more readily.

Although the rumen [K] following feeding was dependent on the K intake, the higher the K intake the greater the rumen [K], the rise in plasma [K] was independent of the K load ingested. However, the rates of excretion of K in the urine, for individual animals, was dependent on the K intake. As the K intake was raised so the maximum rate of K excretion rose attaining levels of 1200  $\mu$ -equiv/min with the maximum K intake. The rate of urinary K excretion increased within 1 to 3 hr of an animal eating and subsequently attained a maximum at between 5 and 12 hr.

Urinary responses to the addition of 400 m-mole of  $\text{KHCO}_3$  or  $\text{KCl}$  to the rumen were not uniform. On 2 occasions using  $\text{HCO}_3$  the maximum kaliuresis was achieved in the third hr after the addition of K to the rumen yet in 4 other experiments the peak of the kaliuresis ranged from 6 to 17 hr. The maximum rate of excretion of K following K loading was  $670 \mu\text{-equiv/min}$ , a figure similar to that found when this quantity of K was ingested in the food. Bicarbonate excretion, as indicated by a rise in urinary pH, rose within 20 min of adding  $\text{HCO}_3$  to the rumen.

Dewhurst et al., (1968) also found a large increase in the urinary excretion of K 2 to 3 hr after feeding. The period before the increase occurred was shortened by the addition of K salts in solution into the rumen. Their explanation of the reduced delay in excretion following the addition of extra K may well explain the variable time of increased excretion in the present experiments. By adding the K salts in 1.0 l. of water they thought that flow from the rumen may have been increased, carrying digesta of increased K content to sites of more rapid absorption. Since in the present investigations, the K salts were added in only 150 ml of water, the outflow from the rumen would be less pronounced, absorption, apart from that across the rumen wall would be reduced and

the rate of excretion retarded. During feeding an increased salivary flow and drinking would produce a greater outflow of K from the rumen.

More than 100% of the quantity of K added to the rumen was recovered in the urine in 24 hr with  $\text{HCO}_3$  as the anion, but less than 75% with Cl though this was in only 1 experiment. When the 24 hr urinary excretion of K without any K supplement was subtracted from the total urinary excretion (with the  $\text{KHCO}_3$  supplement) only 75% of the extra K was excreted in the urine in 24 hr. A value of 65% and 82% recovery in the urine in 24 hr was given by Dewhurst, et al., (1968) using Ac and Cl as the anion respectively.

Comparing the changes in plasma [K] and urinary excretion, both followed a similar pattern though the changes in urinary output were slightly delayed. This delay was in excess of the time for urine to pass from the kidney pelvis through the ureter, bladder, catheter and associated tubing dead space to the collecting tube. Malnic, et al., (1966 a) and Guinnebault and de Rouffignac (1966) proposed that intracellular K is the source of K secreted by the kidney of rats. Assuming a similar process in sheep the delay between rises in plasma [K] and urinary excretion may be due to the period necessary for K to accumulate in the cells before being secreted into



the tubule lumen and excreted.

In spite of increases in the [Na] of plasma which began in the first hr of feeding the urinary excretion of Na did not rise for several hours by which time K excretion had increased and was attaining its maximum. The peak of the natriuresis was normally attained after the peak of the kaliuresis. The magnitude of the kaliuresis depended on the K content of the diet but the magnitude of the natriuresis was quite variable. Dewhurst, et al., (1968) made similar observations but were unable to explain how K loading caused a natriuresis.

Urine flow after feeding tended to follow the same pattern as the rate of K excretion with a slight effect from the natriuresis when it occurred.

Contrary to what might be expected and to the findings of Dewhurst, et al., (1968) there were no marked alterations in Na excretion following an intraruminal load of K salts. The total excretion of Na during the 24 hr following rumen supplementation was not significantly different from that without any K supplement. This may in part be due to the absence of or reduced change in the plasma and rumen [Na] which followed addition of K to the rumen, compared with the changes which

followed normal eating. Presumably the increased salivary flow, drinking and increased intake of ions, including Na, which occur during eating may also alter the absorption and subsequent excretion of K and Na. From these results it does not appear that elevation of the rumen [K] alone (increased OP) produces the increased Na absorption from the rumen, the rise in plasma Na and increased urinary Na excretion which follows feeding.

Part of the increased K excretion following feeding was found, in 2 experiments, to be due to a rise in GFR which coupled with rises in plasma [K] raised the filtered load of K. Assuming that a constant fraction of the filtered load was reabsorbed for all levels of K filtered then the increase in filtered load would have provided a rise in the quantity of K excreted. Thus an increased rate of K excretion accounts in part for the absence of any effect of K intake on the post-feeding [K] of plasma. Another explanation of the absence of an effect of K intake on changes in the plasma [K] following feeding is to be found in the results of Alexander and Levinsky (1968) using rats. Acute K loading of normal rats resulted in large rises in the plasma [K] and death due to K intoxication. If, however, rats were accustomed to a high K diet prior to an acute K load, the initial changes observed

in the plasma [K] were far less, even if the rats were nephrectomised. Alexander and Levinsky interpreted these results as an indication that rats fed a high K diet were capable of absorbing an acute K load intracellularly, thus preventing large changes in the plasma concentration. They attributed this high cellular permeability and absorption of K to an increased secretion of aldosterone or a related mineralocorticoid.

Evidence for an increased aldosterone secretion with increased K intake has already been presented in relation to changes in salivary composition. If the same situation exists in the sheep as in the rat, then the lack of a larger rise in the plasma [K] following an increase in the K intake may, as originally postulated, be due to an increased absorption of K by the cells of the tissues in response to a higher endogenous level of aldosterone.

The sheep would normally have far more tolerance to an acute load of K (rapid feeding) than a rat owing to the naturally high K content of the diet. This may explain the absence of any substantial changes or far more irregular ones in the plasma [K] following intraruminal dosing with 400 m-equiv of K. This quantity of K is well inside the normal range ingested daily by a grazing animal and is not

an acute load for a sheep.

Alterations in absorption of K by tissue cells may also explain the alterations in the maximum rates of K excretion, which occurred at different levels of K intake. Since intracellular K is the source of K secreted in the distal tubule (Malnic, et al., 1966b) an increased intracellular [K] would result in increased K secretion. Hence if the intracellular K content in the distal tubule cells of the sheep kidney increased as the K intake rose so may the secretion and total rate of K excretion rise. It is unlikely, however, that the increased cellular uptake of K was produced by aldosterone because this hormone had no effect on K excretion in the urine of the sheep despite variations in K intake.

