WATER AND ELECTROLYTE TRANSFERS IN RUMINANTS

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
B.P.	blood pressure
CSF	cerebrospinal fluid
DOCA	deoxycorticosterone acetate
ECF	extracellular fluid
Fig	figure
GFR	glomerular filtration rate
gm	gram
Hb	haemoglobin
ICF	intracellular fluid
i/v	intravenous
kg	kilogram
l	litre
m-equiv	milliequivalent
min	minute
ml	millilitre
mosm	milliosmole
M	molar
O.P.	osmotic pressure
PAH	para-amino hippuric acid
PCV	packed cell volume
P.D.	potential difference
RBF	renal blood flow
S.G.	specific gravity
[ ]	concentration of

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PREFACE

At the present time, the economy of New Zealand is largely dependent upon the health and well-being of the ruminant animal. The national loss due to primary water and electrolyte disturbances, and to those secondary to other diseases, is of considerable importance. Deficiencies in our knowledge of water and electrolyte metabolism in the ruminant have become apparent, even of the principal cations sodium and potassium.

The specialized form of nutrition in the ruminant has entailed some changes in the water and electrolyte economy. In adapting to a diet of plant material rich in cellulose, they have developed a large forestomach, the reticulo-rumen, where a symbiotic population of bacteria and protozoa is maintained and exploited. Microbial fermentation breaks down plant cellulose, and converts carbohydrate to volatile fatty acids, principally acetic, propionic and butyric acids, which are rapidly absorbed by the host for use as an energy source. Microbial protein and certain vitamins are also made available further down the gastro-intestinal tract.

The development of the reticulo-rumen has resulted in an increase in the content and daily turnover of gut water and electrolytes. A major source of this content of the reticulo-rumen liquor, which provides a well-buffered medium for the microbes, is the copious salivary flow. The rumen of the sheep may contain 500-800 m-equiv of Na^+, approximately half that in the extracellular fluid. The daily digestive cycle of salivary secretion and later reabsorption may involve double this amount of Na^+.

The maintenance of water and electrolyte balance in the face of this

digestive cycle, coupled with the low Na^+-high K^+ content of the diet, suggests that the ruminant has efficient homeostatic mechanisms in operation. Whether or not these are identical with those seen in other species, or have features unique to the ruminant, is not clear. While the enlarged digestive cycle would appear to impose an extra load on the regulatory mechanisms, the presence of the reticulo-rumen might confer advantages during times of stress, when rumen fluid can be called upon as a reserve of water and electrolyte. During dehydration, the ECF can draw upon gut fluids (Macfarlane, Morris and Howard, 1963; Hecker, Budtz-Olsen and Ostwald, 1964); and the rumen forms a Na^+ store which can be drawn upon during reduced dietary intake (Denton, 1957; Kay and Hobson, 1963). The ability to repair a water deficit of up to 10% body weight (more in camels) within minutes would be an advantage in the natural environment.

Investigation in ruminants of the overall regulation of water and electrolyte metabolism has not been extensive. Most of our knowledge has been derived from studies of man, rodents and the dog. In the ruminant, more commonly, the longer term adjustments to dietary deficiency or supplementation, or to altered environmental conditions have been followed; less often, short-term water and electrolyte redistributions and mechanisms of elimination and conservation in varying physiological situations have been studied.

Some properties of the ruminant digestive tract, particularly regions of net addition and absorption, and the characteristics of rumen epithelial transport have been identified. However, in many cases the particular experimental situations employed make generalization uncertain. Thus, net transport is commonly estimated using a simple solution in an isolated, emptied and washed rumen or rumen pouch, a procedure providing an

abnormal environment for the rumen mucosa, and which can alter its transport properties (Masson and Phillipson, 1951; Armstrong, Blaxter and Graham, 1957; Ash and Dobson, 1963). Studies in the intact organ under physiological conditions present practical difficulties because of the continuous inflow of saliva and outflow to the omasum, and the lack of uniformity of composition of the contents in different regions of the reticulo-rumen. Accurate estimation of the rumen volume at any particular time is not an easy task. Direct measurement by total removal has a limited application, and marker dilution is only accurate during periods of relative constancy. In addition, to calculate a change in total electrolyte content an average ruminal concentration is required, although a uniform electrolyte concentration in the rumen is not usually a physiological reality.

The present thesis is concerned with short-term transfers, especially water and electrolyte movements between the contents of the reticulo-rumen and body fluid compartments. The rumen water and electrolyte status was altered rapidly in two ways: by once-daily feeding whereby there was net gain in the rumen at the expense of body fluids; and by infusion of known amounts of electrolyte. Net gain or absorption from the rumen has been followed by observing changes in urinary excretion and in blood composition. The rumen itself has not been sampled; it was considered that the advantages of direct rumen observations would be outweighed by the experimental errors and by the disturbance to the animal caused by the sampling. In the undisturbed sheep, relative plasma volume estimations can be inferred from the PCV and $[Hb]$.

Urine and blood changes associated with a single daily feed (Chapter 2) have confirmed and extended the observations of Stacy and Brook (1964), and are in agreement with later observations from that group. An attempt was

made to gain further information about the homeostatic mechanisms involved by the use of the diuretic, acetazolamide, and by restriction of drinking water. The relevance of these to variations seen in ad libitum fed animals was examined (Chapter 3).

Prior to the present experiments only isolated water and electrolyte infusions had been performed in ruminants (Sellers and Roepke, 1951; Lysov, 1960; Anderson and Pickering, 1962a) although more reports have appeared while the work was in progress (Potter, 1966, 1968; Kaynes and Harrison, 1967; Dewhurst, Harrison and Kaynes, 1968). A series of intraruminal infusions of water, NaCl and KCl has been carried out (Chapter 4), integrated with the intraduodenal infusion of NaCl (Chapter 5) and the intravenous administration of sodium and potassium salts (Chapter 6). It would appear that, with the exception of sodium, net water and electrolyte movements across the rumen mucosa in physiological situations may be small in magnitude. Should this be so, then sensory receptors in the forestomach may be involved only in regulating the functions of the digestive tract itself, and not in the overall regulation of water and electrolyte metabolism. Thus, the homeostatic mechanisms in the ruminant may more closely approach those in the monogastric than would be likely if the rumen permitted freer exchange with the internal body fluids.

CHAPTER 1

WATER AND ELECTROLYTE METABOLISM IN RUMINANTS

The outstanding anatomical feature of the ruminant digestive tract is the large stomach made up of four compartments - the reticulum, rumen, omasum and abomasum. The reticulo-rumen, commonly referred to for simplicity as the rumen, forms a large fermentation vat. Fermentation continues in the omasum, which also is a propulsive and absorptive organ. The abomasum is the only compartment to have a secretory epithelium, which forms gastric juice as in the monogastric stomach.

This thesis is concerned with the water and electrolyte transfers in sheep consequent upon altering the rumen composition by once-daily feeding or electrolyte administration. This involves movements across the membranes separating several body fluid compartments in the animal. Relevant topics reviewed in this chapter include absorption from the digestive tract, particularly from the reticulo-rumen and omasum, shifts of water and electrolytes between extracellular and intracellular compartments, and the homeostatic mechanisms regulating excretion of the principal electrolytes and body composition. Apart from knowledge of the specialised regions of the ruminant digestive tract, most of this information has been obtained in non-ruminant species.

I. Water and electrolyte transport across the gut wall

In recent years, following the application of more precise criteria, considerable advances have been made in the study of electrolyte transport

across biological membranes. It is now recognized that electrolytes can move across membranes by five mechanisms: passive diffusion, solvent drag, facilitated diffusion, active transport and exchange diffusion. In retrospect, much of the earlier work on water and electrolyte transport in the gastro-intestinal tract is considered inconclusive because of incomplete data on ion activity, electrical potential and the effect of solvent drag. This was the case with many studies on the rumen, where movement against a concentration gradient was considered evidence of active transport.

1. Reticulo-rumen

Net transport of water and electrolytes across the rumen mucosa has usually been detected by one of two methods: measurement of concentration differences in arterial and rumen venous blood; or measurement of changes in composition of rumen fluid. Both these methods must be considered gross and indirect; direct measurements of the characteristics of the epithelial membrane itself have not been possible in vivo. The preparations used have included animals with chronic rumen fistulae, rumen pouches, and in acute experiments the isolated, emptied and washed rumen. More recently, studies of transport across the membrane itself have been attempted, using sheets of stripped rumen epithelium.

While such experiments probably define adequately the characteristics of rumen electrolyte transport, the procedures used would preclude quantitative accuracy. The pre-treatment of the animal or tissue appears to be an important determinant of the subsequent rate of absorption. Fatty acid uptake can be depressed by cannulation of the rumen during the experiment (Masson and Phillipson, 1951), by excessive washing out, by leaving the rumen filled with

saline overnight (Ash and Dobson, 1963) and by starvation (Armstrong et al., 1957). In their studies of isolated rumen epithelium, Ferreira, Harrison and Keynes (1966) found that for a stable preparation the tissue must be removed quickly from a fed sheep, and that VFA's were required in the bathing fluid. Stevens (1964) observed that the method of stripping the rumen epithelium affected the electrical potential across isolated preparations.

Uptake of Na^+ from solutions in the rumen was reported by Danielli, Hitchcock, Marshall and Phillipson (1946), Masson and Phillipson (1951), Sperber and Hydén (1952) and Parthasarathy and Phillipson (1953). Dobson (1959) demonstrated active transport of Na^+ out of the rumen against both a concentration and an electrochemical gradient. Active transport of Na^+ has also been observed in isolated sheets of rumen epithelium of sheep (Ferreira, Harrison and Keynes, 1964), cattle and goats (Stevens, 1964). Ferreira et al. (1964) found the net Na^+ transport across isolated sheep rumen epithelium was greater than indicated by the short-circuit current. An explanation offered by Stevens (1964), who worked with a similar preparation from cattle and goats, is that part of the Na^+ is transported by exchange diffusion or as sodium chloride.

Transfer of K^+ across the rumen epithelium has always been found to be in accordance with the electrochemical gradient. Depending upon the initial concentration, it can be absorbed from the rumen down this gradient (Parthasarathy and Phillipson, 1953; Dobson, 1959; Hydén, 1961), or accumulate in the rumen fluid up to an equilibrium value five times the plasma level (Sperber and Hydén, 1952). Ferreira et al. (1964) observed a net flux of K^+ from blood to lumen sides in the isolated rumen epithelium of sheep.

Chloride can be absorbed from the rumen of sheep and goats against a concentration gradient (Sperber and Hydén, 1952; Parthasarathy and Phillipson, 1953; Dobson and Phillipson, 1958; Hydén, 1961), but by virtue of the electrical potential, this absorption is down the electrochemical gradient (Dobson and Phillipson, 1958). The direction of movement of Cl^- depends upon the concentration in rumen fluid. At low rumen $[Cl^-]$, Cl^- enters the rumen contents (Masson and Phillipson, 1951; Parthasarathy and Phillipson, 1953), but when $[Cl^-]$ exceeds one-third the plasma concentration, Cl^- is absorbed (Parthasarathy and Phillipson, 1953). Some Cl^- uptake may be active: Dobson (1959) reported a small movement of Cl^- against the electrochemical gradient when $[K^+]$ was raised and $[Na^+]$ lowered in rumen fluid; Stevens (1964) noted active movement of Cl^- from the lumen to blood sides in isolated cattle and goat rumen epithelium.

Ash and Dobson (1963) have shown that the rumen epithelium is permeable to both CO_2 and HCO_3^-, which diffuse down their electrochemical gradients in either direction, but the permeability to H^+ is low. They observed that, in the absence of VFA, $[CO_2]$ was greater and $[HCO_3]$ lower in rumen fluid than in plasma. During VFA absorption, HCO_3^- appeared in the rumen, the amount depending on the pH (Masson and Phillipson, 1951; Dobson, 1959; Ash and Dobson, 1963). Ash and Dobson (1963) believe that at near neutral pH, the HCO_3^- is generated in the rumen from buffer CO_2 during the absorption of unionised fatty acid, and does not represent HCO_3^- secreted into the rumen.

Although $^{32}PO_4^=$ can move in either direction across the rumen epithelium (Scarisbrick and Ewer, 1951; Parthasarathy, Garton and Phillipson, 1952), the net flux is small (Scarisbrick and Ewer, 1951; Sperber and Hydén, 1952; Hydén, 1961). Because the absorption of $PO_4^=$ in the rumen was believed

to be small, Hydén (1961) used $PO_4^{\equiv}$ as an index of salivary inflow in studies of rumen water absorption.

Water movement across the rumen wall appears to be influenced by osmotic gradients or coupling to solute movement. Water can be absorbed from hypotonic or isotonic solutions placed in the isolated rumen or rumen pouch (Sperber and Hydén, 1952; Parthasarathy and Phillipson, 1953; Dobson, 1959), whereas hypertonic solutions gain water (Parthasarathy and Phillipson, 1953). However, in sheep and goats the rumen contents can be maintained hypotonic to plasma after several hours of fasting (Engelhardt, 1963; Warner and Stacy, 1965; Ternouth, 1967). In intact sheep unfed for 2-12 hours, Hydén (1961), using polyethylene glycol (PEG) as a rumen marker, estimated the rate of water absorption from the rumen to be of the order of 0.15 l/hr. Using tritiated water, Engelhardt (1963) found that, although the rumen mucosa showed considerable water permeability, the net flux was small.

An electrical potential difference (P.D.) exists across the rumen wall. Under normal circumstances it is of the order of 30 mv, rumen contents negative relative to blood (Dobson and Phillipson, 1958; Dobson, 1959). In vivo, the potential difference can be abolished or reversed by filling the rumen with water (Dobson, 1959); in vitro, both the potential and the short-circuit current across isolated epithelium are abolished in Na^+-free media (Ferreira, Harrison and Keynes, 1966). The magnitude of the potential difference is related to the relative concentrations of Na^+ and K^+ in the rumen fluid. It is increased when rumen $[Na^+]$ is reduced and $[K^+]$ increased by instilled solutions (Dobson, 1959), changing the diet from hay to grass (Sellers and Dobson, 1960), loss of saliva through a parotid fistula, or adrenal steroid therapy (Scott, 1966). Sellers and Dobson (1960) and Scott (1966)

found that the P.D. was negatively correlated with $[Na^+]$ and positively correlated with $[K^+]$.

Although the rumen $[Na^+]$ and $[K^+]$ were themselves negatively correlated, yet Sellers and Dobson (1960) obtained no better fit with a multiple correlation than with P.D. and $[Na^+]$ alone. Ferreira, Harrison, Keynes and Nauss (1966) examined the interaction of rumen $[Na^+]$ and $[K^+]$ on the P.D. by fixing the concentration of one of the ions and varying the other in solutions made isotonic with sucrose. The potential increased linearly with increasing $[Na^+]$ and logarithmically with $[K^+]$, although the effects of changes in $[K^+]$ were greater than changes in $[Na^+]$. These workers suggested the negative correlation of P.D. with $[Na^+]$ reported by Sellers and Dobson (1960) and Scott (1966) could be the result of these interactions of Na^+ and K^+.

2. Omasum and Abomasum

The omasum, with its large surface area, would appear to be an ideal absorbing structure, although its effectiveness in this role has not been established. The extent of omasal water and electrolyte absorption depends upon the amount of digesta retained in the omasum, and the retention time. Digesta leaving the omasum represents a mixture of that passing directly through the organ with that retained for some time, but the relative proportions have been difficult to determine. From the radiological observations of Bensis and Phillipson (1957), the direct flow would appear to be large. Oyaert and Bouckaert (1961) calculated the direct flow to be 66% (using polyethylene glycol as a marker), and as high as 87% (comparing the dry matter content of reticular fluid and omasal outflow). However, in this investigation the

digesta was not returned to the abomasum, resulting in increased omasal outflow and water intake, and reduced rumen dry matter content. Under those conditions, the rate of absorption in the omasum may decrease. Fluid absorption in the omasum has been estimated to be of the same order as the volume of gastric juice added in the abomasum (Hydén, 1960; Hogan, 1964) and as the volume of saliva (Raynaud and Bost, 1957). Omasal absorption may be relatively less important in sheep eating restricted amounts of hay than in sheep eating green fodder or large amounts of hay (Hogan, 1964).

Water absorption from the omasum was inferred from the composition of samples taken from several compartments of the forestomach of slaughtered sheep. Grey, Pilgrim and Weller (1954) used the N/lignin ratio, and Garton (1951) found higher $[P_i]$ and $[$soluble Mg$]$ in the omasum than in the rumen and abomasum. Oyaert and Bouckaert (1964) found omasal outflow had a 10-20% higher polyethylene glycol concentration than rumen liquor. The same authors instilled fluid into the ligated, washed omasum, and showed a large absorption of water, greater from dilute solutions, and small amounts even from initially hypertonic fluid.

An electrical potential exists between omasal contents and the blood, similar in magnitude to that across the rumen wall (Ferreira, Harrison, Keynes and Nauss, 1966). Volatile fatty acids may be absorbed in the omasum (Masson and Phillipson, 1952; Gray et al., 1954). The omasum is also capable of absorption of electrolytes. It is probably a region of recovery of HCO_3^- from the digesta before the addition of HCl in the abomasum: fluid leaving the omasum has a higher pH, higher $[Cl^-]$ and lower $[HCO_3^-]$ than rumen liquor (Masson and Phillipson, 1952; Oyaert and Bouckaert, 1961); and $[Cl^-]$ is higher and $[HCO_3^-]$ lower in omasal than rumen contents of

slaughtered cows (Ekman and Sperber, 1953). Oyaert and Boumkaert (1961) found the absorption of electrolytes from solutions in the ligated, washed omasum depended on their initial concentration. The $[Na^+]$ decreased if initially greater than 100 mg/100 ml, but increased if lower; the $[Cl^-]$ decreased if initially above 140 mg/100 ml, but increased if below, $[K^+]$ and $[CO_2]$ decreased, but all four showed absorption in most cases because of concurrent water absorption.

There is usually net addition of water and electrolytes via the gastric juice in the abomasum, and in the many studies where abomasal outflow is collected, net absorption is assigned to the omasum, and the abomasal absorption is considered small. This is probably justified by such studies as those in man by Heitmeier, Code and Orvis (1957a,b), who showed that the acid secreting mucosa offered a barrier to Na^{22} or Na^{24} absorption, although some took place in the antrum, and that stomach water absorption (labelled with D_2O) was about one-tenth the small intestinal rate.

3. Intestine

Transport properties of intestinal epithelium with respect to water and electrolytes have been established in non-ruminant species by careful experimentation, both in vivo and in vitro. Studies on ruminants have been of a different type, defining overall regions of net absorption or secretion in intact animals fitted with re-entrant intestinal cannulae. In the absence of experimental data, the assumption is made that the transfer characteristics of the epithelium itself are similar to those in the species already studied.

Active transport of Na^+ out of the intestine occurs at all levels

(Curran and Solomon, 1957; Cooperstein and Brockman, 1959; Curran, 1960; Curran and Schwartz, 1960; Clarkson, Cross and Toole, 1961; Schultz and Zalusky, 1964a). In the ileum, Na^+ transport is glucose-dependent (Curran, 1960; Schultz and Zalusky, 1964b), and the transport of sugars and of amino acids interact in stimulating Na^+ uptake (Crane, 1962; Barry, Smyth and Wright, 1965; Schultz and Zalusky, 1965). In the colon, Na^+ transport may be hormonally increased: by pitressin (Blickenstaff, 1954; Ussing, 1957; Aulsebrook, 1961), by deoxycorticosterone (Berger, Kanzaki and Steele, 1960) and by aldosterone (Levitan and Ingelfinger, 1965).