(iv) Effect of hormones on water and electrolyte excretion of sheep on different K regimes

(a) Vasopressin

Smith (1951) in reviewing the cases in which urine flow was reported to increase during the period of neurohypophyseal hormone action considered that such effects were due to anaesthetisation of the test animals, impurity of extracts and variations in water and electrolyte loading in these animals. Since that time, the diuretic effects of vasopressin (as a

pure synthetic compound or pure extract) in the dog and rat have been well documented (Jacobson and Kellogg, 1956; Brooks and Pickford, 1957; Duff, Grinnell and Kramar, 1965; Kramar, Grinnell and Duff, 1966; Grinnell, Kramar, Duff and Lydon, 1968). In the sheep arginine vasopressin or the commercial preparation, Pitressin, have been shown to increase electrolyte excretion and either increase or decrease water excretion according to the initial flow rate (Kinne, Macfarlane and Budtz-Olsen, 1961; Cross, Thornton and Tweddell, 1963; Gans, 1964; Peeters and Debackere, 1963; Macfarlane, Kinne, Walmsely, Siebert and Peter, 1967; Kuhn and Peeters, 1967). Stacy and Brook (1965) failed to find any salutetic effect of vasopressin.

During the present experiments, doses of Pitressin ranging from 0.7 to 6 mU/kg by I.V. injection or 3.5 to 20 mU/kg/hr by I.V. infusion produced the expected kaliuresis and at times, natriuresis in sheep fed lucerne chaff. These doses often produced a small diuresis when initial urine flow rates were below 1.5 to 1.0 ml/min but were normally antidiuretic at rates exceeding 1.5 ml/min.

When sheep F was receiving K supplements of 600 and 1200 m-equiv/day Pitressin did not produce obvious alterations in electrolyte output or urine flow. Any changes which did occur were obscured by normal variations in the excretory

pattern. However, sheep S and W when receiving these K supplements, at times, responded to doses of Pitressin as low as 2 to 10 mU, with increases in K and Na excretion. As the K intake was raised the increase in K output became less pronounced though the absolute rate of K excretion was initially higher. The increases in K excretion which Pitressin produced in sheep S occurred despite large reduction in urine flow.

Although at high K intakes, the effect of Pitressin on Na and K loss was variable, all sheep tested showed an increased excretion of  $H^+$  when receiving the 1200 m-equiv K supplement. Sheep S also increased its  $H^+$  excretion when the K supplement was 600 m-equiv/day. The period and magnitude of the increased  $H^+$  excretion depended on the individual animal.

Similar increases in  $H^+$  excretion following injections of Pitressin were observed in sheep on a low K diet. However, contrary to the findings in normal animals, Pitressin tended to reduce the urine flow even when the initial flow was less than 1 ml/min. Potassium and Na excretion in the low K animals was on occasions increased by Pitressin by as much as 100%. In terms of absolute rates of K excretion these increases were insignificant when compared with the increases produced in normal sheep.

Although stimulation of renal carbonic anhydrase may increase  $H^+$  excretion, it is not likely that this is the action of vasopressin in K supplemented and depleted sheep since it had no effect on urinary pH in sheep on normal K intakes, regardless of the initial urinary pH. Berliner, Kennedy and Hilton (1951) extended Pitts' (1945) theory of urinary acidification and proposed that the distal secretion of  $K^+$  or  $H^+$  was the result of competition between these 2 ions for secretion in exchange for  $Na^+$  being reabsorbed. From present results it is difficult to envisage vasopressin acting at this exchange site.

(b) Aldosterone

Kinne et al., (1961) and Macfarlane (1963) reported that aldosterone infusions had no effects on K excretion of normal sheep though it reduced Na excretion. Similar observations were made during the present investigations despite increases in the K intake and increased rates of urinary excretion. These findings are contrary to those found for the effects of aldosterone in rat, dog and man (Barger, Berlin and Tulenko, 1958; Williamson, 1963; Dawborn and Ross, 1967; August, Nelson and Thorn, 1958) when a kaliuresis often accompanies the reduction in Na excretion. Although aldosterone stimulates distal Na reabsorption this reabsorp-

tion does not take place at the site of Na-K exchange (Barger, et al., 1958; Williamson, 1963; Fimognari, Fanestil and Edelman, 1967). This point is clearly demonstrated by the present results as aldosterone had no effect on K excretion. However, this lack of effect indicates either a different mechanism of action of aldosterone or a different mechanism of K secretion in sheep compared with other species. Whatever the mechanism, aldosterone does not appear to regulate renal K excretion in the sheep.

(c) Cortisol

Just as the effects of aldosterone in sheep were different from those in other species, so were the effects of cortisol. Infusions of cortisol at rates between 60 and 1000  $\mu\text{g/hr}$  had no consistent effects on urine flow or electrolyte excretion of normal or K supplemented sheep. Cortisol produces variable alterations in the renal excretion of water and electrolytes of rats, dogs and man, depending on the initial patterns of electrolyte excretion and flow rate (Lipsett et al., 1961). There is no obvious explanation of the absence of any effects of cortisol on renal function in the sheep, but this lack of effect demonstrates that cortisol, like aldosterone, does not function in the regulation of urinary K loss.



(iv) Effects of pharmacological agents on renal function

(a) Acetazolamide

Acetazolamide appears to have an action on renal function of the sheep similar to that in man, rat and dog (Maren, 1967) since it produces a rise in urinary pH, an increased excretion of K and Na and a small increase in urine flow. Because the urine pH of sheep is often alkaline, the rise in pH is not as marked as in other species where the urine is usually acidic.

The alterations in renal function produced by acetazolamide are effected through its inhibition of the enzyme, carbonic anhydrase. Inhibition of this enzyme results in a reduced intracellular production of  $H^+$  which in turn causes a reduction in  $HCO_3$  reabsorption and an increased  $HCO_3$  excretion. Sodium reabsorption is also reduced following the fall in proximal tubule  $HCO_3$  reabsorption. The increased K excretion is probably the result of a reduced intracellular competition between  $H^+$  and  $K^+$  for secretion in exchange for  $Na^+$  (Maren, 1967).