Most reports indicate passive movement down the electro-chemical gradient for K^+ (Cooperstein and Brockman, 1959; Clarkson and Rothstein, 1960; Gilman, Koelle and Ritchie, 1963; Phillips and Code, 1966) and for Cl^- (Cooperstein and Brockman, 1959; Cooperstein and Hogben, 1959; Curran and Schwartz, 1960; Clarkson et al., 1961; Schultz, Zalusky and Gass, 1964). A few describe active transport of these two ions. Edmonds (1967) believed there was active K^+ secretion into the rat colon in his experiments. Active absorption of Cl^- was reported in the rat ileum in vivo (Curran and Solomon, 1957) and in vitro (Curran, 1960). Tidball (1961) demonstrated active Cl^- secretion in the dog treated with cholinergic drugs.

Active HCO_3^- secretion into the gut lumen may occur. Clarkson, Rothstein and Cross (1964) found no evidence for active HCO_3^- secretion in the jejunum and ileum, but Cooperstein and Brockman (1959) observed active HCO_3^- movement into isotonic saline in the dog colon. The $[HCO_3^-]$ in instilled isotonic saline changes towards the equilibrium concentration of 5 m-equiv/l in the proximal jejunum, and around 75 m-equiv/l in the distal ileum and proximal colon in dogs (D'Agostino, Leadbetter and Schwartz, 1953; Swallow

and Code, 1967).

Water absorption from isotonic or hypotonic solutions appears secondary to solute absorption: it ceased when net solute movement was zero (Curran and Solomon, 1957; Curran and Schwartz, 1960; Curran, 1960); during absorption of isotonic electrolyte solutions, fluid remaining within the intestinal lumen, and that absorbed across the in vitro intestine and into the lymph remained isotonic (Gilman and Koelle, 1960; Lee, 1963).

Water absorption has been observed from hypertonic solutions in the dog colon (Goldschmidt and Dayton, 1919) and from rat small intestine (Parsons and Wingate, 1961; Annegers and Wakefield, 1962). While these adverse osmotic gradients would not normally occur in vivo, these observations seem to invalidate passive water movement. However, Curran (1960) has postulated a serial membrane model which would admit passive water movements against adverse gradients, and linkage to solute movement.

Although both water permeability and bulk water flow are lower in the ileum and colon than in the duodenum and jejunum, the former appear to be absorbing segments, and the latter equilibrating segments (Visscher, 1957; Hindle and Code, 1962; Grim, 1962; Fordtran, Rector, Ewton, Soter and Kinney, 1965). Fluids instilled into the duodenum and jejunum are rapidly equilibrated to isotonicity with little net absorption, but in the ileum and colon net absorption occurs (Hindle and Code, 1962).

Experiments on ruminants have defined regions of net absorption or secretion in intact animals where the secretion of gastric juice, bile, pancreatic juice and succus entericus are taking place simultaneously with the absorption of water and electrolyte. Goodall and Kay (1965a) collected

quantitatively ileal contents and faeces in sheep, and demonstrated that large amounts of Na^+, Cl^- and water were absorbed in the large intestine. These authors (Goodall and Kay, 1965b) have also described the change in $[Na^+] / [K^+]$ ratio at several levels of the intestine of sheep during Na^+ depletion and Na^+ or K^+ supplementation. Hydén (1960) inferred water and electrolyte transfer from comparison of their content in various parts of the intestine of the sheep relative to a polyethylene glycol reference marker. It was concluded that there was net absorption of water and Cl^- in the small intestine, a large uptake of Na^+, K^+, Cl^-, $PO_4^{\equiv}$ and water in the caecum and proximal colon, and further absorption in the rest of the colon.

Bruce, Goodall, Kay, Phillipson and Vowles (1966) collected duodenal and ileal contents from re-entrant cannulae of sheep, and compared the amount of electrolyte passing each site per 24 hours with the amount ingested and in the faeces. Much greater amounts of Na^+, Cl^-, water and phosphorus, and a little greater amount of K^+ passed to the duodenum than was ingested in the feed. Most of the K^+, Cl^-, water and phosphorus was absorbed in the small intestine, and most of the Na^+ and remaining Cl^- and water and a little K^+ were absorbed in the large intestine.

II. Exchange of water and electrolytes between ECF and ICF

Redistribution of electrolytes takes place across cell membranes in a variety of experimental and pathological situations. Such transfers occur during intracellular participation in buffering acid or alkali, during primary electrolyte depletion, and during the administration of electrolytes.

1. Electrolyte shifts associated with acidaemia and alkalaemia

The buffering of metabolic and respiratory acidosis and alkalosis by intracellular and extracellular buffers has been intensively studied in nephrectomized dogs by Swan and Pitts (1955), Swan, Axelrod, Seip and Pitts (1955) and Giebisch, Berger and Pitts (1955). After intravenously infused HCl, H^+ was buffered 43% by the ECF, and 57% by the ICF; of the latter 36% was exchanged for intracellular Na^+, and 15% for K^+. Intravenously administered $NaHCO_3$ was buffered 68% extracellularly and 32% by cells - 26% of this being Na^+ gained by the cells in exchange for H^+. Respiratory acidosis and alkalosis were buffered almost entirely by cell buffers. During the acidosis, 37% was accounted for by cell Na^+ and 14% by cell K^+ exchange for extracellular H^+, and 29% by penetration of Cl^- into erythrocytes. During respiratory alkalosis, 16% of buffering was cell gain of Na^+, and 4% K^+ exchange for cell H^+, 37% was buffered by Cl^- and 35% by lactate. Yoshimura, Fujimoto, Okumura, Sugimoto and Kuwada (1961) and Yoshimura, Fujimoto and Sugimoto (1962) have confirmed the finding for metabolic acidosis in intact dogs and have followed the change in site of buffering with time. After 24 hours, the normal ECF composition was restored and 75% of the infused acid was present in the ICF, from where it was excreted over several days.

In man, balance studies of correction of metabolic acidosis with alkali (Elkington, Squires and Singer, 1951), and of acid therapy of metabolic alkalosis (Elkington, Squires and Crosley, 1950, 1951) also demonstrated intracellular Na^+ loss in acidosis and gain in alkalosis, with less consistent K^+ movements. Intravenously administered hypertonic $NaHCO_3$ was buffered similarly in man as in the dog (Singer, Clark, Barker, Crosley and Elkington, 1955). In rat diaphragm in vitro, acidosis of the external medium decreased

muscle K^+, increased Na^+ and Cl^- content to a similar extent, and left the water content unchanged; external alkalosis increased Na^+, K^+, Cl^- and water content, but Cl^- less than Na^+ (Adler, Roy and Relman 1965).

Considerable attention has been given to the inverse relationship between blood pH and plasma $[K^+]$ seen in both intact and nephrectomised animals (Abrams, Lewis and Bellet, 1951; Darrow, Cooke and Coville, 1953; Keating, Weichselbaum, Alanis, Margraf and Elman, 1953; Swan and Pitts, 1955; Scribner, Fremont-Smith and Burnell, 1955; Spurr and Lambert, 1960). Apparent conflict in some observations has arisen from two sources: long-term changes in total body K^+, and the initial transient increase in plasma $[K^+]$ in acute respiratory alkalosis. This latter appears to stem from a diversity of K^+ transfer responses to lowered pCO_2 in different tissues (Joyner, Davis, Young, Craige and Welt, 1955; Fenn and Asano, 1956; Hickam, Wilson and Frayser, 1956; Spurr and Lambert, 1960; Blesa, González and Cingolani, 1965). During long-term observations, the depressed K^+ excretion in chronic acidosis, and the enhanced excretion in alkalosis, lead to cell K^+ accumulation and depletion respectively (Darrow and Sarason, 1944; Cotlove, Holliday, Schwartz and Wallace, 1951; Black and Milne, 1952; Cooke, Coughlin and Segar, 1952; Evans, Hughes Jones, Milne and Steiner, 1954).

2. Electrolyte shifts associated with Na^+ and K^+ depletion

Primary K^+ depletion, produced by low K^+ intake, adrenal steroid therapy with low K^+ diet, or excessive loss of gastro-intestinal fluids, results in low plasma $[K^+]$, low muscle K^+ content, extracellular hypochloraemic alkalosis, and intracellular acidosis. Muscle K^+ is partly replaced by Na^+ and basic amino acids, particularly lysine, and the ratios of ICF/ECF Na^+, and

ICF/ECF K^+ both increase (Orent-Keiles and McCollum, 1941; Black and Milne, 1952; Cooke, Segar, Cheek, Coville and Darrow, 1952; Gardner, MacLachlan and Berman, 1952; Orloff, Kennedy and Berliner, 1953; Darrow et al., 1953; Eckel, Pope and Norris, 1954; Irvine, Saunders, Milne and Crawford, 1960; Hudson and Relman, 1962; Grantham and Schloerb, 1965). Although Miller et al. (1963) failed to find intracellular acidosis in K^+ deficient muscle, others have done so (Gardner et al., 1952; Irvine et al., 1960; Hudson and Relman, 1962). Balance studies during correction of K^+ deficiency by KCl showed that more K^+ was taken up by muscle than Na^+ was lost, and similar results were obtained by tissue analysis of depleted animals. Cooke et al. (1952) and Orloff et al. (1953) found three K^+ were replaced by two Na^+, while Irvine et al. (1960) calculated four K^+ for three Na^+.

K^+ deficiency was associated with alkalosis when the Na^+ intake was high, but not otherwise (Holliday and Segar, 1957). DOCA only produced K^+ depletion when Na^+ intake was adequate (Seldin, Welt and Cort, 1951; Relman and Schwartz, 1952). During combined nitrogen and K^+ deficiency, normal plasma and muscle electrolyte composition was maintained, probably because cell catabolism released enough K^+ to maintain normal levels (Muntwyler, Griffin and Arends, 1953). During K^+ depletion, Na^+ partially replaced K^+ not only in skeletal muscle but also in cardiac muscle (Orent-Keiles and McCollum, 1941; Meyer, Grunert, Zepplin, Grummer, Bohstedt and Phillips, 1950), kidney (Orent-Keiles and McCollum, 1941) other than medulla (Manitius, Levitin, Beck and Epstein, 1960), less in spleen and lung, and not in liver and bone (Orent-Keiles and McCollum, 1941).

Na^+ depletion may be caused by loss of gastrointestinal fluid, by excessive sweating or by dietary deficiency. Na^+ depletion can readily be

produced in ruminants by loss of parotid saliva (Denton, 1956). In all but the most severe states, loss of Na^+ is principally from the ECF, and is accompanied by loss of water, some of which enters cells, increasing their volume and reducing their osmolality (Darrow and Yannet, 1935; Ladell, 1949; Nabarro, Spencer and Stowers, 1952; Meyer et al., 1950). A large Na^+ deficit, produced over 19 weeks in rats, was accompanied by marked K^+ retention (Orent-Keiles and McCollum, 1940). K^+ infused into Na^+-depleted dogs showed lower exchange with ICF Na^+, producing greater increments in plasma $[K^+]$ than in normal dogs (Laragh and Capeci, 1955; Anderson and Laragh, 1958), probably because of cellular K^+ accumulation.

3. Electrolyte shifts associated with electrolyte administration

The reported effects of K^+ administration are by no means uniform, as might be expected from the widely differing experimental conditions employed. Thus, different species of intact and nephrectomized animals have been used, there has been oral and intravenous administration of a variety of loads and at different rates, and acute and chronic experiments have been performed. It might be that large K^+ loads delivered rapidly, particularly in nephrectomized animals, would have different effects from chronic high K^+ intake.

Dogs fed large amounts of KCl (162 m-equiv) over two days had increased ECF Na^+ without overall change in Na^+ balance, and higher $[Cl^-]$ and lower $[HCO_3^-]$ in the ECF. Two K^+ entered the cells for one Na^+ displaced (Laragh and Capeci, 1955). Intravenous KCl in dogs similarly lowered plasma pH and $[HCO_3^-]$, and alkalinised the urine (Roberts, Magida and Pitts, 1953). On the other hand, Lambie, Relman and Schwartz (1959) fed rats 40 m-equiv/kg KCl over two days with little or no effect on electrolyte balance or plasma

CO_2 content. KCl injected into normal rats lowered the ECF pH, decreased ICF pH a little, and raised muscle $[K^+]$, but less than 20% of the K^+ entered cells compared with 40% in K^+ depletion (Irvine et al., 1960). KCl (or RbCl) in nephrectomized, normal rats produced ECF alkalosis and ICF acidosis (Hudson and Relman, 1962). High K^+ intake over 8 weeks in white rats raised $[K^+]$ in all tissues without changing $[Na^+]$ (Meyer et al., 1950). Increased dietary K^+ in normal men caused little K^+ to be retained in the cells (Tarail and Elkington, 1949).

Following the intravenous infusion of hypertonic saline in the dog (Elkington and Winkler, 1944), the ingestion of dry NaCl in man (Gamble, 1947), and the administration of hypertonic Na_2SO_4 in the dog (Elkington and Winkler, 1944; Elkington, Winkler and Danowski, 1948), K^+ moved into the ECF, presumably from the ICF. Meyer et al. (1950) found high Na^+ intake in white rats increased $[Na^+]$ and $[K^+]$ slightly in all tissues. Dogs fed a high NaCl intake excreted in the urine intracellular K^+ (Reinhardt and Behrenbeck, 1967).

Extracellular acidosis may result from NaCl administration if there is rapid expansion of the ECF volume. In intact dogs, infusion of large volumes of isotonic saline (Shires and Holman, 1948), or in nephrectomised dogs infusion of lesser volumes of isotonic saline (Keating et al., 1953) and hyperosmolar NaCl or mannitol (Winters, Scaglione, Nahas and Veroaky, 1964) lowers ECF $[HCO_3^-]$ while CO_2 production continues, resulting in lowered pH. The term "dilution acidosis" for this phenomenon was coined by Shires and Holman (1948), who showed the acidosis did not occur with a similar load of $NaHCO_3$. It might be expected, then, that the ICF pH would rise during dilution acidosis; some support for this is given by the observation of raised CSF pH

(Winters et al., 1964).

4. Water shifts

Water moves between the ICF and ECF when osmotic gradients are set up across the cell membrane. Cells shrink when the external osmolarity rises, and swell when it falls, but rarely show the changes expected of an ideal osmometer. In addition, tissues vary in their response to changing external osmotic pressure. McCance and Robinson (1950) found such differences in rats dehydrated by hypertonic NaCl. Woodbury (1956) lowered ECF $[Na^+]$ by intraperitoneal instillation and subsequent withdrawal of 5.5% glucose solution: the brain lost K^+, gained Na^+ and showed no change in hydration, the muscles increased their water content and showed reduced $[Na^+]$ and $[K^+]$, but the skin lost water. Ames, Isom and Nesbett (1965) incubated isolated rabbit retina in a range of osmolalities, and over a range ± 50 mosm/kg from isotonicity observed water movement predictable from the osmotic gradient, but Na^+ movement with the water, and reciprocal K^+ movement. Erythrocytes show osmometric behaviour, judged from PCV changes (Le Fevre, 1964).

III. Renal excretion of water and electrolytes

Regulation of ECF composition is brought about chiefly by the excretory activity of the kidneys. In this section, the function of the renal excretory processes for water and electrolytes will be described. This is a background to the discussion in Section IV of the factors controlling renal excretion and the other homeostatic mechanisms involved in maintaining normal body composition.

The functional units of the kidney are the nephrons. Each nephron

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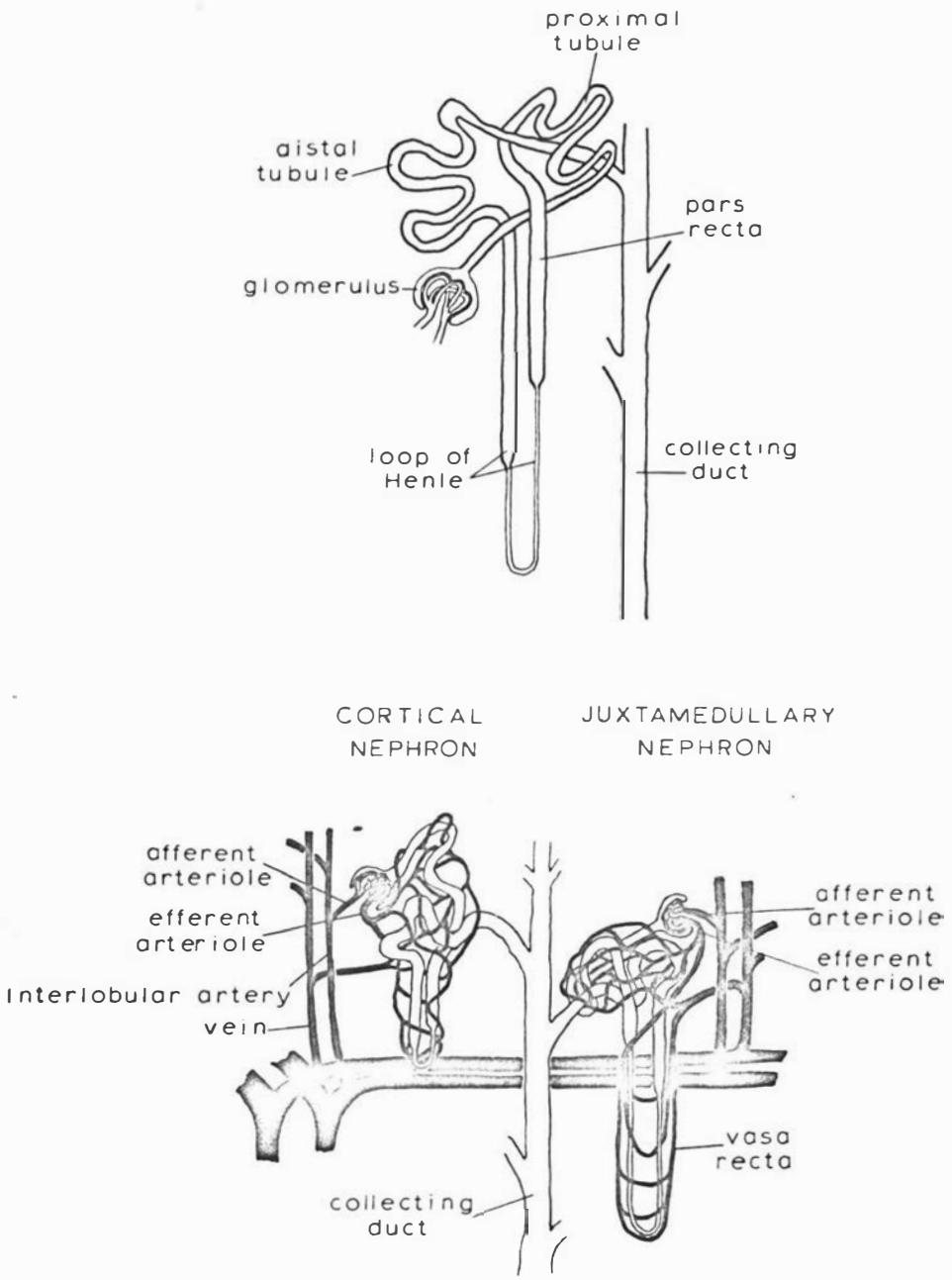

Fig 1. Arrangement of the nephron and its blood supply. The upper diagram shows the parts of an individual nephron described in the text. The lower diagram illustrates the difference in blood supply to the parts of the two types of nephron: cortical and juxta-medullary.

consists of a glomerulus, proximal convoluted tubule, pars recta, loop of Henle, distal convoluted tubule and collecting duct. The convoluted tubules lie in the cortex, the loop and collecting duct in the medulla and papilla. Most of the loop of Henle is thin walled, except for the thick ascending limb in the outer medulla. The arrangement of the nephron and its blood supply are shown diagrammatically in fig. 1. Two types of nephron can be distinguished, depending on the form of the loop: juxta-medullary glomeruli give rise to long loops with very long thin portions penetrating deep into the inner medulla; short loops arise from superficial glomeruli and have very little thin loop.