In a K depleted sheep an infusion of acetazolamide produced a significantly greater loss of Na than K. This suggests that in the normal sheep, part of the Na delivered

to the distal tubule is reabsorbed in exchange for K but in the low K animal there is a reduced Na-K exchange and a larger rise in Na and smaller rise in K excretion than normal.

Partial inhibition of carbonic anhydrase must account for the high urine pH of normal sheep. This inhibition would also allow a greater secretion of K in the distal tubule and could provide part of the mechanism of regulation of K excretion by the kidney. Although there is some form of relationship between urine pH and [K], the K excretion may increase to very high levels without the pH rising. Furthermore, increasing the dose of acetazolamide beyond 100 mg does not increase either the pH or the rate of K excretion beyond certain limits, but the period of action is prolonged. The maximum rate of K excretion produced by acetazolamide may be exceeded by both normal and K supplemented sheep. Therefore, it is unlikely that the mechanism controlling carbonic anhydrase activity is the major regulatory factor in the control of K excretion by the kidney though it must play some part.

(b) Cyclothiazide

It has been proposed that the thiazide diuretics like the organomercurial compounds act

in both the proximal and distal tubule. This results in depression of distal and possibly proximal Na reabsorption and an increase in distal K secretion (Foulkes, 1965).

Cyclothiazide did not cause as significant a rise in Na, K and water excretion in the sheep as it does in the rat, dog and man. Similar findings were made by Cross and Thornton (1966a) using related thiazide compounds and two of the compounds they tested were almost completely ineffective. Thus it may be concluded that the thiazide diuretics either do not affect electrolyte movement in the tubules of the sheep kidney as they do in other species, or the mechanism which they do affect is not very important in the process of Na reabsorption and K secretion. This may indicate that the mechanisms of Na and K transport in the tubules of the sheep kidney vary in some way from those in other species.

(c) Ethacrynic acid

In common with the kidney of dog and man, the sheep kidney proved to be quite responsive to small doses of ethacrynic acid. This compound produced a massive natriuresis, chloruresis and diuresis, a small kaliuresis and a sharp reduction in urinary pH. It was originally postulated that ethacrynic acid produced its effects by inhibition of distal

and proximal Na reabsorption (Beyer, Baer, Michaelson and Russo, 1965). Komorn and Cafruny (1965) proposed that this inhibition occurred through the binding of active -SH groups. Although ethacrynic acid may partially inhibit tubular Na reabsorption the major part of the large natriuresis and diuresis observed in dog and man is due to inhibition of Na reabsorption in the loop of Henle (Goldberg, 1967). This inhibition removes the high OP of the medullary interstitium necessary for concentration of the urine. The current hypothesis of the mechanism of inhibition of Na reabsorption is that ethacrynic acid (and frusemide) inhibits the Na-K-ATPase of the tubules (Duggan and Noll, 1965).

The magnitude of the natriuresis and diuresis which followed the administration of ethacrynic acid to sheep suggests that it also inhibits Na reabsorption in the loop of Henle in the sheep. Nothing can be inferred from the effects of ethacrynic acid, about the mechanism and regulation of K secretion in the sheep kidney.

(d) Frusemide

The diuretic, frusemide, like ethacrynic acid, is reported to inhibit Na reabsorption in the loop of Henle of the kidney of dog, rat and man (Buchborn and Anastasakis, 1964; Deetjen, 1965; Berliner, Dirks and Cirksena, 1967). Frusemide

when administered to sheep produced an extremely large diuresis, natriuresis and chloruresis, a moderate kaliuresis and an increased  $H^+$  excretion. This suggests that its action in the sheep kidney is similar to that in the dog or rat kidney.

The increased  $H^+$  loss following ethacrynic acid or frusemide administration is probably the result of the increased Cl excretion produced by these compounds. Laragh, Cannon, Stason and Heinemann (1966) claimed that both diuretics inhibit Cl reabsorption in the proximal tubule, which results in an enhanced  $NaHCO_3$  reabsorption and increased  $H^+$  secretion. Laragh, et al., (1966) also suggested that this situation lends itself to superimposition of carbonic anhydrase blockage with a resultant increase in  $NaHCO_3$  excretion. Such an increase was found in the sheep following an injection of 100 mg of acetazolamide during a frusemide infusion. In addition, urine flow and K excretion were enhanced, suggesting that frusemide does not alter Na-K exchange in the distal tubule.

The increases in RPF and GFR observed in the sheep during the action of ethacrynic acid and frusemide were similar to those found in man by Vorburger (1964) and Hook, Ludens, Brody and Williamson (1966). It is not apparent

what part the alterations in RPF and blood redistribution in the kidney play in the effects of these diuretics (Birtch, Zakheim, Jones and Barger, 1967) though they may be related to the decrease in proximal Na reabsorption reported by Ullrich, Baumann, Leoschke, Rumrich and Stolte (1966). Berliner, Dirks, and Cirksena (1967) found that ethacrynic acid and frusemide had no effect on proximal Na reabsorption.

The magnitude of the electrolyte losses produced by infusion of ethacrynic acid and frusemide were such as to reduce plasma [K] and [Na], despite decreases in plasma volume (as shown by rises in PCV and plasma protein concentration). Plasma electrolyte concentrations and volumes were suppressed for some time after the end of an infusion and slowly returned to normal values. During this time, RPF, GFR and urine flow fell to very low levels before they too began to return to normal. This reduction in RPF and GFR illustrates an adaptive ability of the sheep kidney to control water and electrolyte loss when there are alterations in the ECF volume and concentration. Such a mechanism may function following feeding, when there are substantial changes in the ECF, and may in part regulate the excretion of K at this time.

(e) Epsilon amino caproic acid

Epsilon amino caproic acid and lysine did not produce hyperkalemia and a large kaliuresis in the sheep as

they do in dogs and rats (Carroll and Tice, 1966; Dickerman and Walker, 1962). <sup>Although</sup> it was hoped that these compounds would provide a method of acutely depleting tissues, in particular the kidney, of K, EACA did provide some useful information about K metabolism in the sheep.

Epsilon amino caproic acid produced an initial sharp transitory rise in RPF and GFR with a resultant transitory rise in K and Na excretion. Renal plasma flow, GFR and K and Na excretion then declined to below pre-infusion levels but rates of urine flow usually continued to rise with a resultant increase in free water clearance. The initial increase in renal excretion of K was insufficient to account for the marked decrease in plasma [K] from 4.5 - 5.0 m-equiv/l. to as low as 2.8 m-equiv/l. This decrease also occurred despite a large decrease in PV and ECV as shown by large rises in PCV, Hb concentration and plasma protein concentration. The rise in plasma [Na] of 9-11 m-equiv/l. could be accounted for by the fall in PV and ECV.

To explain the large change in plasma [K] it is necessary to postulate that whereas in dogs and rats, EACA displaced K from cells and produces hyperkalemia (Carroll and Tice, 1966; Claus, Skoza and Johnson, 1966) in the sheep it displaces K in the plasma forcing the displaced

K into tissue cells and reducing the plasma  $[K]$ . Since EACA and K are supposed to replace each other, the plasma  $[K]$  may not have been a true estimate of the "effective  $[K]$ " and it should be related to the  $[K]$  plus the  $[EACA]$ . The "effective  $[K]$ " may therefore have been quite high as may the effective K excretory rate. Estimates of the excretory rate of EACA were not made, but in dog and man, EACA is rapidly excreted in the urine (McNicol, Fletcher, Aikjaersig and Sherry, 1962).

Overall, these effects of EACA appear to confirm the postulated ability of the tissues of the sheep to absorb large K loads, such as occur after feeding, thus preventing too great a rise in extracellular  $[K]$ . Similarly the increased renal excretion of K following the cessation of EACA infusions and while the plasma  $[K]$  was returning to normal, may be analogous to the large kaliuresis which occurs after feeding.

The adaptive ability of the sheep to reduce RPF and GFR when the ECV decreases was again demonstrated after EACA infusions. These reductions in RPF and GFR were unlikely to be due to changes in ECF electrolyte concentrations since with EACA plasma  $[Na]$  rose whereas with frusemide and ethacrynic acid it fell. It thus appears that volume changes were effective in lowering the renal filtration.



Although the reductions in PV during frusemide and ethacrynic acid infusions were probably the result of the large urinary water loss, the much larger reduction in PV during EACA infusions was likely to be the result of an increased capillary permeability as well. This compound has been shown to produce these changes in dogs (Tice, Redisch and Carroll, 1965). Some circulatory failure resulting from the large reduction in PV would account for the large increase in pulse and respiration rate which occurred during and following administration of EACA. Increases in cellular  $[K]$  and "effective plasma  $[K]$ " may also have increased these rates in the same way as K infusions produced rises in pulse and respiration rate.

(vi) Combined effects of pharmacological agents and vasopressin on renal function

From the results obtained using frusemide and ethacrynic acid it would appear that both these drugs act by removing the high OP gradient of the medulla, thus preventing osmotic water reabsorption from the distal tubule and collecting duct.

This concept was substantiated by the inability of vasopressin to reduce the high urine flows produced by both these diuretics. Vasopressin is thought to increase

the permeability of the distal convoluted tubule and collecting duct to water and the resultant reabsorption of water is a passive process dependent on the medullary osmotic gradient (Wirz, 1961). Frusemide and ethacrynic acid by removing the high medullary osmotic gradient prevented vasopressin from increasing water reabsorption though it may still have increased tubule permeability.

In spite of the failure of vasopressin to reduce high rates of urine flow, the increase in excretion of K normally observed under the action of vasopressin in ruminants still persisted. Increases in K excretion were not as large as usual, but the excretory rate was already elevated due to the action of the diuretics. An injection of 200 mU of Pitressin during one frusemide infusion, as well as increasing K excretion, produced a further rise in Na and Cl excretion and a small but significant rise in water output. Pitressin had no effect on the low urine pH produced by either diuretic.

The increase in K excretion caused by vasopressin during a frusemide or ethacrynic acid diuresis substantiates the proposal of Cross and Thornton (1966a) that the effects of vasopressin on water and electrolyte excretion in the sheep may be dissociated. Furthermore, the sites of action of vasopressin in the kidney are likely to be separate from those of

frusemide and ethacrynic acid. That is, vasopressin does not reduce Na absorption in the loop of Henle. Likewise, vasopressin and cyclothiazide are unlikely to have the same sites of action since in 2 experiments, vasopressin was able during a cyclothiazide infusion to increase K, Na and water output. These results were inconclusive since the dose of cyclothiazide used was not the maximum effective dose and cyclothiazide itself proved to be relatively ineffective in altering urine flow and electrolyte output.

and Tweddel

Cross, ~~and~~ Thornton (1963) postulated that vasopressin increased electrolyte output by increasing the quantity of Na reaching the distal Na- K - H exchange site. The increased electrolyte output was then expressed predominantly as an increase in excretion of either Na or K depending upon the pattern of electrolyte excretion at the time of administration of vasopressin.

Their later hypothesis (Cross and Thornton, 1966a) concerning the production of the increase in distal delivery of Na is still open to question. They made several assumptions based on findings in the rat (Cross, 1964; Cross and Thornton, 1966b) and on the fact that a high [Na] occurs in the papillae of sheep excreting concentrated urine (Schmidt-Nielsen and O'Dell, 1961). Furthermore, the

results they obtained in rats were in contrast to those found using dogs (Boylan and Asshauer, 1962; Goldberg, 1967).  
 and Tweddell's  
 Cross, ~~and~~ Thornton (1963) original hypothesis that vasopressin increases the distal delivery of Na still appears functional, though on the basis of the present results there are some difficulties associated with the proposal that the increased distal delivery of Na then results in an increased Na-K exchange.

Three points arising from the present results which seriously question this proposal are (1) the delay between the peaks of K and Na excretion produced by vasopressin when the rates of K excretion are low (2) the reduced effects of vasopressin when the rate of urinary K excretion was very high and (3) the absence of any effect of vasopressin on urinary pH in the normal sheep though a variable reduction in pH in "high" or "low" K sheep.

The delay between the peaks of the kaliuresis and natriuresis is difficult to explain if the increased K secretion is the result of an increased distal delivery of Na and an increased Na-K exchange. Both increases in excretion should be simultaneous unless there is an initial increase in Na-K exchange preventing a rise in Na excretion and reducing the cellular level of K.