The overall functioning of the kidney was deduced from the early studies of clearances during diuresis, antidiuresis and solute loading. The newer techniques of stop-flow, push-flow, micropuncture, microperfusion of single nephrons and measurement of transtubular electrical potentials have characterized the activity of regions of the nephron itself.

1. Sodium

Normally only a small amount of filtered Na^+ is lost in the urine. A large, fairly constant proportion is reabsorbed in the proximal convoluted tubule, and a variable, regulatory amount in the distal tubule and collecting duct, where steeper gradients may be set up. Reabsorption in the loop of Henle creates the medullary hypertonicity necessary for the formation of hypertonic urine.

Sodium transport throughout the nephron appears to be active, as indicated by several experimental observations. In contrast to the usual situation, a concentration gradient can be established across the proximal

tubule (tubule fluid $[Na^+]$ lower than plasma $[Na^+]$) during mannitol diuresis; this can be maintained in the loop of Henle and increased in the distal tubule and collecting duct (Windhager and Giebisch, 1961a). An adverse electrical gradient for Na^+ transport is present throughout the nephron, but greatest in the distal tubule (Solomon, 1957; Giebisch, 1958, 1960, 1961; Clapp, Rector and Seldin, 1962; Kashgarian, Stöcklo, Gottschalk and Ullrich, 1963; Malnic, Klose and Giebisch, 1964, 1966a). Active Na^+ transport has been observed in the short-circuited proximal tubule of Necturus (Eigler, 1961) and of the rat (Windhager and Giebisch, 1961b; Giebisch, Klose, Malnic, Sullivan and Windhager, 1964), and in the distal tubule of Necturus during stop flow microperfusion (Maude, Shehadeh and Solomon, 1966). There is a linear relationship between renal oxygen consumption and tubular Na^+ reabsorption (Deetjen and Kramer, 1960; Thaysen, Lassen and Munck, 1961; Thurau, 1961; Kiil, Aukland and Refsum, 1961).

In the proximal convoluted tubule about two-thirds of filtered NaCl and water are reabsorbed (Walker, Bott, Oliver and MacDowell, 1941; Lassiter, Gottschalk and Mylle, 1961, 1964; Litchfield and Bott, 1962; Ullrich, Schmidt-Nielsen, O'Dell, Pehling, Gottschalk, Lassiter and Mylle, 1963; Giebisch, Klose and Windhager, 1964; Gertz, Kennedy and Ullrich, 1964). Water movement is dependent on reabsorption of NaCl (Windhager, Whittembury, Oken, Schatzmann and Solomon, 1959; Windhager and Giebisch, 1961a; Giebisch, Klose et al., 1964). Proximal tubular fluid remains isosmotic with plasma under many experimental conditions (Wirz, 1956; Gottschalk and Mylle, 1959; Clapp, Watson and Berliner, 1963a) and there is normally no gradient between tubular fluid and plasma (Walker et al., 1941; Windhager and Giebisch, 1961a; Ullrich et al., 1963).

Proximal tubule Na^+ reabsorption does not proceed to completion although 65% of filtered Na^+ is reabsorbed normally in the 65% of the tubule able to be sampled. Therefore, the rate of reabsorption must be lower in the pars recta, possibly because limiting concentrations may be set up there, or else the pump is less efficient. Under non-diuretic conditions no such limiting concentration is set up in the 65% studied. However, during osmotic diuresis, or microperfusion with isosmotic saline + mannitol or raffinose, a concentration can be reached below which there is net entry of Na^+ into the tubule (Windhager and Giebisch, 1961a,b; Ullrich et al., 1963; Marsh and Solomon, 1964).

An exception to the constancy of the fraction of filtered Na^+ reabsorbed in the proximal tubule has been established by recent micropuncture studies during saline loading. Isosmotic expansion of the ECF volume reduces the fractional Na^+ reabsorption (Cortney, Mylle, Lassiter and Gottschalk, 1965; Cirksena, Dirks and Berliner, 1965; Dirks, Cirksena and Berliner, 1965; Watson, 1966; Landwehr, Klose and Giebisch, 1967), and the more rapid the expansion, the greater the renal effect (Levinsky and Lalone, 1963). Earley and Friedler (1965a,b) reached the same conclusions concerning Na^+ diuresis under the influence of ethacrynic acid and chlorothiazide. The mode of action appears to be humoral, but may be a haemodynamic change.

Plasma protein concentration has been suggested as a determinant of the rate of Na^+ excretion by influencing proximal transtubular fluid movement. However, micropuncture studies have indicated that the colloid osmotic pressure gradients will have only minor effects. Perfused Necturus (Whittembury, Oken, Windhager and Solomon, 1959) and mammalian (Giebisch et al., 1964) tubules reabsorb water despite a reversal in the normal colloid osmotic pressure gradient. Probably any effects related to changes in plasma protein

concentration operate indirectly through a change in blood volume, or GFR, or another factor.

In the loop of Henle active Na^+ reabsorption is in excess of water reabsorption, since fluid entering the distal convoluted tubule is hypotonic to plasma (Gottschalk and Mylle, 1959; Wirz, 1956; Gottschalk, Lassiter, Mylle, Ullrich, Schmidt-Nielsen, O'Dell and Pehling, 1963). When Na^+ reabsorption is reduced in the proximal tubule, loop Na^+ reabsorption usually increases along with medullary blood flow (Thurau, Deetjen and Kramer, 1960; Windhager and Giebisch, 1961a; Cortney et al., 1965).

The capacity of the distal tubule for Na^+ transfer appears to be limited, but large Na^+ gradients can be established during Na^+ deficiency. The distal nephron is the site where adrenal steroids increase Na^+ reabsorption (Vander, Wilde and Malvin, 1960, 1961; Wilde and Howard, 1960). In the collecting ducts, the urine can be rendered virtually free of Na^+ (Windhager and Giebisch, 1961a; Malnic et al., 1966a), and although large amounts are not transported, steep gradients can be set up.

It would appear from successive reports from laboratories using these ultramicromethods that relatively minor changes in the experimental conditions or technique result in large changes in the results obtained. Clapp et al. (1963a) concluded that vasopressin increased proximal tubule water reabsorption, hence Na^+ reabsorption, from greater $TF/P\ [inulin]$ during antidiuresis than during water diuresis; Davis, Knox and Berliner (1967), using recollection micropuncture, found $TF/P\ [inulin]$ was unchanged when a water diuresis was interrupted by a vasopressin infusion. Giebisch, Klose and Windhager (1964) reported a constant fractional Na^+ reabsorption

during isotonic and hypertonic saline loading, but Landwehr, Klose and Giebisch (1967) found isotonic saline loading depressed the fractional reabsorption of Na^+. It would appear that the last word is not yet written even on apparently well documented aspects of tubular function.

2. Chloride

It is generally believed that overall proximal tubular chloride reabsorption is passive, since both the electrical and chemical gradients favour Cl^- reabsorption. When there is preferential reabsorption of HCO_3^-, or NH_3 addition, $[Cl^-]$ in proximal tubule fluid may exceed that of plasma (Walker et al., 1941; Giebisch, Windhager and Pitts, 1960; Litchfield and Bott, 1962; Gottschalk, 1963; Kashgarian et al., 1963). The tubular $[Cl^-]$ depends on the degree of acidification (Clapp et al., 1963a). While the possibility of active Cl^- transport has not been eliminated, so far only passive movement has been observed. The short-circuit current in the perfused amphibian kidney is not altered by $SO_4^=$ substitution for Cl^- (Eigler, 1961), and in the rat kidney the short-circuit current agrees with the rate of Na^+ transport (Windhager and Giebisch, 1961a; Giebisch et al., 1964).

In the distal tubule, usually the steeper electrical gradients are adequate to account for Cl^- reabsorption (Kashgarian et al., 1963), although Rector and Clapp (1962) found they were not large enough for the low $[Cl^-]$ seen in rats during $SO_4^=$ infusion. Active Cl^- transport probably occurs in such cases. It seems certain in the terminal collecting ducts, where Cl^- moves against large concentration gradients and with only small favourable electrical gradients (Hilger, Klümper and Ullrich, 1958; Gottschalk, 1961; Marsh and Solomon, 1963; Windhager, 1964).

3. Potassium

Renal handling of K^+ is complex: filtration, tubular reabsorption and secretion all occur. Usually urinary K^+ is less than the filtered load, indicating net reabsorption, but tubular secretion, i.e. K^+ clearance in excess of the simultaneous inulin clearance, can be demonstrated in particular circumstances. The first such observations were made by McCance and Widdowson (1937) in a patient with alkalosis and dehydration, and by Keith, King and Osterberg (1943) in normal subjects receiving KCl, but their validity was doubted by the authors. Tubular secretion was first demonstrated experimentally in dogs by Berliner and Kennedy (1948) during mercurial diuresis and hypertonic KCl infusion, and by Mudge, Foulks and Gilman (1948) during urea diuresis and after KCl infusion.

The participation of K^+ secretion at normal levels of excretion, rather than functioning only as a reserve for high rates of excretion, was suggested in 1950 by Mudge, Ames, Foulks and Gilman. The dissociation of excreted K^+ from the filtered load of K^+ after kaliuretic drugs originally formed the basis for their hypothesis that filtered K^+ was almost completely reabsorbed proximally and secreted distally, so that urinary K^+ was almost entirely of secretory origin. This hypothesis was adopted by Berliner and others, and has been supported by later experimental evidence.

Micropuncture studies have confirmed extensive reabsorption of K^+ in the proximal tubule of Necturus, the rat and the dog. A variety of chemical and photometric methods has resulted in a range of estimates of TF/P $[K^+]$ above and below the critical level for active reabsorption. Most studies in rats under various diuretic and non-diuretic conditions suggest K^+

reabsorption in the proximal tubule is an active process (Wirz and Bott, 1954; Litchfield and Bott, 1962; Bloomer, Rector and Seldin, 1963; Malnic and Giebisch, 1963; Marsh, Ullrich and Rumrich, 1963; Khuri, Flanigan and Oken, 1963; Watson, Clapp and Berliner, 1964; Malnic et al., 1966a). There is no indication that proximal reabsorption is active in Necturus (Bott, 1962; Oken and Solomon, 1963; Khuri et al., 1963; Watson et al., 1964). However, Watson et al. (1964) believe there may be active K^+ reabsorption in the dog during mannitol diuresis. Whether proximal tubule K^+ reabsorption is active or passive, the extensive fluid reabsorption results in a large fraction of filtered K^+ being reabsorbed.

Further K^+ reabsorption takes place in the loop of Henle and probably the early distal tubule. In low K^+ states, the urine $[K^+]$ may be lowered below the plasma $[K^+]$ in its passage through the distal tubule and collecting ducts (Mateer, Greenman, Peters, Gow and Danowski, 1949; Lowe, 1953; Evans et al., 1954). Although these more distal areas may be sites of net reabsorption, it is more usual for them to be sites of net secretion (Kessler, Hierholzer, Gurd and Pitts, 1959; Sullivan, Wilde and Malvin, 1960; Jaenike and Berliner, 1960). Vander (1961) believed K^+ reabsorption in the distal tubule may occur simultaneously with K^+ secretion. Participation of the collecting ducts in K^+ secretion has been observed by Hilger, Klümper and Ullrich (1957), Jaenike and Berliner (1960), Hierholzer (1961), Vander (1961) and Malnic et al. (1966a).

The phenomenon of tolerance to K^+ loading, first observed in the rat by Thatcher and Radike (1947), is often employed to demonstrate K^+ clearance in excess of filtered K^+. After oral administration of K^+ salts for several days, the renal response to a K^+ load is far greater than normal,

and plasma $[K^+]$ does not rise very markedly.

There are many instances of K^+ excretion being influenced by Na^+ intake or Na^+ excretion. Berliner, Kennedy and Orloff (1951) put forward the hypothesis that K^+ secretion in the distal tubule was coupled to Na^+ reabsorption to explain these observed interactions of the two cations. Cation exchange was demonstrated in the presence of the non-reabsorbable anion, ferrocyanide (Berliner, Kennedy and Hilton, 1950). Inadequate supply of Na^+ to the exchange mechanism was invoked for the diminished K^+ secretion during renal arterial constriction and reduced GFR, and which could be restored by a diuretic (Davidson, Levinsky and Berliner, 1958), and for reduced K^+ secretion in the modified stop-flow experiments of Walker, Cooke, Payne, Baker and Andrew (1961). On the other hand, Na^+ depletion does not prevent excretion of a K^+ load in dogs (Laragh and Capeci, 1955; Anderson and Laragh, 1958).

It has become apparent that Na^+-K^+ coupling may not simply be carrier mediated cation exchange. The micropuncture and microperfusion studies of distal tubule K^+ secretion, Na^+ reabsorption and electrical P.D. over a range of metabolic K^+ states of Malnic et al. (1966a,b) confirmed many earlier findings of reduced K^+ excretion when Na^+ excretion was low, and increased excretion when Na^+ was high or when impermeant anion was present. However, in no case did they find that Na^+ was limiting for K^+ exchange on a 1:1 basis, and normally the amount of Na^+ reabsorbed greatly exceeded K^+ secreted; the kaliuretic effect of raised intratubular $[Na^+]$ was not explicable in terms of saturating a previously unsaturated carrier system. They also confirmed in mammals the observation of Vogel and Teervooren (1964) that in frog kidneys there was no fixed exchange ratio between Na^+ reabsorption and K^+ secretion.

Malnic et al. (1966a) therefore proposed that Na^+-K^+ coupling was electrical rather than carrier mediated. Raised transtubular P.D., produced by raised $[Na^+]$ or impermeant anion, would favour increased K^+ secretion. Berliner (personal communication to Malnic et al., 1966b) reported that the substitution of HCO_3^- for Cl^- in the distal tubule fluid, either by i/v HCO_3^- loading, or by depressed reabsorption at a more proximal site, increased K^+ secretion, perhaps in response to the raised transtubular P.D. observed during HCO_3^- loading (Clapp et al., 1962).

Distal tubular P.D. is but one of the factors influencing K^+ secretion, since at unaltered P.D. K^+ excretion is reduced on low Na^+ diets (Malnic et al. 1966a) and after adrenalectomy (Hierholzer, Wiederholt, Holzgreve, Giebisch, Klose and Windhager, 1965). At comparable intratubular $[Na^+]$, less K^+ is excreted during isotonic than in hypertonic NaCl loading (Landwehr, Klose and Giebisch, 1966). This effect could be mediated through a change in cell $[K^+]$, which has been suggested to be a regulating factor rather than plasma $[K^+]$, since K^+ excretion is related to plasma $[K^+]$ only when this parallels cell $[K^+]$ (Mudge et al., 1950).

A number of observations of an inverse relationship between urinary K^+ excretion and acidification led to the hypothesis that K^+ and H^+ compete for secretion along a common pathway (Berliner et al., 1951). Carbonic anhydrase inhibition enhances K^+ excretion (Berliner et al., 1951; Maren, Wadsworth, Yale and Alonso, 1954; Counihan, Evans and Milne, 1954). The administration of K^+ may cause the urine to become alkaline, depending upon which is more depressed, ECF $[HCO_3^-]$ or renal tubular H^+ secretion, since the balance of the two determines the final urine pH. Whereas in the dog and man the urine becomes alkaline (Loeb, Atchley, Richards, Benedict and Driscoll, 1932;

Winkler and Smith, 1942; Berliner et al., 1950), in the rat the rapid drop in plasma $[HCO_3^-]$ prevents this (Berliner, 1961). Orloff and Davidson (1959) infused K^+ into one renal portal vein in the chicken; the direct effect of raised peritubular $[K^+]$ increased K^+ excretion and raised the pH of the urine; the lowered general plasma total CO_2, and resulting lower peritubular $[K^+]$ caused the other kidney to excrete acid urine and less K^+.

There appears to be no compulsion for either K^+ or H^+ to be excreted in an unfavourable metabolic state. During K^+ depletion combined with acute alkalosis or carbonic anhydrase inhibition, a urine of low K^+ and high pH was excreted (Evans et al., 1954). The excretion of both ions may be increased by infusion of Na^+ and the non-diffusible anions $SO_4^=$ and $PO_4^=$ (Schwartz, Jenson and Relman, 1955; Malvin and Wilde, 1960; Bank and Schwartz, 1960).

4. Acid, bicarbonate and pH

Regulation of renal excretion of H^+ and HCO_3^- is essential in restoring the acid-base balance of the body. Renal activity is such that HCO_3^- reabsorption is consequent upon H^+ secretion, so that alkaline urines are rich in HCO_3^- and acid urines are virtually free of HCO_3^-.

In 1942, Höber infused carbonic anhydrase inhibiting sulphonamides into frogs, and concluded that the enzyme was involved in HCO_3^- reabsorption. A series of experiments by Pitts and colleagues (Pitts and Alexander, 1945; Pitts, 1945; Pitts and Lotspeich, 1946; Pitts, 1948; Pitts, Lotspeich, Schiess and Ayer, 1948; Pitts, Ayer and Schiess, 1949) investigated H^+ and HCO_3^- excretion in mammals. They demonstrated the occurrence of H^+ secretion in acidotic dogs infused with large amounts of buffer as phosphate or creatinine; the rate depended on the amount of buffer available. Titratable

acid increased linearly with the rate of excretion of buffer, and was greater with those of higher pK'. Sulphanilamide administration inhibited HCO_3^- reabsorption to a large extent, and also reduced titratable acidity. On the basis of such observations, Pitts proposed that H^+ generated in the distal tubular cells by the dissociation of carbonic acid under the enzymatic catalysis of carbonic anhydrase, was secreted into the tubule fluid, perhaps in exchange for Na^+. The blockage of a large fraction of HCO_3^- reabsorption by sulphanilamide was caused by a failure of H^+ to react with filtered HCO_3^-, forming CO_2 which back diffused into the cells. This hypothesis is the basis of present concepts of renal acidification.

Secreted acid may be disposed of in three ways: it may result in HCO_3^- reabsorption, be taken up by urinary buffers, principally phosphate, or react with ammonia produced by the tubule cells. Pitts et al. (1948) followed the changing acid and HCO_3^- excretion in subjects with metabolic acidosis receiving a $NaHCO_3$ infusion when brought first into normal acid-base balance, then into alkalosis. In the acidotic phase, as plasma $[HCO_3^-]$ rose to near 20 mM/l, the rate of excretion of titratable acid and ammonia was maintained at an almost constant high level; from 20-26 mM/l the rate of excretion of titratable acid and ammonia progressively declined, but virtually no HCO_3^- was excreted; above 26 mM/l further increase in plasma $[HCO_3^-]$ greatly increased HCO_3^- excretion, while titratable acid and ammonia fell to very low levels. It was apparent that only when titratable acid and ammonia excretion were small was there significant HCO_3^- excretion.

Recent experimental work has defined the location in the nephron of acid secretion, HCO_3^- reabsorption, and NH_3 formation. In mammals, but not in amphibians, H^+ secretion takes place firstly in the proximal convoluted

tubule (Gottschalk, Lassiter and Wylle, 1960; Bank, 1962; Litchfield and Bott, 1962; Clapp et al., 1963a,b; Young and Edwards, 1964; Rector, Carter and Seldin, 1965) where the bulk of HCO_3^- may be reabsorbed against a low gradient and causing only a small drop in pH. Acidification in the distal tubule, and particularly in the collecting duct, takes place against steeper gradients, and the pH may fall markedly (Gottschalk et al., 1960; Hierholzer, 1961; Hilger et al., 1958; Ullrich and Rigler, 1958). In these more distal areas, virtually all remaining HCO_3^- may be reabsorbed. It is difficult to conclude that all HCO_3^- reabsorption results from H^+ secretion and that none occurs via ionic reabsorption. Acetazolamide, the most potent carbonic anhydrase inhibitor, does not completely block HCO_3^- reabsorption in the doses used; the alternatives remain that there was still some residual enzyme activity, uncatalysed hydration of CO_2 can supply a significant amount of H^+, or some HCO_3^- is ionically reabsorbed.

During the excretion of acid urine, NH_3 is formed in the tubule cells from amino acid precursors, principally glutamine (Van Slyke, Phillips, Hamilton, Archibald, Futcher and Hiller, 1943; Pitts, Pilkington and de Haas, 1965, and others). Ammonia production is inversely related to the urine pH, but in chronic acidosis the NH_3 production and glutaminase activity are enhanced (Pitts, 1948; Davies and Tuffin, 1952; Rector, Seldin and Copenhaver, 1955). Ammonia formation may occur in the proximal tubule as well as in more distal areas (Glabman, Klose and Giebisch, 1963; Clapp, Owen and Robinson, 1965; Hayes, Owen and Robinson, 1966).

Ruminants excrete HCO_3^- in the urine at normal mammalian plasma pCO_2 and $[HCO_3^-]$ levels (Anderson and Pickering, 1962b). The renal threshold, usually at a plasma $[HCO_3^-]$ of 24-28 mM/l, varies with the species, the plasma

pCO_2 and $[Cl^-]$, and the body K^+ status. Probably the underlying primary factor is the cell $[H^+]$ which varies inversely with $[K^+]$ in many circumstances. H^+ secretion is increased by raised plasma pCO_2 (Pitts, 1953; Portwood, Seldin, Rector and Cade, 1959; Rector, Seldin, Roberts and Smith, 1960), hypokalaemia (Fuller, MacLeod and Pitts, 1955) and lowered plasma $[Cl^-]$ (Bank and Aynedjian, 1965).

Direct information on the nature of the $H^+ - K^+ - Na^+$ interaction in the distal nephron is scarce. The inverse $H^+ - K^+$ relationship has been discussed above. The amount of Na^+ reaching the more distal H^+ secretory area has been claimed to be limiting to acid secretion in some circumstances. Keeler and Pearson (1966) found that the lowered rate of H^+ secretion in rats with metabolic acidosis and suprahepatic caval constriction could be increased by augmented Na^+ excretion due to osmotic diuresis or aldosterone-blocking steroid. There may be direct coupling as proposed by Pitts and Alexander (1945), or there may be electrical coupling as suggested by Malnic et al. (1966a) for Na^+ and K^+. Studies of the characteristics of H^+ transport across the urinary bladder of the water turtle by Steinmetz (1967) and Steinmetz, Omachi and Frazier (1967) showed that H^+ transport was not dependent upon that of any other electrolyte, it was decreased by acetazolamide, and increased slightly by the potential created by Na^+ transport. If this also holds for renal H^+ transport, then the relationship with Na^+ may be through a change in transtubular potential difference.

5. Concentrated urine and urea

Urine of a wide range of osmolality is produced by Na^+ transport and variable water permeability through the operation of the hairpin countercurrent

system, formed by the loops of Henle and associated collecting ducts in the renal medulla. The basic principle of a countercurrent multiplier system is that two parallel streams moving in opposite directions multiply small exchanges of material between them, and although only small gradients exist at any level, considerable longitudinal gradients can be established.

This concept of urinary concentration originated in the papers of Kuhn and Ryffel (1942), Hargitay and Kuhn (1951) and Wirz, Hargitay and Kuhn (1951), who suggested the driving force was either water movement from the descending to ascending limb of the loop, or solute transport in the reverse direction. Their hypothesis has received strong support from cryoscopic studies of tissue slices, tissue analyses and micropuncture and microperfusion studies, which have established the operation of a countercurrent multiplier system in the loop of Henle and collecting ducts, and that the driving force is active Na^+ transport from the ascending limb. The vasa recta, arising from juxtamedullary glomeruli, function as countercurrent exchangers preventing the dissipation of the established gradients. In its passage through the loop of Henle the glomerular filtrate is first concentrated, then diluted, and finally concentrated again in the collecting ducts.

In the proximal convoluted tubule about 80% of the glomerular filtrate is isosmotically reabsorbed (Walker et al., 1941; Wirz, 1956; Lassiter et al., 1961; Ullrich et al., 1963).

Active Na^+ transport in the water impermeable ascending limb creates a hypertonic medullary interstitium. Osmotic gradients across the descending limb of the loop of Henle result in the removal of water and a progressive rise in osmolality as the tubular fluid passes deeper into the medulla. The

hairpin arrangement allows a gradient of osmotic pressure from the cortex to the tip of the papilla (Wirz et al., 1951; Ullrich, Drenckhahn and Jarausch, 1955; Ullrich and Jarausch, 1956), in the loops of Henle and also in the medullary interstitium and in the vasa recta. These gradients are greater in antidiuresis than during a water diuresis: the osmolality at the tip of the loops and vasa recta was around 2200 mos/kg H_2O in antidiuretic rats compared with 500 mos/kg in rats with diabetes insipidus (Gottschalk, Lassiter, Mylle, Ullrich, Schmidt-Nielsen, Pehling and O'Dell, 1960; Wirz, 1953).

Knowledge of activities in the ascending limb is limited because of the considerable technical difficulties involved in sampling and in measurement of electrical potentials. Active Na^+ transport out of the thick ascending limb, which progressively dilutes the fluid, causes a hypotonic fluid to be delivered to the distal convoluted tubule (Wirz, 1956; Gottschalk and Mylle, 1959). Histologically, only the thick ascending limb would appear capable of active transport, yet the gradient of osmotic pressure increases to the tip of the loops through the region of thin segments. The driving force for the gradient in these segments is uncertain. Marsh and Solomon (1963, 1965) favour active Na^+ transport only in the thick segment, Gottschalk's group favour both segments, while Ullrich suggests it occurs in the thick ascending limb and the collecting duct.

In an animal forming hypertonic urine, the urine becomes isotonic during its passage through the distal tubule and cortical collecting duct (Gottschalk and Mylle, 1959; Gertz et al., 1964), and then hypertonic after losing water as it once more traverses the hypertonic medulla in the collecting ducts (Ullrich, 1960). ADH is generally believed to render the

epithelium of the distal tubule and collecting duct water permeable, thus allowing osmotic equilibration. The solutes transported out of the loop in excess of water are responsible for the reabsorption of solute-free water ($T^{c}_{H_2O}$) in the collecting ducts of antidiuretic animals during equilibration of collecting duct fluid with that in the loop of Henle and vasa recta at the same level (Gottschalk, 1961). During a water diuresis these segments are relatively water impermeable, and solute loss not accompanied by water creates a urine of low tonicity. A slightly hypertonic urine may be excreted in the absence of ADH during reduced tubular flow after arterial clamping, suggesting some diffusion along the gradient across the collecting ducts may occur (del Greco and de Wardener, 1956; Berliner and Davidson, 1957).

During a water diuresis there is marked reduction in the medullary hypertonicity, and a failure of creatinine to become concentrated at the tip of the papilla (Ullrich and Jarausch, 1956). The reduced hypertonicity is usually explained as a washout of solute secondary to increased medullary blood flow (Clapp et al., 1963a; Thurau, 1964). However, Sabour, MacDonald, Lambie and Robson (1964) examined electron micrographs of kidneys of rats in states of hydration and dehydration, and found the most striking morphological difference was not in the collecting ducts, but in the basement membrane of the descending loop of Henle. This was markedly thickened during a water diuresis compared with its state in dehydrated animals or those given pitressin. They suggested that this thickening prevents water loss, and hence osmotic equilibration, in diuretic animals, and as a consequence, breakdown of the multiplying function of the loop. It was proposed that ADH caused the descending limb of the loop to be water permeable, allowing the counter-current to function and create a medullary hypertonicity which would then

drew water from the collecting ducts.

Urea has an important role in the renal countercurrent, since its presence in the medulla increases the loss of water from the collecting ducts. In many species where it is the principal urinary solute, maximum urine concentration and $T^{c}_{H_2O}$ are reduced at very low rates of urea excretion which occur when low protein diets are ingested (Epstein, Kleeman, Pursel and Hendrikx, 1957; Levinsky and Berliner, 1959; Levinsky, Davidson and Berliner, 1959; Crawford, Doyle and Probst, 1959; Jaenike, 1964).

Urea appears to be excreted by a process of glomerular filtration and passive movement across the tubular epithelium. Filtration and passive reabsorption were concluded from early observations in the dog (Shannon, 1936, 1938) and in man (Chasis and Smith, 1938) since urea clearance was independent of plasma [urea], but varied with the urine flow. The rate of urea excretion was proportional to the plasma [urea].

Within the nephron the situation is a little more complex. In non-diuretic rats, micropuncture studies have shown that about 50% of filtered urea is reabsorbed in the proximal tubule, but an amount equivalent to the filtered load reaches the distal tubule. Since only a small amount is excreted in the urine, it would appear that urea recycles from the collecting duct back to the loop, and back to the collecting duct (Ullrich, 1960; Gottschalk, 1961; Lassiter et al., 1964). Diffusion equilibrium is not reached in the medulla, urea concentration being highest in the collecting ducts, next in the vasa recta and lowest in the loop (Lassiter, Mylle and Gottschalk, 1966). During hypertonic saline and water diuresis, the urea loss in the proximal tubule is similar to that in non-diuretic animals, but there is no gain of

urea in the loop, and very little loss from the distal tubule and collecting ducts. Vasopressin has been described as increasing the permeability of these areas to urea (Jaenike, 1961; Gardner and Maffly, 1963; Lee, Cross and Thornton, 1967).

A sudden increase or decrease in urine flow produces exaltation or abatement of the urea clearance. This has been explained (Schmidt-Nielsen, Osaki, Murdaugh and O'Dell, 1958; Thomas, 1964) by a sudden rise in urine flow causing decreased $[\text{urea}]$ in papillary tissue and washout of urea into the urine; and conversely, a sudden fall in flow rate causes the $[\text{urea}]$ to increase in papillary tissue and urea clearance to drop.

The role of tubular regulation of urea excretion in most species is still uncertain. Tubular reabsorption or secretion has been demonstrated in certain circumstances in rodents, amphibians and fish (Kempton, 1953; Forster, 1954), and has been suggested to occur in other species, particularly when the diet is low in protein. Higher concentrations of urea in the vasa recta or renal tissue than in collecting ducts have been reported in protein deficient rats (Bray and Preston, 1961; Truniger and Schmidt-Nielsen, 1964; Clapp, 1966; Lassiter et al., 1966), and sheep (Schmidt-Nielsen and O'Dell, 1959), in dogs undergoing a diuresis during urea loading (Goldberg and Ramirez, 1965) and in rats on a high protein diet during saline loading (Kleinman, Radford and Torelli, 1965). The high tissue $[\text{urea}]$ is usually seen at the level of the inner stripe of the outer medulla, corresponding with the thick portions of the ascending loops, suggestive of, but not conclusive evidence for, active transport of urea. A complicating factor is the capability of the kidney to produce urea metabolically (Carlisky, Brodsky and Huang, 1962).

IV. Regulation of Water and Electrolyte Metabolism

In regulating overall water and electrolyte metabolism, animals have to adjust loss through urine, faeces, skin and respiratory tract to the intake in food and water. The diet of the ruminant is rich in K^+ but low in Na^+, so that in the natural environment the most likely problem would be the maintenance of Na^+ balance. In arid regions there is the possibility of dehydration. The large digestive cycle of fluid and electrolytes can at times stress the overall regulatory mechanisms.

1. Acid-base

Excess acid or alkali is rapidly neutralized by ECF and ICF buffers (see II., 1 above). Restoration of the normal level of the acid component of the buffers (pCO_2) takes place through adjustment of respiratory elimination of CO_2; the salt component (HCO_3^-) is restored by variation in the rate of renal acid excretion. Raised blood pCO_2 stimulates respiration through the peripheral chemoreceptors and by a direct action in the brain stem; the reverse is the case when pCO_2 falls. Final adjustment of the altered buffer content of the ECF is made through variation in renal acid and HCO_3^- excretion (see III., 4 above): during alkalosis HCO_3^- rich urine is excreted, and during acidosis the HCO_3^- loss is negligible, but titratable acid and NH_3 excretion is high (Pitts et al., 1948). Renal cell $[H^+]$ appears to be an important factor governing the rate of acid excretion.

2. Sodium

Since Na^+ is the principal extracellular cation, its homeostatic regulation is essential for the maintenance of normal ECF volume and tonicity.

The diet of the sheep dictates that Na^+ conservation is usually required to maintain the normal body Na^+ content. In sheep, the overall balance can be maintained on a very low Na^+ intake, when urinary Na^+ loss is very small, and faecal loss can be reduced to 1-5 m-equiv/day (Denton, 1957). Cutaneous loss in suint is small, about 0.5 - 1.0 gm of suint being produced daily (Daly and Carter, 1955; Stacy, Brook and Short, 1963) with a Na^+ content of 1.3% (Farnworth, 1956). Stress can be placed on the sheep's Na^+ regulating mechanism, especially on low Na^+ diets, when the internal requirements are increased by pregnancy, lactation and by hot weather when increased cutaneous loss occurs, and the ECF volume may nearly double (Macfarlane, Morris, Howard and Budtz-Olsen, 1959).

In ruminants, the internal digestive cycle involves a large volume of Na^+-rich fluid: salivary Na^+ enters the rumen and is absorbed back into the ECF, about half through the rumen wall, and half from the omasum and small intestine (Dobson, 1959; Bruce et al., 1966). The stress of expanding this digestive cycle is reflected in the Na^+ conservation mechanisms: change of diet from grass to hay increased the rumen volume in the sheep, and caused marked urinary Na^+ retention (Dobson, Scott and Bruce, 1966). On the other hand, this cycle functions as a Na^+ reserve. In circumstances such as increased rumen volume, excessive loss of Na^+ through a parotid fistula or dietary deficiency, K^+ partially replaces Na^+ in saliva, and hence in rumen liquor. The fall in $[Na^+]$ and rise in $[K^+]$ of parotid, submaxillary and inferior molar saliva is brought about by the action of aldosterone (Denton, 1956; Kay, 1960; Dobson, Kay and McDonald, 1960; Denton, Goding, Sabine and Wright, 1964; Scott and Dobson, 1965).

The well known Na^+ appetite of ruminants has been studied under

laboratory conditions by Denton and co-workers. On moderate Na^+ intake, their sheep showed little interest in drinking solutions containing Na^+, but during Na^+ depletion the voluntary Na^+ intake approximated the deficit (Denton and Sabine, 1961; Beilharz, Denton and Sabine, 1962), even when the concentration of the solution was varied considerably. They failed to identify the appetite mechanism involved; it was not the result of low plasma $[Na^+]$ (Beilharz et al., 1962), the angiotensin or aldosterone content of the blood perfusing the brain (Denton and Sabine, 1961; Bott, Denton and Weller, 1967), the rumen $[Na^+]$, and the gustatory sense was not implicated (Beilharz and Kay, 1963).

Although loss of Na^+ in the faeces is variable (Goodall and Kay, 1965b), changes in renal excretion of Na^+ account for the greatest variation in Na^+ output from the body. Faecal Na^+ may be very low in Na^+ deficiency, when the K^+:Na^+ ratio may be raised, probably due to the action of aldosterone (Davis, Bahn, Yankopoulos, Kliman and Peterson, 1959).

Renal Na^+ excretion can be altered by a change in the filtered load, or in tubular reabsorption, or both. The evaluation of the role of GFR is limited by the insensitivity of present clearance methods. At constant tubular reabsorption, small, barely detectable changes in GFR can alter the daily Na^+ output markedly; Pitts (1963) has calculated that a 5% increase in GFR would double the Na^+ loss. There appears to be a species difference in the extent to which acute variations in Na^+ loss are mediated by changing the GFR. Adaptive increases in GFR appear to play a large part in eliminating the high intake of Na^+ in sheep drinking saline water (Potter, 1961; 1963), and in dogs on a diet of high Na^+ content (Ladd and Raisz, 1949). Potter (1966) reported that Merino sheep infused with hypertonic saline showed no

consistent change in GFR, however, in a subsequent paper (Potter, 1968) increased GFR was reported in Dorset Horn sheep under similar conditions.

Tubular reabsorption of Na^+ appears to be sensitive to a variety of parameters associated with ECF volume and composition. It is not surprising that the principal regulation of the excretion of Na^+ should be complex, and involve multiple receptor and effector mechanisms. Nor is it unexpected to find that these predominantly originate from the ECF volume and composition, of which the body Na^+ is a major determinant. Although many of these factors have been established to be, or at least implicated as, regulators of tubular Na^+ reabsorption, it is uncertain which are major and which are minor controlling systems, how they interact, or in which particular circumstances each operates.

The plasma $[Na^+]$ has been reported to affect Na^+ reabsorption, but the reports are apparently contradictory. The absolute rate of Na^+ reabsorption increases in dogs following plasma $[Na^+]$ (Bresler, 1960; Toussaint and Vereerstraeten, 1962; Kamm and Levinsky, 1964), yet, when the GFR was reduced by clamping, hypernatraemia appeared to inhibit the fractional Na^+ reabsorption (Blythe and Welt, 1963; Kamm and Levinsky, 1965).

The plasma protein concentration has been suggested as a controlling mechanism for urinary Na^+. In many, but not all, circumstances Na^+ retention accompanies increased [plasma protein], and natriuresis accompanies a falling level (Bojesen, 1954; O'Connor, 1955, 1958). Some workers did, and some did not, observe Na^+ retention during the infusion of hyperoncotic albumin solution during water or osmotic diuresis (Goodyer, Peterson and Relman, 1949; Orloff and Blake, 1950; Orloff, Welt and Stowe, 1950; Welt

and Orloff, 1951; Elpers and Selkurt, 1963). It is possible that a correlation between [plasma protein] and Na^+ retention does not indicate a direct causal relationship, but that both are separately related to a common factor, the intravascular volume.

Renal haemodynamics also influence the rate of Na^+ excretion. Stahl (1965) found a correlation between Na^+ excretion and the cardiac output, but not with blood pressure unless this paralleled the cardiac output. Saline loading in hypertensive patients produces a much greater increase in Na^+ excretion than does a comparable loading in normal persons (Birchall, Tuthill, Jacobs, Trautman and Findley, 1953; Cottier, Weller and Moobler, 1958; Baldwin, Biggs, Coldring, Hulet and Chasis, 1958; Papper, Belaky and Bleifer, 1960). Increased perfusion pressure increases the Na^+ excretion of isolated kidneys (Selkurt, 1951; Selkurt, Womack and Dailey, 1965; McDonald and de Wardener, 1965a). In stop flow experiments on isolated kidneys, the $[Na^+]$ in distal tubule fluid is directly related to the perfusion pressure before occlusion (Tobian, Coffee, Ferreira and Meuli, 1962, 1964).

Efforts to assess the precise role of aldosterone in the physiological regulation of Na^+ balance have been hampered by the difficulty in measuring hormone levels. In the past, the excretion of an acid-released urinary metabolite, representing about 5% of secreted hormone, has been assayed, but more recently direct estimation of adrenal venous aldosterone levels in transplanted glands has been undertaken. The most likely role of aldosterone is to provide a suitable background for maximum Na^+ retention, particularly under conditions of Na^+ deficiency. Perhaps the strongest evidence in support of this contention is the observation that adrenalectomized subjects cannot maintain Na^+ balance on a very low Na^+ intake, but can

do so on moderate intakes. Aldosterone increases distal tubular Na^+ reabsorption (Vander et al., 1960, 1961), and as well causes a decrease in Cl^- and a small increase in K^+ or H^+ excretion (Bartter, 1956; Mills, Thomas and Williamson, 1960, 1961; Yunis, Bercovitch, Stein, Levitt and Goldstein, 1964). Renal tubular effects are difficult to detect in normal dogs, but are very evident when aldosterone is administered to adrenalectomised dogs (Berger, Berlin and Tulenko, 1958).