This in turn may reduce K excretion and allow Na excretion to rise to a maximum (Several other objections to K-Na exchange being the primary means of distal K secretion have been raised elsewhere).

Malnic, Klose and Giebisch (1966a) seriously queried the idea that increasing the distal tubule [Na] should increase Na-K exchange as Na is not the limiting factor in the rate of K secretion and Na-K exchange does not occur on a one-to-one basis. Potassium secretion into the distal tubule depends on the transtubular potential and consists of passive transfer from cell to lumen and carrier mediated, active uptake from the lumen into cells. Active Na extrusion out of the cells and K uptake into the cell is postulated at the peritubular side of distal cells (Malnic, et al., 1966a,b,; Windhager, 1969). Alterations in K secretion produced by vasopressin could thus result from alterations in the passive transfer of K into the tubule, a reduction in active reabsorption from lumen into cells or an increased uptake of K by cells from the plasma.

Stimulation of the passive entry of K into the lumen is likely if there is a rise in the electronegativity of the tubule or an increase in the intracellular [K]. Intracellular K is the source of secreted K (Black, Davies,

Emery and Wade, 1956; Orloff and Davidson, 1959; Malmic, et al., 1966a). Vasopressin by increasing the rate of delivery of Na to the distal tubule would increase the electronegativity of the distal tubule and increase the rate of passive K secretion. This mechanism would explain the reduced effects of vasopressin on K excretion in K supplemented and depleted sheep. In the "high" K sheep, the passive entry of K into the distal tubule lumen may be near a maximum. Thus vasopressin would be unable to produce a very significant rise in K secretion. Conversely in the "low" K sheep the source of K for passive secretion, intracellular K, may be reduced and hence vasopressin could not increase the rate of K secretion to any great extent. Alternatively, any rise in K secretion in the distal tubule may have been reduced by the large rates of K reabsorption in the collecting duct, which it is necessary to postulate to account for the observed rates of K loss in "low" K sheep.

Although this mechanism may explain some of the observed facts it does not exclude the possibility of vasopressin directly altering the peritubular transport of K, the luminal membrane permeability to K, the rate of K reabsorption in the distal tubule or collecting duct, or the rate of Na-K exchange in the distal tubule even though not on a one-to-one exchange basis.

None of these alterations provide an explanation of the increased  $H^+$  excretion found in both the "low" and "high" sheep during vasopressin administration. In "low" K sheep intracellular K may be limiting and hence alterations in distal transtubular potentials whether produced by an increased distal delivery of Na or otherwise may have resulted in an increase in active  $H^+$  secretion. Such an explanation would not account for the increase in  $H^+$  secretion in "high" K sheep when the supply of intracellular K was unlikely to be limiting, unless different Na-K-H exchange systems are involved in K supplemented and K depleted sheep.

From the present results, it is clear that vasopressin produces more changes in renal tubular function than had previously been visualised. How these alterations are brought about is still uncertain. Nevertheless vasopressin does possess the ability to increase K excretion in sheep, the extent of the increase depending on the initial rates of K loss and the K status of the animal. It is the only hormone so far tested which does significantly alter K excretion and the possibility therefore still exists that as well as assisting in the maintenance of renal water loss it functions in the regulation or maintenance of K excretion in the sheep.

#### IV SUMMARY

As pointed out by Ward (1966) in his review of potassium metabolism in domestic ruminants, "ruminant animals, subsisting as they do on a high-roughage diet, may have a potassium intake throughout their lifespan which is many times their dietary requirement". Until recently this large potassium intake was studied in terms of possible toxic effects. Little was known concerning the true maintenance requirements of potassium in ruminants, what effects the normally large potassium intakes had on water and electrolyte metabolism as a whole or what physiological mechanisms allowed the ruminants to tolerate and excrete the excess potassium ingested. Therefore, a study was undertaken to investigate the tolerance of sheep to alterations in dietary potassium and the effects of these alterations in potassium intake on body fluid and electrolyte metabolism. Alterations in urinary excretion and possible mechanisms of potassium secretion and their regulation in the kidney were studied. in association with these investigations.

Increasing the daily potassium intake of sheep over the range of 450 to 2000m-equiv/day produced an almost linear increase in water intake, turnover and urine volume. Presumably as a result of increased water turnover there was a rise in total body water,



total body water as a percentage of body weight, extracellular volume and extracellular volume as a percentage of body weight. Plasma volume was not altered. The plasma sodium concentration and osmotic pressure decreased as the potassium intake rose, though the plasma K concentration remained unchanged. Since the extracellular volume rose the total quantity of sodium in the extracellular fluid remained constant whereas the total quantity of potassium rose.

Determinations of salivary composition in 2 sheep prior to feeding and 4-6 and 8-10 hr after feeding commenced showed a reduction in the Na:K ratio with an increased potassium intake. This reduction was less in the sheep which had continual access to salt. These results were taken to indicate that although there was no demonstrable sodium deficiency, a high potassium intake may produce a "sodium deficiency type effect" and that aldosterone or related mineralocorticoid secretion may have been increased.

Increasing the potassium intake produced large alterations in rumen electrolyte concentrations, the prefeeding rumen sodium concentration declined while the potassium concentration rose. At the maximum level of potassium supplementation rumen sodium concentration was 27-35 m-equiv/l. and the rumen potassium concentration 115-120 m-equiv/l. Somewhat similar, though much less marked, alterations occurred in the rumen of

unsupplemented sheep following feeding. In supplemented sheep feeding also caused a further fall in rumen sodium concentration and rise in potassium concentration but these changes were smaller than those in the normal sheep. It was concluded that the decline in rumen sodium concentration, which resulted from an increased potassium intake in the food was due in part to stimulation of sodium reabsorption by the high rumen potassium concentration.

The increased absorption of electrolytes from the rumen and possibly lower gut segments, following feeding, resulted in increases in plasma concentrations of sodium and potassium and plasma osmotic pressure. These increases in plasma concentrations persisted from 8 to 18 hr depending on the period of eating.

An increased potassium uptake from the rumen was demonstrated using  $^{42}\text{K}$ . Equilibration of  $^{42}\text{K}$  between the rumen and blood was rapid following the addition of 400 m-equiv of potassium to the rumen. However, similar additions of potassium produced no detectable alterations in the uptake of  $^{24}\text{Na}$  from the rumen.