Even the evidence for a role for aldosterone in Na^+ depletion is controversial. Ross and Winternitz (1960) found that the renal response to a low Na^+ diet was delayed when aldosterone activity was inhibited by spironolactone. Thorn, Ross, Crabbé and van't Hoff (1957) observed an inverse relationship between the urine Na^+ excretion and acid-released hormone in random urines from normal subjects. However, Crabbé, Ross and Thorn (1958) were unable to relate urinary Na^+ output to acid-released aldosterone during Na^+ deprivation, and Mills (1963) was unable to correlate diurnal variation in Na^+ excretion with aldosterone levels.

It would seem that, although aldosterone may play an important part in long term adjustments to low levels of Na^+ intake, short-term changes in Na^+ excretion are not aldosterone dominated. This is strongly supported by the time course of the renal response to its administration. Berger, Berlin and Tulenko (1957) and Ganong and Mulrow (1958) injected d-aldosterone directly into one renal artery, and observed the characteristic effect on Na^+ and K^+ excretion simultaneously in both kidneys: there was a time lag of 30-60 minutes, and the maximum effect occurred after 4 hours. Adrenalectomised or Addisonian patients maintained on adequate steroid replacement, in whom there can be no induced changes in the circulating aldosterone level, show the normal

renal response to both rapid posture change and pooling of blood in the legs (Rosenbaum, Papper and Ashley, 1955; Epstein, 1956).

Aldosterone secretion can be influenced by a number of factors, but the exact physiological importance of many of these remains to be determined. The strongest stimuli are of haemodynamic origin, but others identified include ACTH, plasma electrolytes and perhaps a pineal factor. Although largely independent of ACTH (MacLean, Lipsett, Li, West and Pearson, 1957), aldosterone secretion is stimulated for a short time by the trophic hormone (Davis, Carpenter, Ayers and Bahn, 1960; Mulrow, Ganong, Cera and Kuljian, 1962; Blair-West, Coghlan, Denton, Goding, Wintour and Wright, 1963), probably through increased production of hormone precursors. Low plasma $[Na^+]$, and particularly high plasma $[K^+]$ stimulate the adrenal gland directly (Denton, Goding and Wright, 1959; Davis, Urquhart and Higgins, 1963; Blair-West et al., 1963). Farrell (1958, 1959, a, b) has produced evidence for a role of the pineal gland in stimulating aldosterone secretion, but this has not been confirmed by others (Davis et al., 1959; Wurtman, Altschule, Greep, Falk and Grave, 1960; Blair-West et al., 1963).

Changes in ECF volume, but not of ICF volume, and particularly changes in the intravascular volume rather than the interstitial component, are potent stimuli for aldosterone secretion (Bartter, Liddle, Duncan, Barber and Delea, 1956; Bartter, Mills, Biglieri and Delea, 1959). The hyperaldosteronism of cardiac failure, hepatic cirrhosis and nephrosis, which results in excessive Na^+ retention and oedema, can be mimicked experimentally by haemodynamic changes induced by inferior vena caval constriction (Davis, Goodkind, Pechet and Ball, 1956; Ball, Davis and Goodkind, 1957), renal ischaemia (Goldblatt, Lynch, Hansal and Summerville, 1934) or pulmonary artery stenosis (Davis, 1962).

Cross circulation experiments have shown that the aldosterone stimulating activity is humoral (Denton et al., 1959; Yankopoulos, Davis, Kliman and Peterson, 1959; Davis, Carpenter, Ayers, Helman and Bahn, 1961); it has been identified to be renin secreted by the juxtaglomerular apparatus (Cook and Pickering, 1959; Hartroft, Sutherland and Hartroft, 1964).

A natriuretic factor is associated with rapid expansion of the ECF volume. The magnitude of the renal Na^+ response appears to be related to the rapidity and magnitude of the initial expansion (Levinsky and Lalone, 1963; Shuster, Alexander, Lalone and Levinsky, 1966). The origin of the increased Na^+ excretion has been shown not to be a change in GFR, RPF, filtered Na^+, plasma $[Na^+]$, [plasma protein], PCV, adrenal steroids or vasopressin (Mills, de Wardener, Hayter and Clapham, 1961; de Wardener, Mills, Clapham and Hayter, 1961; Blythe and Welt, 1963). The site of action in the kidney is the proximal tubule, where tubular reabsorption is decreased (Cortney et al., 1965; Cirksena et al., 1965; Dirks et al., 1965; Watson, 1966; Landwehr et al., 1967). Two mechanisms have been proposed; one group favours a direct haemodynamic effect (e.g. increased perfusion pressure), while the other favours a humoral factor secreted in response to the stimulation of "volume receptors". Both may be involved, but one may predominate in any particular experimental study. McDonald and de Wardener (1965 a,b) perfused an isolated dog kidney with blood from a saline loaded donor; the natriuresis in the isolated kidney was closely related to the perfusion pressure, but that in the donor was associated with only a small rise in B.P., although several times greater in magnitude.

Volume receptors have been proposed to monitor body Na^+ content, and in turn influence aldosterone secretion and also the recently discovered

natriuretic principle associated with ECF expansion. In practice, ECF volume could be metered by pressor receptors, perhaps in a particular region in the interstitial or intravascular compartment. Conclusive evidence for their location has not yet been produced. Bartter, Mills and Gann (1960) describe receptors at the thyro-carotid arterial junction. A cephalad site was suggested by Strauss, Davis, Rosenbaum and Rossmeisl (1952) who observed increased Na^+ excretion in recumbent water loaded subjects by hypotonic expansion of the ECF, but not in seated subjects. Several workers have postulated a receptor in the liver or portal circulation. The reduced proximal tubule Na^+ reabsorption after saline loading can be prevented or reversed by partial suprahepatic inferior vena caval ligation (Cirksena et al., 1965). Levinsky and Lalone (1965) almost abolished the natriuresis by chronic thoracic caval ligation, reduced it by acute ligation, but abdominal vena caval ligation was less effective.

3. Potassium

Dietary K^+ deficiency, in contrast to Na^+ deficiency, is an unlikely occurrence in ruminants on natural diets because of the high K^+ intake. In several breeds of sheep, about 90% of K^+ output is via the urine (English, 1966; Dewhurst and Harrison, 1966; Beal and Budtz-Olsen, 1967). A small K^+ loss occurs in suint, of which 26% is K^+ (Farnworth, 1956), secreted at the rate of 0.5-1.0 gm/day (Daly and Carter, 1955; Stacy et al., 1963). Compared with the complex regulation of Na^+ balance, the control of K^+ homeostasis appears to be simple. Since the principal route of K^+ excretion is in the urine, the kidney would provide the most effective site for regulation.

Urinary K^+ excretion is not directly related to GFR, filtered load

of K^+, or plasma $[K^+]$, but does seem to be influenced by cell $[K^+]$, the distal tubular P.D. and the rate of H^+ secretion. It seems likely that cell $[K^+]$ plays a central role in determining K^+ excretion, but evidence in support of, or against, such a hypothesis is difficult to obtain in practice. The decreased K^+ excretion during water diuresis, and increased excretion during loading with hypertonic solutions has been attributed to changes in cell $[K^+]$ with hydration (Seldin and Tarail, 1949; Mudge, Foulks and Gilman, 1950). The transtubular P.D. and level of H^+ secretion appear only to provide suitable background conditions favouring K^+ secretion, but in some cases low P.D. or high H^+ secretion may be limiting. Competition for excretion between K^+ and H^+ may not be in a carrier mediated system (Berliner et al., 1951), but may reflect the cell $[K^+]$ and $[H^+]$ which are known to be inversely related in many circumstances (Swan and Pitts, 1955; Swan et al., 1955; Orent-Keiles and McCollum, 1941; Cooke et al., 1952; Adler et al., 1965).

It has been suggested that the adrenal steroids may be involved in some way in K^+ homeostasis because plasma $[K^+]$ increases stimulate aldosterone secretion, and decreases inhibit it (Denton et al., 1959; Davis et al., 1963; Blair-West et al., 1963; Gann, Delea, Gill, Thomas and Bartter, 1964), and aldosterone may increase the rate of K^+ excretion (Bartter, 1956; Mills et al., 1960, 1961; Yunis et al., 1964). Kinne, Macfarlane and Budtz-Olsen (1961) infused aldosterone (10-300 µgm for 1 hour) in sheep without effect on K^+ excretion. On present evidence, neither does plasma $[K^+]$ appear to be an important determinant, nor does regulation of K^+ excretion seem a major function, of aldosterone. Keynes and Harrison (1967) have reported preliminary experiments investigating the role of the adrenal hormones in K^+ metabolism in sheep, but these are not sufficiently advanced to reach any

conclusions.

4. Water

Water is ingested in the feed and as free water, and is lost in faeces and urine and through the skin and respiratory tract. Lactating animals also lose water in the milk. Loss through the skin and respiratory tract depends on the environmental temperature and humidity. Since evaporative cooling is an essential temperature regulating mechanism in most mammals, there is a large obligatory water loss in this way during hot weather. When there is an abundant water supply, the important factors regulating water metabolism are the amount of water drunk and the urine flow. Animals on dry feeds with water freely available use more water in short-term adjustments of body fluids than do animals on natural feeds of greater water content, but which drink less.

The hypothalamus appears to contain the integrating centres for water metabolism. Here are the proposed feeding and drinking centres, the temperature regulating centre and the osmoreceptors concerned in ADH secretion.

The amount of water drunk will depend upon water losses, including that required for temperature regulation, and as well on the quantity and composition of the feed, and in some cases on psychological factors. Merino sheep drank twelve times more water in summer than in winter in unshaded yards (Macfarlane, Morris and Howard, 1956). Many species show a constant water: feed intake ratio (Bruce and Kennedy, 1951; Cizek, 1959; 1961). The actual ratio will depend on the particular feed used; in the case of sheep, Riek, Hardy, Lee and Carter (1950) found an average ratio of 9.1 ml/gm feed on a low plane of nutrition, and 2.8 ml/gm on a high plane. Gregerson (1932)

suggested that the increased secretion of digestive juices decreased the ECF volume, creating thirst; Cizek (1961) suggested that thirst was caused by the osmotic effect of the feed drawing water into the gut. In the ruminant, both effects might be expected. Water restriction reduces the feed intake and conversely reduced feed intake lowers the water consumption. Merino sheep stopped eating after two days without water (Macfarlane, Morris, Howard, McDonald and Budtz-Olsen, 1961).

Multiple stimuli are involved in thirst. For a recent discussion of the subject, refer to a symposium "Thirst in the regulation of body water", edited by Wayner (1964). The thirst created by raised plasma osmolality may act through cell dehydration, since administered osmotically active solutes produce more drinking than do comparable amounts of non-active solutes (NaCl compared with urea) (Gilman, 1937; Holmes and Gregersen, 1950; Kanter, 1953; Adolph, Barker and Hoy, 1954; Fitzsimons, 1961a). Other effective stimuli are reduced blood volume (Holmes and Cizek, 1951; Huang, 1955; Strauss, 1958; Fitzsimons, 1961b; Stricker, 1966), and drying of the oral mucosa (Adolph et al., 1954). Satiation of thirst involves oro-pharyngeal metering and stomach distension (Holmes and Gregersen, 1950); in sheep, the passage of water through the lower oesophagus, and the fill of the rumen (Bott, Denton and Weller, 1965), are comparable factors. The administration of pitressin depresses thirst in many species (Holmes and Gregersen, 1950; Adolph et al., 1954; Di Salvo, 1955).

Water loss in the urine includes both obligatory and regulatory fractions. The obligatory fraction is dependent on the minimum volume excreted with the solute, i.e. the maximum concentrating ability; the regulatory fraction is the volume in excess of this. ADH is necessary for the formation

of hypertonic urine. The physiological stimuli to its release from the posterior pituitary appear to be raised plasma O.P. (Verney, 1947; Leaf and Hamby, 1952a, and others) and reduced blood volume (Cisek and Huang, 1951; Leaf and Hamby, 1952b; Cort, 1954; Lemaire, Boura, Dupont, Deiss and Allegrini, 1959). In anaesthetized rats, Dyball (1966) observed that severe haemorrhage was a stronger stimulus than intracarotid injections of 5% NaCl. A series of experiments by Haberich and co-workers (Haberich, 1968) has demonstrated osmoreceptors in the portal circulation, probably in the liver. Ingested water, or water infused into the portal vein, lowers portal blood O.P. and increases the water content of the liver, in many cases buffering systemic effects. The curve of the resulting diuresis parallels the curve of the water content of the liver. A role for left atrial stretch receptors in the control of ADH has been proposed (Henry, Gauer and Reeves, 1956; Gauer and Henry, 1963) but discounted by others (O'Connor, 1962; Lydtin and Hamilton, 1964). Although ADH is usually considered the important factor in water conservation, Macfarlane (1964) believes that in acute heat stress the principal factor in the sudden reduction in urine volume is not ADH but GFR and excretion and tubular reabsorption of solute.

Dehydration may occur if adequate water is not available. Considerable attention has been directed to the study of water balance in a hot, dry environment (see reviews by Schmidt-Nielsen and Schmidt-Nielsen, 1952; Hudson, 1964; Chew, 1965). These adaptations include behaviour patterns such as burrowing and avoiding the heat of the day; sacrificing thermoregulation to maintain water balance; the development of a highly concentrating kidney; and the development of special physiological mechanisms, such as counter-current heat exchangers, the ability to detoxify oxalate, or special mechanisms to

maintain the intravascular volume at the expense of other ECF compartments. Ruminants living in hot, dry regions increase not only the rate of water turnover (Macfarlane, 1965), but also the total body water content (Macfarlane et al., 1959; Morris et al., 1962) and in particular the ECF volume (Macfarlane et al., 1959; Macfarlane, 1965); plasma electrolyte and plasma protein concentrations may fall slightly (Macfarlane, Howard and Morris, 1966). During dehydration amounting to 25% of the total body water, 45% of this loss came from the ECF, and 55% from the gut and cells (Macfarlane et al., 1963). Hecker et al., (1964) found the interstitial fluid and plasma volumes were both reduced, but a large contribution came from the rumen water and other gut fluids during dehydration in the sheep.

V. Conclusions

The above review has shown that, in general, water and electrolyte studies have been carried out in non-ruminant species. Particular aspects have been examined in the ruminant, and it would appear that overall controlling mechanisms are no different from those in other animals. Ruminants have proved useful animals for the study of aldosterone secretion and Na^+ deficiency, since the parotid salivary Na^+/K^+ is sensitive to this hormone, and the adrenal glands can readily be transplanted to the neck. Other studies have principally been on dehydration and adaptation to a hot, dry environment, and the study of water and electrolytes involved in the digestive cycle.

The unique features of water and electrolyte metabolism in the ruminant are related to the large digestive cycle of ECF to saliva to rumen liquor to ECF. The ruminant has the ability to augment renal and intestinal methods of Na^+ conservation by increasing body Na^+ at the expense of Na^+ in

the digestive cycle. The urine is alkaline, and its composition reflects the composition of the diet, being low in Na^+ and rich in K^+. Potassium is usually the principal urinary solute, in contrast to the situation in carnivores where Na^+ and urea predominate. As in non-ruminants, the large movement of water and electrolytes during feeding is reflected in changes in the pattern of urinary excretion.

The present thesis is concerned with the movement of water and electrolytes within the body and their loss in the urine consequent upon acute alterations in the electrolyte status of the rumen. Observations have been made on sheep feeding for a single 2 or 3 hour period daily, in which it might be expected that any effects of feeding would be magnified by sudden fluctuations in the digestive cycle. In an attempt to analyse the mechanisms involved, the diuretic, acetazolamide, has been administered at the time of feeding, and drinking water has been withheld. The same parameters have also been followed during ad libitum feeding. In other experiments, water, NaCl and KCl have been infused into the rumen, NaCl into the duodenum, and NaCl and KCl intravenously. From these, patterns of absorption and renal excretion have been determined, and their interpretation attempted.

CHAPTER 2

CHANGES IN URINE AND BLOOD COMPOSITION UNDER A RESTRICTED FEEDING REGIME

During studies of renal function, feeding or rumination produced a marked reduction in urine flow in goats undergoing a water diuresis (Andersson, 1955), and in polyuric sheep (Schmidt-Nielsen et al., 1958). The antidiuresis seen in sheep fed hay, but not beets or potatoes, by Lysov (1960) was interpreted as a reflex originating from receptors in the gastro-intestinal tract. Kinne et al. (1961) observed acidification of the normally alkaline urine in feeding sheep, and considered it a reflection of the loss of extracellular alkali in the profuse saliva of high HCO_3^- content.

These observations prompted studies of water and electrolyte excretion in sheep trained to eat for only a short period each day. Stacy and Brook (1964, 1965) compared a urine sample collected within 90 minutes of feeding with the mean of three taken in the 90 minutes before feeding. They found that, after feeding, urine flow decreased, specific gravity increased, the urine was acidified, and there was decreased excretion of Na^+, K^+ and total solutes. A transient hyperproteinaemia was observed, but no consistent change in either GFR or RBF. Using water-loaded sheep as assay animals, Stacy and Brook (1965) demonstrated antidiuretic activity in the urine of fed, but not unfed, sheep. A sustained increase in plasma osmolality and $[Na^+]$ occurred after feeding (Stacy and Brook, 1965; Warner and Stacy, 1965; Stacy and Warner, 1966; Ternouth, 1967).

The renal and plasma changes appeared to result from the demands on extracellular water and electrolytes for digestive secretions during feeding. The antidiuresis was a consequence of the increased plasma osmolality releasing antidiuretic hormone, while the acid urine resulted from loss of extracellular alkali. A brief report of decreased blood pH after feeding has appeared in an abstract (Stacy, 1967). The mechanism promoting Na^+ conservation was not identified, but it was suggested that reduced Na^+ excretion, combined with increased H^+ excretion, uncovered competition of H^+ and K^+, resulting in lowered K^+ excretion.

Rumen electrolyte changes have been studied under similar feeding conditions. In the fasted animal, rumen contents were hypotonic to plasma (Parthasarathy and Phillipson, 1953; Engelhardt, 1963; Warner and Stacy, 1965; Ternouth, 1967), but increased to near 400 mos/kg upon feeding (Warner and Stacy, 1965; Ternouth, 1967). When water was withheld, hypertonicity persisted for many hours, but when drinking was permitted, after three hours the contents returned to hypotonicity (Ternouth, 1967). After feeding, rumen $[K^+]$ increased markedly, but $[Na^+]$ fell (Reid, 1965; Warner and Stacy, 1965; Stacy and Warner, 1966; Ternouth, 1967), however, the absolute amount of both electrolytes increased (Reid, 1965). The volume of fluid in the rumen increased after feeding in cattle (Reid, 1965) and in sheep (Ternouth, 1967; Warner and Stacy, 1968b). Ternouth observed a second peak of rumen volume 5-6 hours after feeding, but this has been attributed by Warner and Stacy (1968a) to errors in the experimental technique.

From changes in the concentration of a rumen marker, Na^+ and K^+, and making assumptions about salivary flow, Stacy and Warner (1966) concluded that net water influx into the rumen after feeding was small, and that Na^+

absorption was enhanced by the hypertonic conditions. Ternouth (1967), who estimated rumen volumes at various times after feeding, and apportioned salivary flow according to the ruminating activity at the time, calculated that 20% of the rumen volume one hour after feeding originated from transruminal influx of water. The conflicting conclusions could in part be due to the different times needed for consuming the feed (4 hours and 1 hour respectively). However, both workers made assumptions which are hard to justify; Ternouth, in particular, used marker dilution techniques of questionable accuracy.

At the time the present work was commenced, only the first paper of Stacy and Brook had been published. Their results were clearly far from complete: the period studied was very short, information on plasma changes was scant, and urine samples were not obtained by catheterization. The work reported in this Chapter was therefore undertaken as further study of the post-prandial changes in urine, plasma and erythrocyte electrolytes was clearly warranted. Particular emphasis has been placed on using quiet, loosely restrained sheep, accustomed to their surroundings and to the experimental procedure, and in which samples have been collected from previously inserted urinary catheters and jugular cannulae. The changes in urine and body fluid composition during and after one short feed a day have been compared with those seen during superimposed restriction of drinking water and the administration of the diuretic, acetazolamide. Acetazolamide was chosen because in other species its effects, namely increased urine flow, raised urine pH, and increased urinary loss of Na^+ and K^+, are opposite to the conservation mechanisms observed during feeding in the sheep.

MATERIALS AND METHODS

Experimental design

Animals were fed for a period of 2-3 hours in the morning, and samples of blood and urine were collected before, during and after feeding. There were six experimental situations, according to whether or not the animals received an injection of acetazolamide, and to the availability of drinking water during and after feeding. These six conditions are listed below:

A. No acetazolamide
   (a) water available ad libitum
   (b) no water during feeding, but available before and after
   (c) no water before, during or after feeding

B. Intravenous acetazolamide
   (a) water available ad libitum
   (b) no water during feeding, but available before and after
   (c) no water before, during or after feeding

Feed

One batch of chaffed, dehydrated lucerne was used throughout. Routinely, the sheep were fed from 10 a.m. to either noon or 1 p.m. In the initial experiments 600 gm were fed for 2 hours, but as the animals grew older this was increased to 900 gm given over 3 hours. The sheep became accustomed to the short feeding time, and unless water was withheld, usually all was consumed in the allotted time. For the earlier experiments a salt lick was provided, but later 5 gm NaCl was added to the feed. Water was provided

in plastic buckets.

Experimental animals

Nine young Romney ewes and one Romney-Cheviot cross ewe were used, their body weights ranging from 22 to 43 kg, but mostly falling between 25 and 35 kg. All remained healthy and gained weight on the feeding regime. They were shorn at approximately 6 week intervals. Thiabendazole was administered when the sheep were first brought indoors and at times later; faecal egg counts usually showed very low worm burdens.

The sheep were housed in a room in which the temperature and humidity were not accurately controlled, but in which heaters were used in the winter, and care was taken not to increase the humidity by hosing during experiments. Relative humidity varied from 50% to near 100%. Over the year, the temperature in the room ranged from 11° to 26°C, but over any 24 hours the fluctuation was 2° to 6°C. The sun shone directly into the room for only a short time each day, but not on to the animals. The sheep were confined in loose sacking slings in metabolism crates so they could stand normally, but not sit on the floor of the crate. They were thoroughly accustomed to this form of restraint, and head-stocks were not needed.

Self-retaining Foley catheters (size 16 Fr) were used for urine collection. Both the urinary catheter and the jugular vein cannula were inserted aseptically on the afternoon or evening prior to the first experiment in the week, and left in place for 3 (occasionally 4) days. In selecting experimental animals, any which could not tolerate the presence of a urethral catheter without discomfort were discarded. The urine delivered from the catheter was clear, and on the rare occasion when the urine became cloudy or

the animal showed any discomfort, the experiment was terminated.

A polythene Sterivac cannula (size 2) was inserted into the jugular vein through a large bore needle. After withdrawal of the needle, a short piece of rubber cannula blocked by a plastic bung was fitted over the other cannula, and a loop of thread was tied around the junction of the two and stitched to the skin. The cannulae were inserted downwards towards the heart, they were filled with saline containing a small amount of heparin between sampling, and artery forceps were used to block off the rubber cannula when the bung was not in place. These precautions minimized blood leakage into the cannula, and clotting rarely occurred.

In three sheep, one carotid artery had been exteriorized into a skin flap to form a carotid loop. Blood was collected directly from the artery into a needle and syringe after injection of local anaesthetic.

Sample collection

Sampling procedures caused a minimum of disturbance to the animals, and even during collection of jugular blood samples the sheep were not held. They took little notice, and some continued feeding.

Urine samples were collected by gravity each 30 or 60 minutes into tared, collapsed plastic vaccine bags which fitted tightly into the end of the catheter, allowing anaerobic collection for pH and HCO_3^- estimations. Since the end of the catheter was clamped close to the sling, the bags could be changed without touching the sheep. After the bag was weighed, samples for pH and HCO_3^- were taken out through the wall of the bag into a needle and syringe. The rest was poured out into a measuring cylinder and diluted in

the usual manner. The usual sampling period of 30 minutes was selected as a compromise between the number of samples to be analysed and good resolution of the time course of urinary excretion.

Eight 8-10 ml blood samples were collected at hourly intervals, and an additional one 15 minutes before feeding. The heparinized saline in the cannula was aspirated into a syringe before the collection of the sample into a separate syringe wetted with heparin solution ('Pulmin'). The sample was protected by sealing the tip of the syringe with a cap. Blood pH and CO_2 content were estimated immediately, and samples drawn off for PCV and $[Hb]$ determination. The rest was then centrifuged for plasma and erythrocyte samples. Usually the sample for pH was collected into a separate syringe.

Acetazolamide administration

Acetazolamide, dissolved in distilled water at 100 mg/ml, was injected over 30-60 seconds through the jugular cannula. The dose rate ranged from 5 mg/kg to 16 mg/kg. The time of administration was usually 10 minutes before feeding, but doses were given up to 30 minutes after feeding started.

Preparation of plasma and lysed erythrocytes

Plasma was prepared by centrifugation for 30 minutes at 3500 rpm (radius 6 inches). In the earlier experiments open centrifuge tubes (internal diameter 16mm) were used, but later the bulk was placed in tubes of 8 mm internal diameter, and the rest in the larger tubes. In these latter experiments, the blood sample was placed under a paraffin layer through a long needle and the plasma separated anaerobically. Solutions of lysed erythrocytes

were made from the packed cells in the smaller tubes, using only the lower two-thirds of the column of cells, which were diluted and lysed in distilled water. Both plasma and packed erythrocytes were rinsed into the volumetric flask with the diluent.

Analytical methods

(i) Urine volume. Usually the weight of urine was estimated as (sample weight - tare of bag) to the nearest 0.1 gm, and the volume calculated as weight x S.G. In a very few experiments, the volume was obtained directly by collection into graduated measuring cylinders.

(ii) Urine specific gravity. S.G. was measured with a small hydrometer (Clay-Adams Urinometer), usually on undiluted urine, but for a high S.G., or very low volume, a dilution of 1 in 2 with distilled water was used. S.G. could be estimated to ± 0.002, or ± 0.004 if diluted.

(iii) pH. Urine and blood pH were determined anaerobically on the Beckman model 76 expanded scale pH meter with the microblood pH assembly (46850), glass electrode (39045) and reference electrode (39070) (Ag-AgCl), mounted in the Beckman Thermomatic Constant Temperature Block. The apparatus was duplicated, one for blood samples and one for urine. The temperature was maintained at 38° ± 1°C as much as possible, although sometimes the temperature moved out of this range for part of the determination. These fluctuations did not cause any apparent alteration in pH. The buffer used for standardisation was Sørensen buffer, pH 6.84 at 38°C, made of equal amounts of $^{M}/_{40}$ KH_2PO_4 and $^{M}/_{40}$ Na_2HPO_4. The two solutions were stored in the refrigerator and mixed freshly daily.

A standard procedure was evolved for using the equipment. It was observed that a single sample of blood introduced into the assembly gave an initial low reading which rose over 1-2 minutes. Further drift occurred when a second sample was introduced, but the final reading from this sample appeared to be stable, for it could be repeated when a third sample was injected. The procedure adopted for blood, therefore, was to make 3 injections of 2 ml, and in each case readings were taken at 30 second intervals for 2 minutes. The correct reading was taken as the final reading for the third sample. The same procedure was used for urine, except that only 2 injections were usually required. Each sample was washed out with saline, water, then saline, and buffer readings were taken between each blood sample on one instrument, and between each 2 or 3 urine samples on the other pH meter. These methods allowed repeatable readings of pH to ± 0.02 units for urine, and to ± 0.01 for blood (using the expanded scale). After a number of samples of either blood or urine had passed through the electrode assembly, the time taken to reach final readings became longer; the electrodes were regenerated by washing in $^{N}/_{10}$ acid and alkali alternately.

(iv) Bicarbonate and total CO_2. The method of Conway (1957) was used with some minor modifications. Because of the high CO_2 content of some urine samples, the $Ba(OH)_2$ concentration was doubled, and the volume used in the centre well was 1 ml for 1 ml of blood, and 1.5 ml for 0.5 ml of urine. In spite of this, some urine samples generated so much CO_2 after the initial mixing with H_2SO_4 that the lids lifted and the seal broke. Duplicate estimations were always performed, and where these did not agree within 5%, the sample was discarded. Estimations of blood CO_2 usually involved less variation than 5%. The CO_2 content was calculated as total CO_2 for blood,

and as HCO_3^- for urine, since urine CO_2 content was large only at alkaline pH when the fraction as CO_2 or H_2CO_3 was 5% or less.

(v) Osmolality. A Fiske Osmometer, model C-62 was used. Great care was taken to standardize all procedures; over the entire range duplicates agreed to 1% or less. A calibration curve was established by using 10 NaCl solutions of known osmotic activity, ranging from 100 mosm/kg H_2O to 1800 mosm/kg. It was found that plasma duplicates, and successive determinations on the one sample, differed at the most by 1 mosm/kg. The standard procedure was to make two estimations on the one sample. For urine the same procedure was adopted, except that for osmolalities above 1600 mosm/kg, a 1 in 2 dilution was used, and the result doubled, although this would give a slightly high value through an increase in the coefficient of ionisation upon dilution.

(vi) Sodium and Potassium. Na^+ and K^+ were estimated on plasma, urine and lysed erythrocytes using an E.E.L. Flame Photometer (Evans Electroselenium Ltd.) Mark II, supplied by propane gas and compressed air. Mutual interference of Na^+ and K^+ was encountered only for low urine $[Na^+]$. Because of the wide range of dilutions required for maximum sensitivity, ($^1/_{10}$ to $^1/_{1000}$), no attempt was made to avoid the small amount of over-estimation of very low urine $[Na^+]$ by adding K^+ to the Na^+ standards. Plasma and erythrocyte Na^+ and K^+ were always estimated in duplicate with a maximum difference of two scale divisions being tolerated (2%).

(vii) Chloride. The potentiometric endpoint titration of Sanderson (1952) was used, employing the Gallenkamp Potentiometric Microtitration apparatus. Duplicates differed by only 1 m-equiv/l (1%). Not all

samples were routinely done in duplicate. Occasionally a particularly concentrated urine sample had to be diluted before titration.

(viii) Urea. Urine [urea] was determined by the method of Conway (1957). Duplicates varied by 5% for low concentrations, and 2% for high concentrations.

(ix) Packed Cell Volume. Wintrobe tubes were centrifuged for 60 minutes at 3500 rpm. Duplicates differed by 2% or less.

(x) Haemoglobin. Blood was diluted and converted to cyanmethaemoglobin with the Cyanmethaemoglobin Reagent of Diagnostic Reagents Ltd. Initially the [Hb] was read on an E.E.L. Hb meter, calibrated in gm Hb/100ml, using commercial standards (3 and 18 gm/100 ml, Diagnostic Reagents Ltd.). The scale on the instrument was found to be incorrectly calibrated, so later results were read at 540 mμ on a Unicam SP500 spectrophotometer. A conversion graph for the two methods of reading was constructed, and the earlier results corrected from this. Duplicates agreed to 1%.

(xi) Plasma protein. Plasma protein was estimated by the Biuret method of Gornall, Bardawill and David (1949). 1 ml of diluted plasma (1 in 10) was reacted with 4 ml of Biuret reagent and read at 540 mμ after 30 minutes on a Unicam SP500 spectrophotometer. A standard curve was constructed using bovine serum albumin. Duplicates agreed within 2%.

(xii) Plasma volume (Evan's Blue method). Evan's Blue dilution space was measured as a check of calculated relative plasma volumes (see below). A priming dose of 1 ml of 0.5% Evan's Blue (5 mg) was injected into the jugular vein of each sheep 17 hours before the determination to saturate

the reticulo-endothelial system. A 10 ml sample of blood was withdrawn prior to this injection for a blank. For the estimation of the plasma volume, a preinjection blank was again taken, then 3 or 4 ml of 0.5% Evan's Blue was injected. Samples of blood were taken at 10, 20, 30 and 40 minutes. Preinjection blanks, samples and standards were read at 620 mμ on a Unicam SP500 spectrophotometer against the original blank from the day before. Samples were corrected for the preinjection blank, plotted on semilogarithmic paper, and the curve obtained was extrapolated to zero time. From this value, and from the optical density of the standards, the volume of dilution was calculated.

Calculated Parameters

(i) Relative plasma volume. The plasma volume relative to that of the first sample of the day was calculated:

$$\frac{PV_2}{PV_1} = \frac{[Hb]_1}{[Hb]_2} \times \frac{1-PCV_2}{1-PCV_1}$$

where PV_2 = plasma volume of sample

PV_1 = plasma volume of first sample

$[Hb]_2$ = [Hb] of sample

$[Hb]_1$ = [Hb] of first sample

PCV_2 = PCV of sample

PCV_1 = PCV of first sample

The first plasma volume was taken as 1.00, and the others a fraction of this.

(ii) Erythrocyte volume. This was estimated from PCV/ [Hb].

(iii) Erythrocyte electrolyte content. The electrolyte concentration was multiplied by the calculated erythrocyte volume.

(iv) Electrolyte excretion rate was calculated from the urine volume and concentration of the electrolyte in the sample, and expressed on the basis of excretion rate / 30 minutes. No correction was made for the "dead space" in the bladder and catheter which would tend to delay the excretion of solutes but not the volume of urine collected. However, in sheep this dead space is only of the order of 4-8 ml (Greenway, personal communication), which would only produce insignificant effects except when urine flow was very low and composition changing rapidly.

(v) Osmolar clearance and Solute-free water reabsorption.

These were calculated:

$$C_{osm} = \frac{U_{O.P.} \; V}{P_{O.P.}}$$

where $U_{O.P.}$ = urine O.P.

$P_{O.P.}$ = plasma O.P.

V = urine volume

$$T^{c}_{H_2O} = C_{osm} - V$$

Where solute-free water reabsorption ($T^{c}_{H_2O}$) is negative, it is frequently called Free water clearance (C_{H_2O}).

Presentation of Results

Neither the preceding excretion pattern nor the response under any particular experimental condition was uniform, even from day to day in the same sheep. The pattern obtained on one day could not be regarded with confidence as the control for a different treatment 1 or 2 days later. In interpreting the results, therefore, the whole group of replicates for each experimental condition has been compared and contrasted with those in the other groups.

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Table 1. Feed intake under different experimental conditions for 3 sheep.

Experimental conditions	Sheep	Av. intake and S.D. (gm)	No. of days ate all 900 gm offered	Total No. of days
Water ad lib.	1	874 ± 38	6	9
	2	832 ± 55	3	10
	3	874 ± 67	6	9
No water during feed	1	731 ± 126	0	6
	2	734 ± 204	1	6
	3	824 ± 77	1	4
Water ad lib. acetazolamide	1	800	0	1
	2	900	1	1
	3	900	1	1
No water during feed acetazolamide	1	635 ± 78	0	2
	2	798 ± 91	1	3
	3	772 ± 125	1	3

Table 2. Feed and water intake of sheep feeding for 3 hours daily.

	Sheep				
	1	2	3	9	10
Av. feed intake (gm)	810	855	905	580	650
Range (gm)	400-1100	665-1100	390-1100	50-1100	145-900
No. of obs.	45	50	50	52	52
Av. water intake during feeding (ml)	2930	1660	2195	1600	2110
Range (ml)	1880-3660	1090-2655	620-3840	95-2060	900-3460
No. of obs.	48	50	50	50	52
Av. water:feed during feeding (ml/gm)	3.62	1.94	2.43	2.76	3.25
Correlation coeff. (r) feed & water intake each feed	r= 0.48 ($p < .01$)	r= 0.19 (not signif.)	r= 0.50 ($p < .01$)	r= 0.81 ($p < .01$)	r= 0.74 ($p < .01$)
Av. water intake for 24 hrs. after feeding (ml)	70	80	120	785	85
Range (ml)	20-250	15-515	5-640	90-1645	15-740
No. of obs.	40	39	36	43	42

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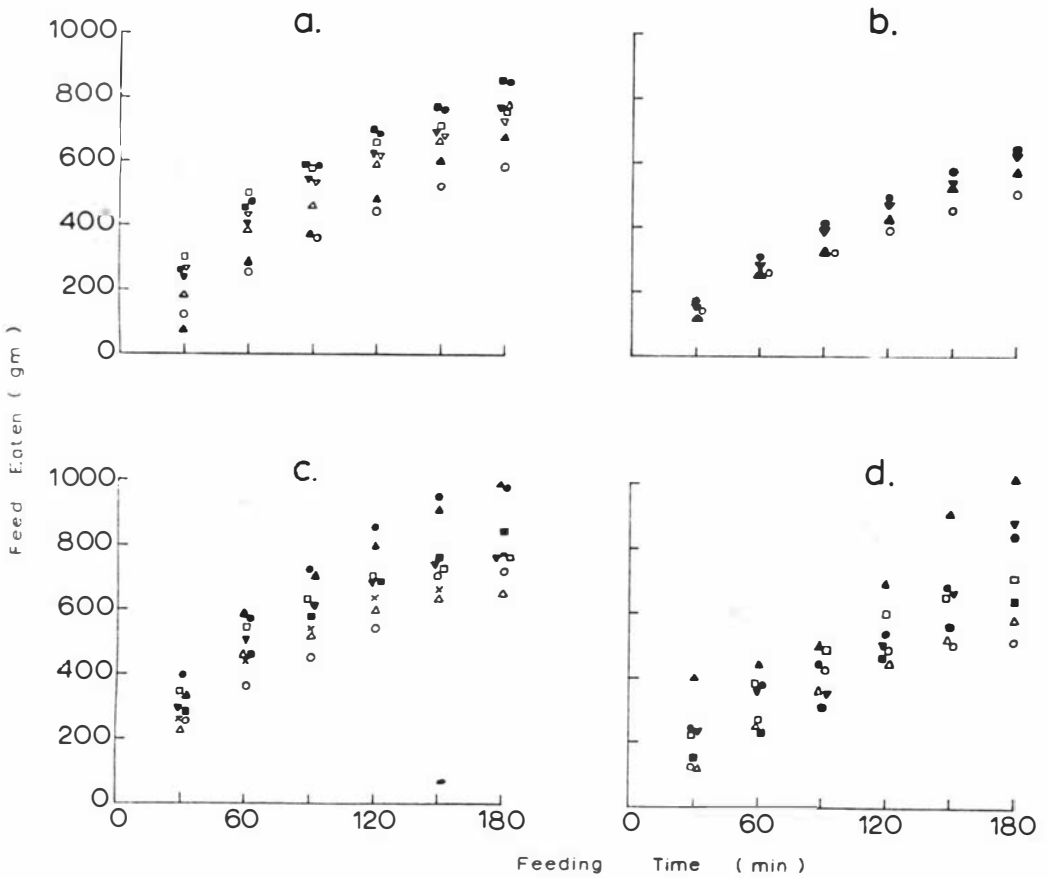

Fig 2. Effect of no drinking water during a 3 hour feed, on the half-hourly feed intake in 4 sheep. Note the reduced intake beginning around 90 minutes and becoming progressively more prominent (a - sheep 2; b - sheep 10; c - sheep 3; d - sheep 1; ● ▲ ▼ ■ water ad libitum; ○ ▽ □ △ × no drinking water).