TOH uptake from the rumen was used to assess whether the addition of potassium to the rumen altered water movement to and from the rumen. Although there was some reduction in total water movement following the addition of potassium to the rumen, it

was not statistically different from normal. Nevertheless, a positive relationship between rumen volume and total water flux was derived. The mean total water movement in 14 measurements on 6 sheep from a mean rumen volume of 5.58 l. was 2.64 l./hr.

It was demonstrated that the rumen wall provided a much greater barrier to the movement of  $^{42}\text{K}$ ,  $^{24}\text{Na}$  and TOH than does the epithelium of the duodenum, ileum, and upper and lower colon. Notwithstanding, it was shown that rumen transepithelial water movement was considerable and could account for much of the equilibration of TOH between blood and rumen. Considerable variation in rumen wall permeability was also observed.

As the water intake and water turnover rose with increases in the quantity of ingested potassium, so did urine volume. The urinary excretion of potassium was maintained at 97% or more of the total urinary plus faecal potassium loss, and urinary potassium loss was proportional to the potassium intake. Due to the rise in urine volume there were no large changes in urine potassium concentration, though sodium concentration fell. However, the total output of sodium in the urine was proportional to the sodium intake. Individual animals showed considerable variation in water turnover and urine volume and hence potassium concentration, but this was not due to a limitation

of the kidney to concentrate potassium in the urine. Faecal excretion of potassium was unaltered by changes in potassium intake and the faecal sodium excretion only rose very slightly. Raising the potassium intake did produce a rise in faecal water excretion but no significant alteration in dry matter digestibility.

The pattern of urinary potassium loss following feeding was similar to that of plasma potassium concentration. Although the rise in plasma sodium concentration was independent of rises in potassium intake, the post-feeding kaliuresis increased in magnitude. Lack of correlation between the post-feeding rise in plasma potassium concentration and potassium intake could partially be accounted for in terms of the increased urinary loss, but the resting rate of urinary potassium excretion was higher in potassium supplemented than normal sheep when plasma potassium concentration was the same. It was, therefore, postulated that at the higher levels of potassium intake tissues were able to absorb an increased quantity of potassium. This prevented unduly large rises in plasma potassium concentration.

This hypothesis was extended to include the kidney. Since intracellular potassium is the source of secreted potassium, this may have aided the production of the higher resting and post-feeding rates of potassium excretion in potassium supple-

mented animals. There is no apparent explanation of why the cellular absorption of potassium should be increased in potassium supplemented animals unless it is due to the proposed rise in aldosterone secretion or some other hormone. It is unlikely that aldosterone is the hormone responsible, since it does not increase renal potassium excretion in the sheep.

Support for the postulate of increased potassium uptake by cells came from investigation of the effects of epsilon-amino-caproic acid. Infusions of this compound (an analogue of L-lysine, which in dogs and rats replaces potassium in cells causing hyperkalemia and a large kaliuresis) caused a 30-50% reduction in the plasma potassium concentration and reduced potassium excretion in sheep. Presumably potassium was displaced from the plasma and absorbed intracellularly.

Urinary excretion of electrolytes following an intraruminal load of potassium was irregular compared with the changes after feeding. A kaliuresis did occur but not at any regular time after the addition of potassium to the rumen. Once again these were rises in plasma potassium concentration, but these too were irregular. Urinary sodium loss was unaffected by the addition of potassium to the rumen yet a natriuresis occurred after feeding. This difference in sodium excretion appeared to be related to changes in rumen and plasma sodium concentration,

which were altered by feeding, but little affected by potassium loading.

Various pharmacological agents and natural hormones were administered to the sheep to investigate the possible mechanisms and control of renal potassium excretion. The carbonic anhydrase inhibitor acetazolamide was found to produce variable increases in the urine pH depending on the initial pH and enhanced excretion of sodium, potassium and water with reduced chloride excretion. It appeared that when the urine pH of sheep is high there must be considerable inhibition of carbonic anhydrase in the kidney, which allows a greater rate of potassium excretion presumably by increasing sodium:potassium exchange in the distal tubule. However, increases in potassium excretion produced by large doses of acetazolamide did not produce rates of potassium excretion as high as those found in potassium supplemented sheep or after feeding. Therefore, although alterations of carbonic anhydrase activity may play some part in regulating potassium it is not a major regulatory mechanism.

In common with other thiazide diuretics, cyclothiazide administration produced only small alterations in the renal excretion of potassium, sodium and chloride and water. These compounds are either less effective in inhibiting sodium and potassium reabsorption or in enhancing potassium secretion in the

renal tubules of the sheep than in other species or the tubular mechanism involved in sodium and potassium reabsorption or potassium secretion in the sheep kidney are different to those in other species. Either conclusion suggests a difference between renal function in the sheep and that of dog, rat or man.

Both the diuretics, ethacrynic acid and frusemide increased the electrolyte and water loss in sheep as they do in other species, apparently by inhibiting sodium reabsorption in the loop of Henle and thus removing the high medullary osmotic gradient necessary for urine concentration. Support for this mechanism was gained by the observation that the administration of vasopressin, during infusions of either compound, did not decrease the high urine flows they produced.

Although vasopressin did not reduce the high urine flow produced by ethacrynic acid or frusemide it still slightly enhanced the already elevated rate of potassium excretion. Thus the well documented effects of vasopressin on water and electrolyte excretion can be dissociated. It is likely that vasopressin acts to increase potassium excretion by direct stimulation of tubular secretion or inhibition of tubular reabsorption. The possibility that sodium-potassium exchange is stimulated cannot be excluded but is unlikely. Of the 3 natural hormones tested, vasopressin was the only one

to alter potassium excretion. Aldosterone increased sodium reabsorption and decreased sodium excretion while cortisol had no consistent effect on urinary excretion of either water, or electrolytes regardless of potassium intake.