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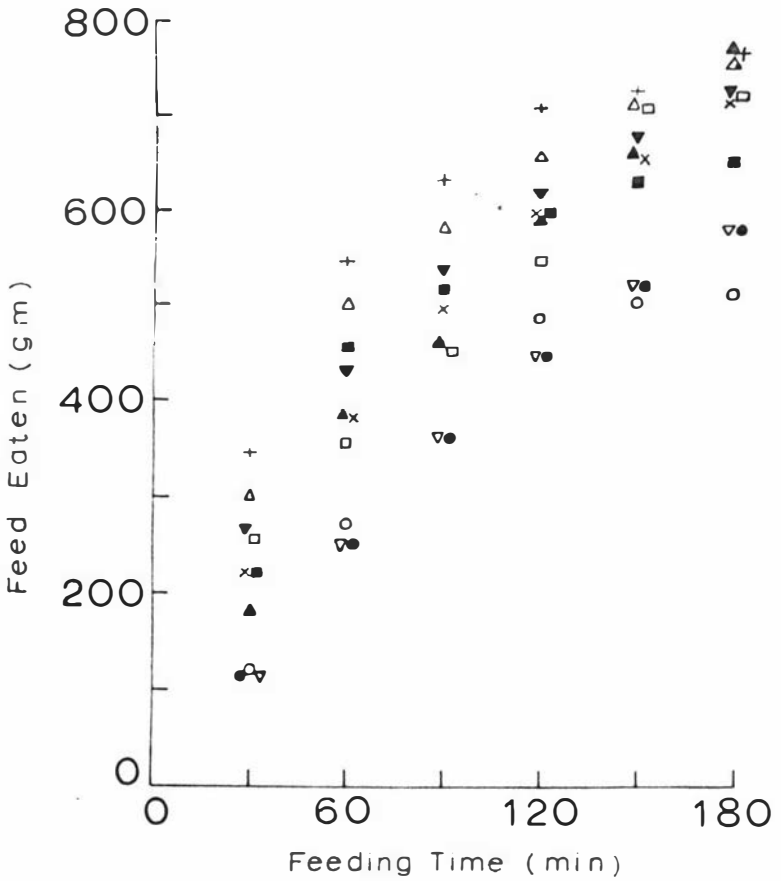

Fig 3. Lack of effect of acetazolamide on the half-hourly feed intake; no drinking water.
(Acetazolamide injected:
▲ - sheep 2, 12.5.66
▼ - sheep 2, 15.6.66
● - sheep 1, 22.6.66
■ - sheep 3, 8.6.66
no acetazolamide:
△ - sheep 2, 3.5.66
▽ - sheep 2, 13.6.66
○ - sheep 1, 20.6.66
× - sheep 1, 3.5.66
□ - sheep 3, 7.6.66
\+ - sheep 3, 3.5.66)

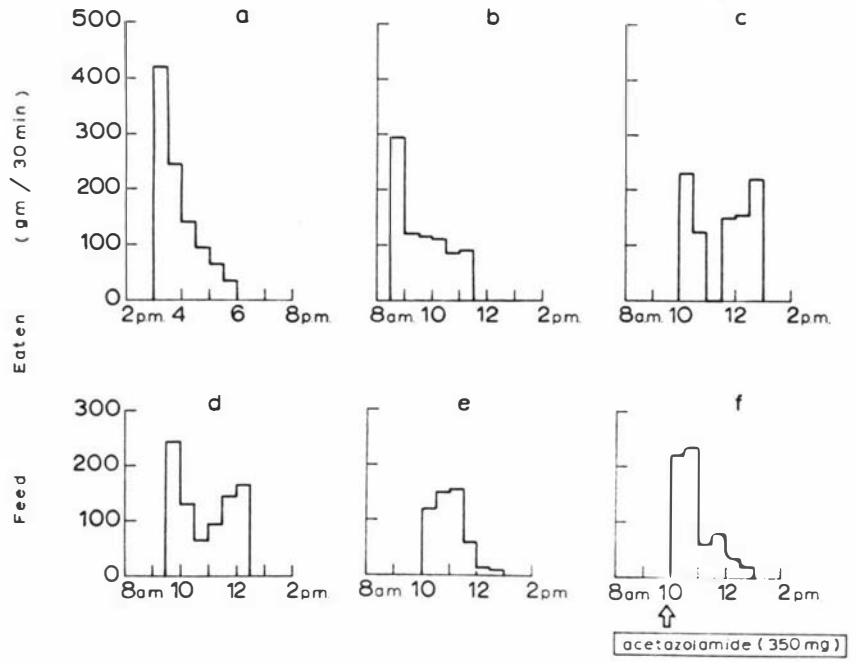

Fig 4. Feed intake patterns during a 3 hour feed. a,b - Type I; c,d - Type II; e,f - unusual patterns during feeding with no water when the intake is reduced in the first 30 minutes (a - sheep 3, 21.4.66; b - sheep 3, 22.4.66; c - sheep 1, 28.4.66; d - sheep 1, 20.4.66; e - sheep 1, 20.6.66; f - sheep 3, 8.6.66).

RESULTS

Feed Intake

The absence of drinking water reduced the feed intake (Table 1, Fig 2). This first became apparent after 1½ hours, and thereafter the divergence became progressively greater (Fig 2). Acetazolamide had no effect on feed intake, whether water was available or not (Table 1, Fig 3).

The rate of feed intake varied through the feeding period. When water was available, the highest half-hourly intake was always in the first 30 minutes. In 3 out of 4 of these, there was a successive decline (Type I) (Fig 4a, b), but in the remainder, there was a period of reduced intake in the middle of feeding (Type II) (Fig 4c, d). Type II was seen on 9 days, 6 of them in one animal (sheep 1). When no water was provided, the intake patterns were similar to those above on 7 of 12 occasions, but on the other 5 the intake in the first 30 minutes was not the highest, and was lower than usual for that sheep (Fig 4e, f). This appears to be a conditioned response as all cases occurred at the end of the series.

Water Intake

An intimate relationship between feed and water intake was evident. Almost all of the water intake for the day was drunk during the feeding period (Table 2). For 4 sheep the feed and water intake during each feeding period showed highly significant correlation (Table 2); the exception was sheep 2, which also had the lowest average water : feed ratio. In sheep 9 and 10, a wide range of feed and water intakes was obtained by measuring these almost from the time the sheep were first brought indoors.

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Table 3. Frequency of observation of the 3 water intake patterns during feeding.

Sheep	Water intake pattern (No. of obs.)		
	A	B	C
1	1	5	11
2	5	3	10
3	7	8	2
9	1	4	0
10	0	5	1

Table 4. Association of feed and water intake patterns.

Feed intake pattern	Water intake pattern (No. of obs.)		
	A	B	C
I	11	8	3
II	0	4	4

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Table 5. Extent of prefeeding diuresis in 8 sheep.

Feeding time	Maximum prefeeding 30 min urine volume (no. of obs.)				Total no. of obs.
	< 50 ml	> 50 ml	> 100 ml	> 150 ml	
10 a.m.	19	44	21	7	63
After 10 a.m.	0	8	7	3	8

Table 6. Urine volumes for latter 2½ hours of feeding.

Conditions during feeding	No. of obs.	Mean volume & S.D. (ml)	Range (ml)
Water ad lib.	16	124.3 ± 23.6	78.0–174.9
No water	13	119.6 ± 28.5	77.1–188.9
Water ad lib. + acetazolamide	3	138.3 ± 34.5	100.0–167.0
No water + acetazolamide	8	149.5 ± 34.7	100.5–196.7
All no acetazolamide	29	122.2 ± 25.5	77.1–188.9
All acetazolamide	11	146.4 ± 33.3	100.0–196.7

Footnote: Experiments performed between November 1965 and June 1966.

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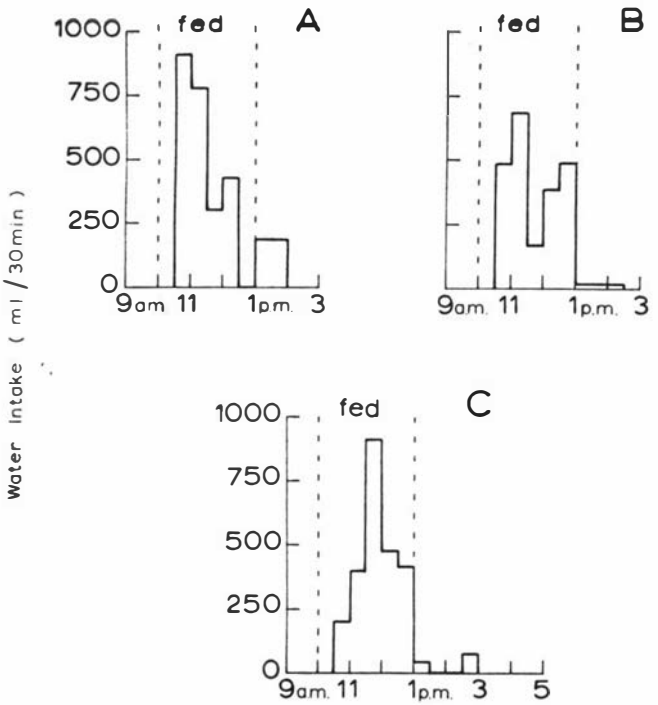

Fig 5. Water intake patterns during a 3 hour feed. Type A (sheep 3, 17.3.66); Type B (sheep 2, 10.3.66); Type C (sheep 1, 23.11.65).

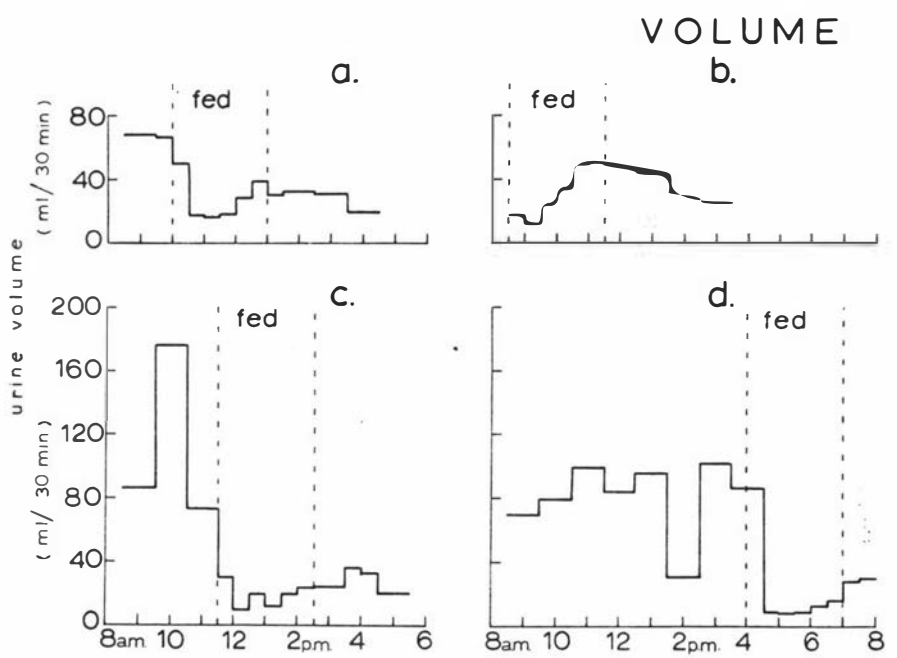

Fig 6. Urine volume relative to a once-daily 2 or 3 hour feed at different times of the day; water ad libitum. Note the prefeeding diuresis and the antidiuresis during feeding in each case (sheep 3 a - 14.3.66; b - 15.3.66; c - 16.3.66; d - 31.3.66).

Little or no water was drunk during the first 30 minutes of feeding: on 44 of 63 occasions no water at all was consumed, and on a further 6,100 ml or less was drunk. The pattern of intake on most days during feeding was readily classified into one of 3 types, designated A,B,C (Fig 5). The frequency with which each type occurred in 5 sheep is shown in Table 3. On 4 days when acetazolamide was administered these same patterns of water intake were observed. The association of the 3 water intake patterns with 2 feeding types is shown in Table 4.

If water was withheld until 1 p.m., within a few minutes the sheep drank 1-2 litres. Sheep 2 usually consumed enough in this short time to satisfy its thirst for the day, but sheep 1 and 3 drank again during the afternoon. The total intake for sheep 2 and 3 was near that when water was available during feeding, but sheep 1 drank about 1 litre less than usual.

When the water was not offered until 4.30 p.m., the sheep all drank immediately, but less than if it were returned at 1 p.m., on some days as little as half.

Urine Volume

A prominent feature of these experiments was the occurrence of a diuresis in the period before the animals were fed. The magnitude of this diuresis varied (Table 5). It did not occur at all in some cases; more commonly, it provided the highest urine flow rates during the experiment. The cause was probably excitement arising from the anticipation of being fed. If feeding was postponed, a profuse diuresis persisted until the food was given (Table 5, Fig 6). On the other hand, the diuresis appeared to be reduced under circumstances in which it might be expected that excitement was less marked.

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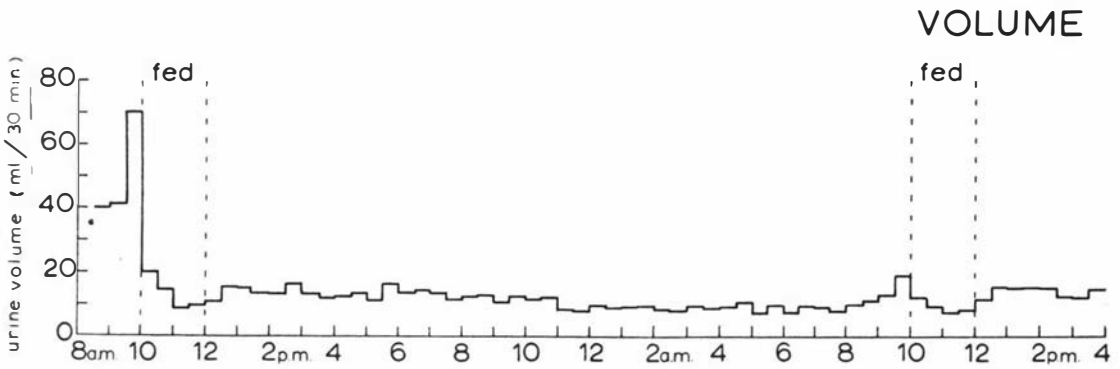
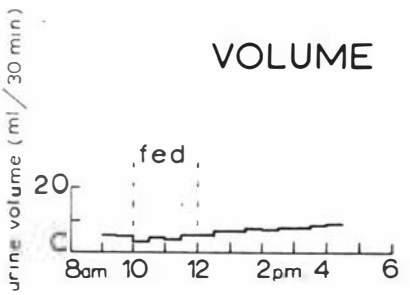


Fig 7. Urine volume relative to a once-daily 2 hour feed; water ad libitum. Note the prefeeding diuresis, less marked before the second feed; the antidiuresis during feeding; a comparable minimal flow 8-12 hours prior to the diuresis (sheep 3, 22.6.65 - 23.6.65).

Fig 8. Urine volume relative to a once-daily 2 hour feed; water ad libitum. Note absence of prefeeding diuresis and thus less prominent antidiuresis during feeding (sheep 3, 12.5.65).



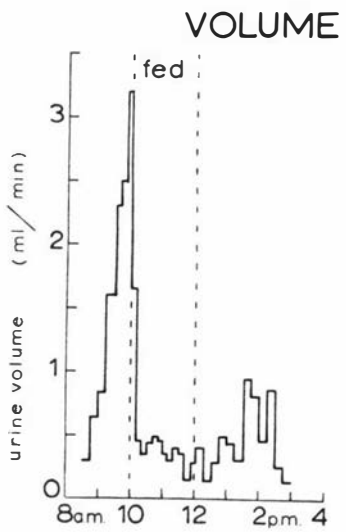


Fig 9. Urine volume relative to a once-daily 2 hour feed; water ad libitum. Note onset of antidiuresis in first 10 minutes, and almost minimal levels in the second (sheep 1, 21.10.65).

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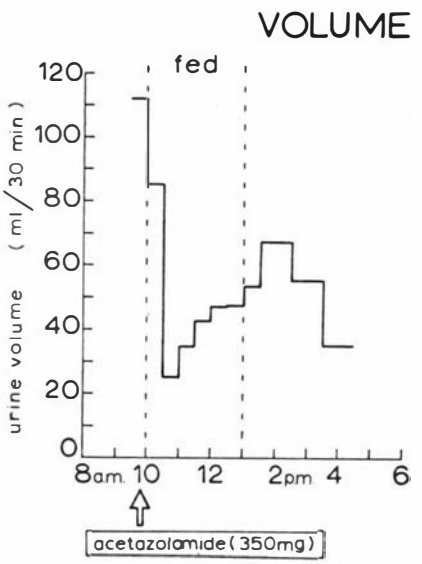

Fig 10. Urine volume relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note particularly high flow rate during feeding (sheep 3, 2.3.66).

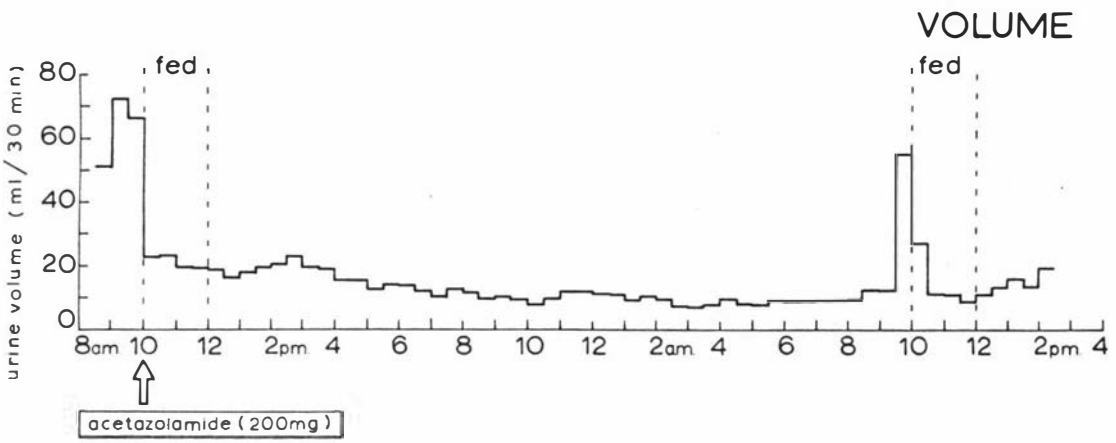

Fig 11. Urine volume relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note the less intense prefeeding diuresis before the second feed (sheep 3, 3.8.65 - 4.8.65).

Thus, in experiments lasting 32 hours and involving two feeds, and during which the animals were receiving continual attention, the diuresis preceding the second feed was less than that preceding the first (Fig 7, 11).