The kaliuretic effect of vasopressin was reduced as the potassium intake/day rose or when sheep were potassium depleted. Initial rates of potassium excretion, except in potassium depleted animals largely determined the magnitude of the kaliuresis produced by vasopressin. The higher the rate of potassium excretion prior to vasopressin administration the smaller was the kaliuresis. This suggested that vasopressin stimulated potassium secretion and that when the rate of potassium secretion is already high further stimulation results in only a small increase in secretion. Potassium depletion did not remove the kaliuretic effects of vasopressin but responses were irregular and much smaller than normal. No unitary theory to explain the increased hydrogen ion excretion caused by vasopressin in potassium depleted or potassium supplemented sheep is apparent.

The actual mechanisms responsible for the increased electrolyte excretion produced by vasopressin remain unknown. Nevertheless, vasopressin is capable of enhancing electrolyte, in particular potassium, excretion in the sheep and hence may play a small part in the regulation of K excretion.



Therefore, although it was shown that alterations in potassium intake of the sheep do affect body fluids and electrolyte metabolism and it appeared that the tissue cells were capable of increased potassium absorption during acute potassium loading other mechanisms by which sheep may tolerate large and often acute loads of potassium remain for future investigation.

### Conclusion

Sheep are able to tolerate quite successfully large daily intakes of potassium. This tolerance extends to levels of potassium beyond those likely to be ingested by the grazing ruminant. The reason for this tolerance is not fully understood, but depends in part on the fact that:-

- 1) when the potassium intake was raised there was a proportional rise in water intake, water turnover and urine volume
- 2) as a result of the increased water turnover, there was an increase in the total body water due to an expansion of the extracellular volume
- 3) an apparent aldosterone response to a high potassium turnover may have produced alterations in salivary composition and caused the expansion of extracellular volume
- 4) a high potassium intake increased potassium and sodium uptake from the rumen. Alterations in sodium absorption appeared to depend on potassium ingestion by feeding rather than by direct addition to the rumen.

- 5) a high rumen potassium concentration produced by direct addition of potassium salts to the rumen did not discernably alter the water flux in the rumen. Nevertheless a considerable transepithelial movement of water was detected.
- 6) much of the excess potassium absorbed from the digestive tract at high potassium intakes was finally excreted in the urine. This was achieved without undue concentration of the potassium in the urine since the increased water intake produced by a high potassium intake, allowed a larger urine volume
- 7) at high potassium intakes there was greater storage of potassium in tissues prior to excretion. Epsilon-amino-caproic acid (which behaved differently in sheep from rats and dog) caused transfer of potassium from the plasma into cells.
- 8) Vasopressin caused an increased potassium excretion in normal, potassium depleted and potassium supplemented sheep. This effect persisted during the maximum saluresis and water diuresis induced by frusemide and ethacrynic acid.
- 9) Vasopressin produced an increased K output during frusemide and ethacrynic acid administration without reducing water flow so that these two actions appear to be separate in the sheep.
- 10) Vasopressin appears to be the only known hormone affecting potassium excretion in the sheep, since aldosterone and cortisol are without effect.

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## A D D E N D U M

### EXAMINERS' COMMENTS

The study of the renal response to certain drugs and hormones yielded results of considerable interest. The effects of vasopressin in provoking either an increase or decrease in urine volume are clarified in relation to the pre-dosing rate of urine formation, and a kaliuretic effect of this substance was clearly demonstrated. The candidate concludes that vasopressin was the only hormone of those tested which affected potassium excretion, for neither cortisol nor aldosterone were shown, under the experimental conditions of this study, to influence potassium excretion.

I feel this conclusion is inadequately discussed and it raises a number of questions:

1. Does the candidate regard the evidence of these infusion experiments to be definitive?
2. For how long were aldosterone infusions sustained? (This information is not given).
3. Was the duration of infusion long enough for the effects of the exogenous hormone to become manifest, as evidenced, for example, by an induced alteration in the salivary sodium:potassium concentration ratio?

4. With respect to the actions of endogenous hormone, what effect on urinary potassium excretion have aldosterone antagonists such as spiro lactones?
5. There is no description of the calibration and standardization of the continuously recorded pH, Na and K measurements in urine. Presumably some checks were made of the calibration during or at the end of a period of measurement to determine the presence or absence of drift.
6. On p. 60 the candidate describes the use of sublimed water in estimations of tritiated water. The Shorter Oxford Dictionary defines sublimed as: "that has undergone the chemical process of sublimation", and Chambers Technical Dictionary defines sublimation as: "vaporisation of a solid without the intermediate formation of a liquid". It seems therefore incorrect to use the word sublimed in the context of this assay when presumably it was condensed water that was used.
7. With reference to the electrolyte estimations, the candidate gives no indication of how long the samples were stored at  $-5^{\circ}\text{C}$  until analysed (p. 51) (urine at  $+5^{\circ}\text{C}$ , p. 53). It is possible that some of the variations in plasma K (p. 68) and in other parameters could have been caused by varying periods of storage of the samples and this would also depend on the type of bottle (polythene or glass) used and the method of sealing the containers.

8. The method used for chloride estimations appears to have been omitted.
9. P. 69 - Did the candidate find any differences in haematocrit values which would produce variability in the estimations of whole blood potassium.
10. P. 82. - The plasma (K) of 7.7 seems very high for normal sheep even when fed high K. Unfortunately, the candidate gives no indication of the likely error of his analyses nor even if they were duplicate or single estimations. These facts will obviously influence the reliability of the experimental observations.
11. The method of K salt administration is not clearly stated. In some experiments the lucerne hay was apparently treated (p. 54) with varying quantities of KCl,  $\text{KHCO}_3$  or KAc solutions. Was this sprayed on to the hay and at what concentration? In later experiments (p. 89) K salts were added to the rumen - and it is only in the discussion that one is told that the salts were given in 150 ml of water.
12. In these experiments (p. 90) it is not clear if the sheep were fed following the dosing with K salts.
13. In Figure 18 the scale for plasma (K) appears to be too big i.e. 40 instead of 4.

14. P. 93 - It is probably more correct to say that no osmotic gradient existed in the gut solutions, unless the composition was the same as plasma.
15. P. 101. - refers to an infusion and injections of pitressin illustrated in Figures 34 and 35 but the infusion appears to have been omitted.
16. P. 102. - "her urine output and flow rate were always higher".
17. It is most unfortunate that the candidate relied on a commercially available product, Pitressin, for his studies of the action of vasopressin. This product contains varying amounts of lysine and arginine vasopressin and can also have oxytocic activity. It would seem that the interpretation of the results as a product of vasopressin activity is weakened by the use of this variable product. It is also weakened, as the candidate appears to recognise, by the results obtained on sheep F (Fig. 36) where 100 m.u. of pitressin caused an initial fall in Na, K and chloride excretion.

ANSWERS TO QUESTIONS AND QUERIES OF EXAMINERS

1. & With respect to the experimental conditions under which  
2. aldosterone and cortisol were administered, I would regard the experimental evidence as definitive.

Aldosterone and cortisol infusions were sustained for periods of from 2 to 4 hours, periods sufficient to provide for a substantial reduction in sodium excretion. As pointed out in the discussion, previous reports have also stated that although aldosterone produces a fall in sodium excretion it does not cause an increase in potassium loss in the urine (Kinne, Macfarlane and Budtz-Olsen, 1961; Kinne, 1963; Macfarlane, 1963), such as occasionally occurs in rat and dog.

3. Although changes in the salibary potassium:sodium ratio were not measured during aldosterone infusions, Blair-West and co-workers (1963) have shown that this ratio changes within 1 to 2 hours of the start of infusions. A similar delay is seen when aldosterone reduces renal sodium excretion. Therefore the periods of infusion would appear to have been sufficient for any effects on potassium excretion to have become manifest.

4. The effects of aldosterone antagonists such as spironolactone were not tested although an attempt to suppress endogenous aldosterone secretion was made (p. 105).

Overall there are still a number of problems relating to the possible effects of aldosterone on tissue potassium levels which require



further research. Unfortunately, the hypothesis, that aldosterone (or some other hormone) may be increased by a high dietary intake of potassium and that this in turn may alter the cellular absorption of potassium, was only considered in the latter stages of the investigation, when the period of time required for further detailed work was limited.

5. The pH meter and chart recording of pH was standardized by passing standard pH buffer solutions through the pH electrode cell. Checks on the calibration were normally carried out before and after experimental periods and no drifting was detected provided the pH electrode and cell were cleaned regularly.

Since the sodium and potassium electrodes were only used to provide an indication of the alterations in urine sodium and potassium concentration, no definite calibration of these electrodes was made (all absolute concentrations were determined later by emission or absorption flame spectrophotometry). Electrodes were checked for change in concentration response by using either sodium or potassium standard solutions alone or combined.

6. Sublimed water or the term sublimation, as used in the text, does have the same meaning as that given in the Shorter Oxford Dictionary and Chambers Technical Dictionary.

In the process of preparing tritiated water from tritiated whole blood a sublimation procedure was used. The method involved the

freezing of tritiated whole blood (solid) in one arm of an evacuated, inverted "U-tube". The second arm of the tube was placed in liquid nitrogen. Heating the frozen whole blood, by exposing this arm of the tube to room temperature, produced tritiated water vapour, which re-solidified as tritiated "ice" in the much colder second arm of the tube. On removal of the tube from the liquid nitrogen the tritiated ice melted to produce sublimed water.

7. It was realised that prolonged storage of samples could possibly cause variations in the plasma potassium concentration or other parameters. For this reason all samples were analysed as soon as practically possible after collection. Plasma protein concentration, as measured by refractive index, was determined on the day of collection. All analyses of a particular sample type, e.g. plasma, were carried out together to minimise further possible variations due to storage.
8. All chloride determinations were carried out using a Cotlove potentiometric chloridometer.
9. Consideration was not given to the possibility that part of the variability in whole blood potassium concentration was due to alterations in haematocrit. There were considerable differences in haematocrit between sheep, so it is likely that part of the variation in the concentration of whole blood potassium between sheep was due to these differences.

10. The plasma potassium concentration of 7.7 m-equiv./l. was exceptionally high but when estimates such as this were obtained analyses were repeated. Duplicate estimates of plasma and whole blood electrolytes were made and when there was a large discrepancy between duplicates, analyses were repeated.

Referring to the value of 7.7, it should also be noted that this sheep had the lowest water intake of all animals and responded least to increases in potassium intake (related to total potassium intake). Also in this particular instance, the plasma osmotic pressure and sodium concentration were the highest measured.

11. In the pilot experiment single salts of potassium were sprayed onto the chaff. Unfortunately I do not have on hand the concentrations of the solutions used.

When all three salts of potassium were added together, a mixture of the dry salts was added directly to the lucerne chaff in the mechanical mixer. The hygroscopic nature of potassium acetate provided sufficient moisture for a ready adsorption of all three salts to the ground material.

The method of addition of potassium salts directly to the rumen is given in the methods, although the volume (150 ml) is not expressly stated.

12. The omission of the fact that animals were not fed after the addition of potassium salts to the rumen is an oversight on my part. It was intended that the quantity of potassium added to the rumen would be "equivalent" to that ingested during rapid feeding.
13. The scale is in error and 40 should read 4.0 (etc.).
14. I agree that osmotic or perhaps isosmotic would be a more correct term.
15. On the original Fig. 35 an infusion was illustrated but when it was decided to show the effects of Pitressin during a saline diuresis, this segment was deleted and the word infusion was not removed from the text - i.e. it should read, "effects of injections of varying ----".
16. A grammatical error and "were" should have been used.
17. Although Pitressin does contain both lysine and arginine vasopressin, it is standardized, with regard to pressor activity, in the same manner as pure lysine or arginine vasopressin. Therefore, since both lysine and arginine vasopressin have identical effects

on renal function in the adult sheep, when administered separately, and oxytocin is without effect (Kinne, Macfarlane and Budtz-Olsen, 1961; Kinne, 1963; Macfarlane et al, 1967), the conclusions drawn with respect to vasopressin (Pitressin) seem completely valid.

With regard to the results using sheep F (Fig. 36), it is true that they did not demonstrate any clear cut effect of vasopressin, especially when the animal was receiving potassium supplements. However, as pointed out and perhaps misinterpreted by the examiner, random fluctuations in urine flow made it difficult to observe a specific response to vasopressin. In view of the continued low urine output of this sheep during potassium supplementation and the much smaller decrease in urine pH following vasopressin injections, at the maximum potassium intake (cf. sheep S and W), it can be suggested that the endogenous secretion of vasopressin was high and hence masked the effect of endogenous vasopressin.

References given are included in the list of References in the thesis.