Feeding decreased urine flow in all experiments, whether water were available or not, whether or not the diuretic, acetazolamide, had been administered. The decrease was slight when the pre-feed urine flow had been small (Fig 7, second feed; Fig 8) but marked when a pre-feed diuresis had been established (Fig 6; 7, first feed). The results of the two 32 hour experiments (Fig 7, 11) would suggest that the minimum flow during feeding was comparable to the flow during the 8-12 hours immediately prior to the pre-feed diuresis. Characteristically, the onset of the antidiuresis was rapid. Thus, in an experiment where 10 minute collection periods were used, the high flow rate present immediately prior to feeding was halved during the first 10 minutes of feeding, and was reduced to almost minimal levels in the next 10 minutes (Fig 9). When water was freely available, the lowest urine volume during feeding was in the second half hour on about 50% of the days, and in the third or fourth sample equally often on most of the other days. When there was no drinking water, the minimum volume was observed most frequently in the third half hour. When acetazolamide was given, the minimum volume was later in feeding.

That the antidiuresis was a true feeding response and not a diurnal rhythm was demonstrated by feeding three sheep at different times of the day. In each case, the antidiuresis occurred during feeding (Fig 6).

Urine excretion during the last 2½ hours of feeding under the different experimental conditions is compared in Table 6. The large volume

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Table 7. Urine volume in 3½ hours after feeding.

Conditions of experiment		No. of obs.	Mean volume & S.D. (ml)
During feeding	After feeding		
Water ad lib.	water ad lib.	13	207 ± 59
No water	water ad lib.	4	225 ± 50
No water	no water	9	197 ± 66
Water ad lib. + acetazolamide	water ad lib.	3	216 ± 87
No water + acetazolamide	water ad lib.	3	197 ± 44
No water + acetazolamide	no water	5	218 ± 89

Footnote: Experiments performed between November 1965 and June 1966.

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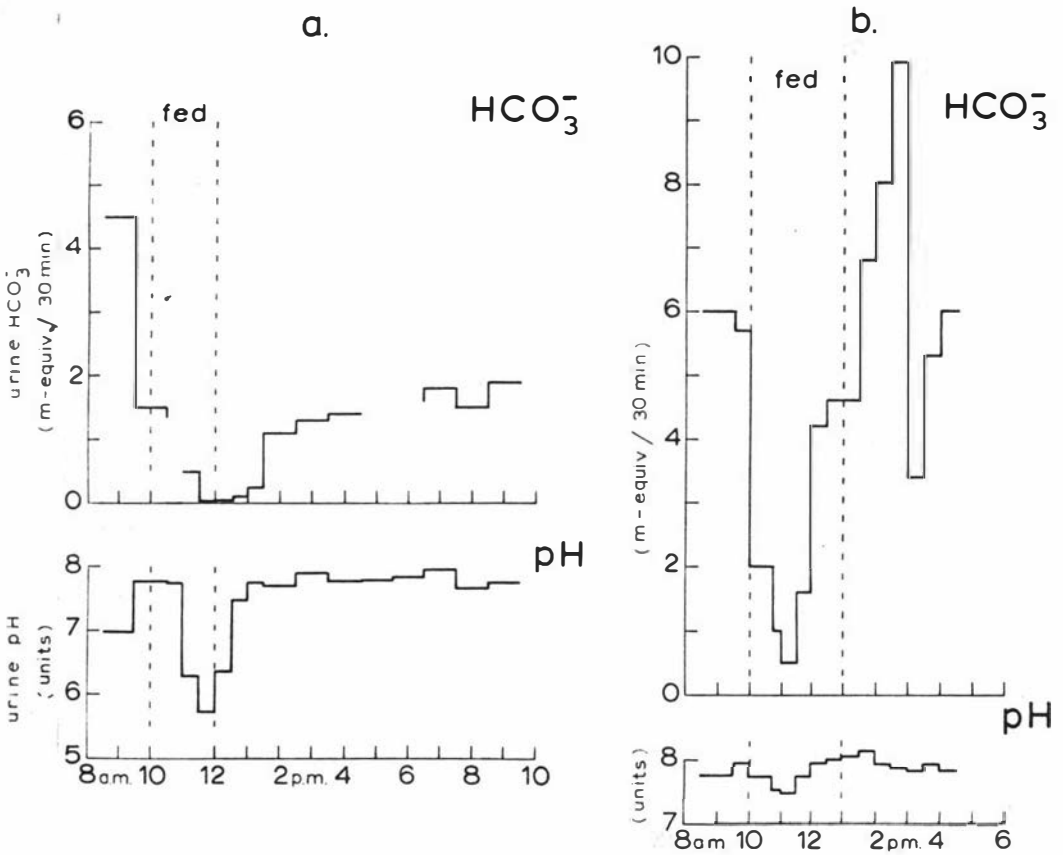

Fig 12. Urine pH and HCO_3^- excretion relative to a 2 or 3 hour once-daily feed; water ad libitum. a - usual observation of urinary acidification and reduction of HCO_3^- excretion almost to zero during feeding; b - atypical day on which HCO_3^-, although markedly lowered, failed to reach very low levels and the urine remained alkaline (a - sheep 4, 17.8.65; b - sheep 5, 7.12.66).

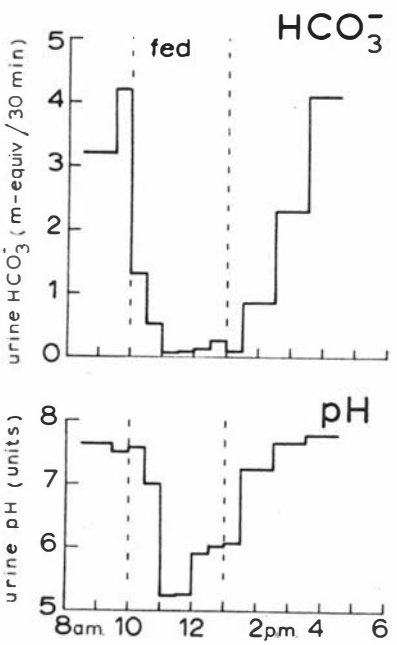

Fig 13. Urine pH and HCO_3^- excretion relative to a once-daily 3 hour feed; no water all day. Usual response of urinary acidification and almost negligible HCO_3^- excretion during feeding (sheep 1, 10.2.66).

during the first 30 minutes was not included, since this might be regarded as a transition period. The presence or absence of drinking water made little difference to the urine volume during feeding: in the two groups without a diuretic, the mean volume was only 4% greater when water was provided. Acetazolamide appeared to cause an increase of about 20% in urine flow. However, both low and high flow rates were encountered in individual experiments (Fig 10, 11).

After feeding, urine flow usually increased substantially. In two 32 hour experiments (Fig 7, 11) the post-feeding increase was followed by a slow decline to the low flow rates seen 8-12 hours before the next feed. Urine volume during 3½ hours after feeding under the different experimental conditions is shown in Table 7. The largest difference appeared to be a 10% greater volume when water was offered at the end of feeding after feeding without water.

In the early experiments when the sheep were younger and smaller, and consumed less feed, urine volumes were lower than in later experiments (Fig 6-8, 11).

Urine pH and Bicarbonate excretion

Bicarbonate excretion was reduced during feeding, whether water was available (8 experiments) or not (7) (Fig 12, 13). The minimum excretion rate was less than 0.1 m-equiv/30 min on 11 days, compared with the usual rate of 2-8 m-equiv/30 min before feeding. Administration of acetazolamide, in all doses used, resulted in high HCO_3^- excretion during feeding, usually above 2 m-equiv/30 min (Fig 14). If acetazolamide was given before feeding, HCO_3^- excretion increased, but subsequently fell during feeding, although not always

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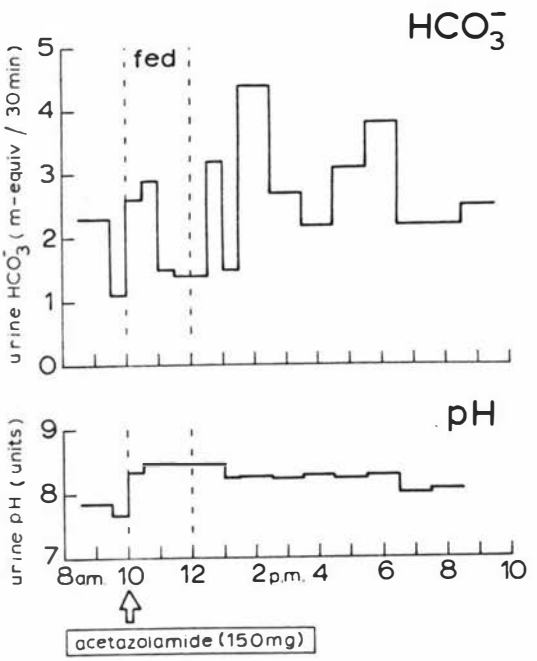

Fig 14. Urine pH and HCO_3^- excretion relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note high HCO_3^- excretion during feeding and alkaline urine (sheep 4, 19.8.65).

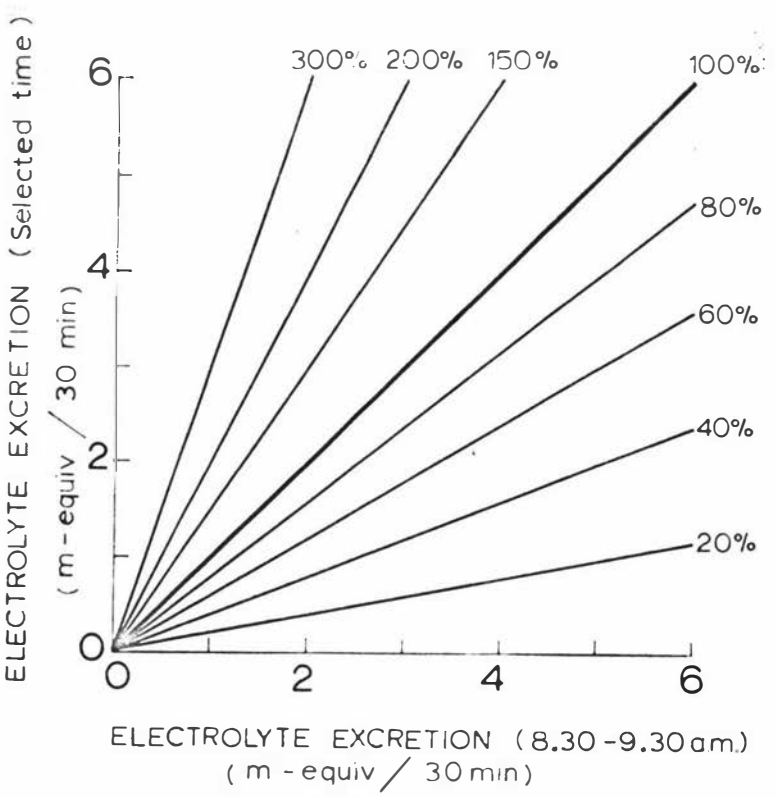

Fig 15. General diagram showing regression lines of electrolyte excretion at a selected time (feeding or prefeeding) on that in the first prefeeding period (8.30-9.30 a.m.). Lines shown correspond with excretion of 300%, 200% down to 20% of the prefeeding rate. The 100% line (45° slope for equal scales on the two axes) represents unchanged excretion.

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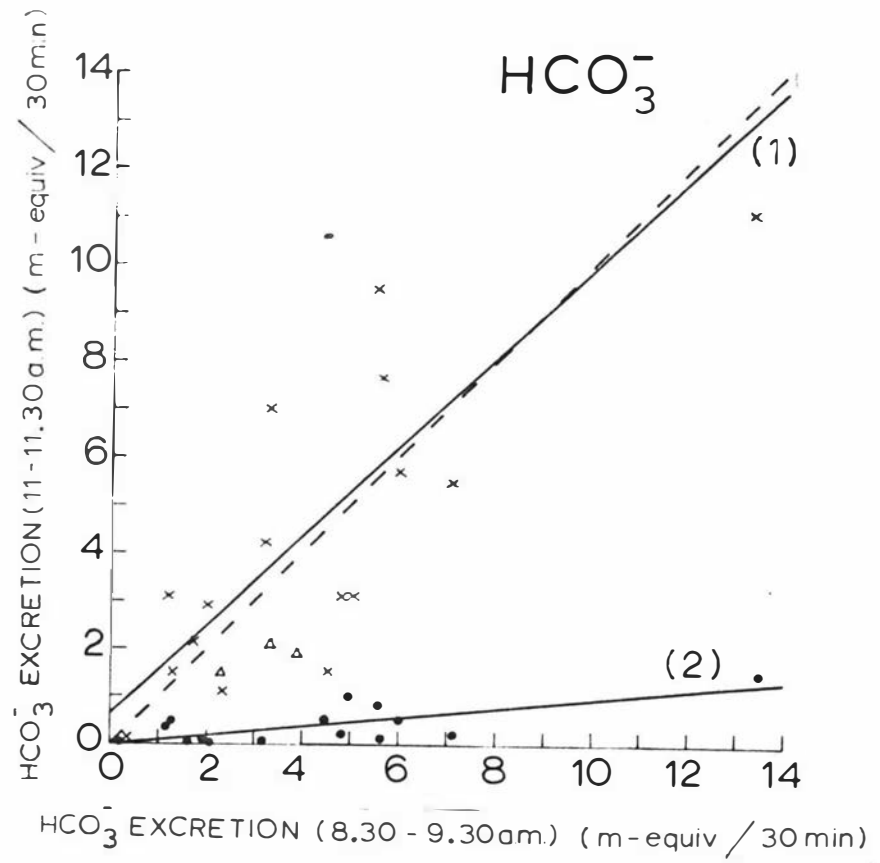

Fig 16. Effect of the prefeeding diuresis, feeding and acetazolamide on HCO_3^- excretion. Regression lines have been calculated for 3 groups of points (1)× - prefeeding 2 (9.30-10 a.m.) vs prefeeding 1 (8.30-9.30 a.m.); (2)● - feeding (11-11.30 a.m.) vs prefeeding 1, no acetazolamide; (3)△ - feeding (11-11.30 a.m.) vs prefeeding 1, acetazolamide. Significant lines: (1) $y = 0.601 + 0.919x$ ($p < .001$), slope not different from 45^0 ($p > .5$); (2) $y = -0.007 + 0.096x$ ($p < .01$), slope significantly different from 45^0 ($p < .001$).

to levels below that before injection.

The changes in excretion of any electrolyte in all experiments have been combined in a single graph by a modification of the method of Cross and Thornton (1966). This method is useful for the demonstration of changes in excretion rate under conditions where it fluctuates spontaneously throughout the experiment, and when the basal excretion rate varies widely from experiment to experiment. The excretion of the particular electrolyte during the second prefeeding period (9.30-10 a.m.) was plotted against that during the first (8.30-9.30 a.m.) to give a series of points for the different experiments. The regression line through these points describes the tendency of the excretion rate to increase or decrease spontaneously during the prefeeding period. A line with a slope of 45° would indicate unchanged excretion, a line above and to the left of this increased excretion, and one below and to the right decreased excretion (Fig 15). The excretion rate during a selected feeding period (e.g. 11-11.30 a.m.) was then plotted against that in the first prefeeding period to give another regression line. Two such lines were plotted, one for the control group, and one for the group receiving acetazolamide; since the availability of water had no effect on electrolyte excretion, no distinction was made on this basis. The effect of the prefeeding diuresis, of feeding and of acetazolamide could then be seen from the difference in slope of these 3 lines, which could be subjected to statistical analysis.

Bicarbonate excretion has been presented in this manner in Fig 16. Excretion was unchanged by the prefeeding diuresis, since the regression line was not statistically different from one of 45° slope. By contrast, after feeding had proceeded for an hour, HCO_3^- excretion had decreased to 10% of the initial rate. A regression line could not be calculated for the acetazolamide

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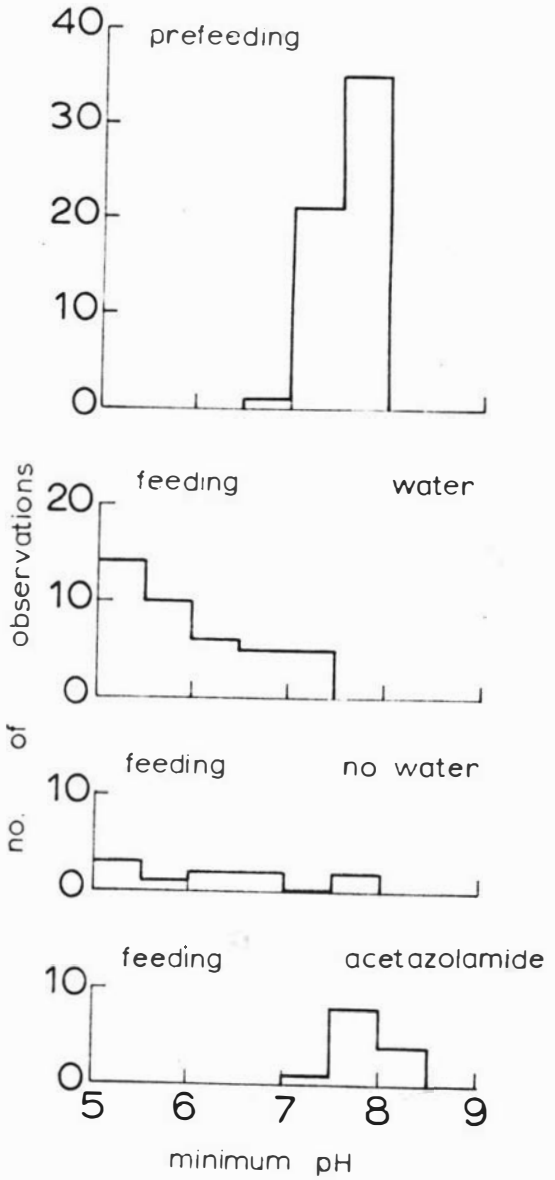

Fig 17. Distribution diagram of the minimum urine pH before feeding, and during feeding under the different experimental conditions. The pH in the first 30 minutes of feeding has been excluded. Note the smaller proportion of very low pH values with no water during feeding, and the prevention of acidification by acetazolamide.

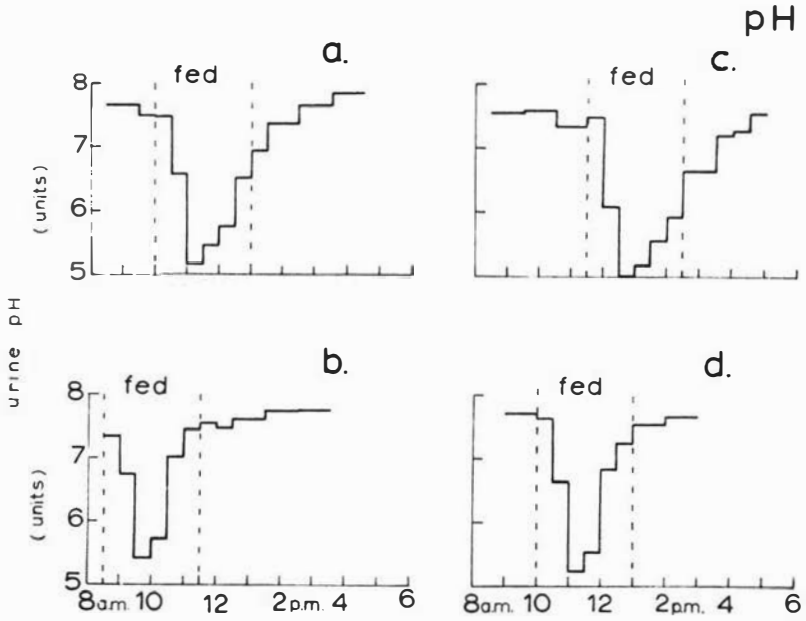

Fig 18. Urine pH relative to a once-daily 3 hour feed; water ad libitum, feeding at varying times. Note acidification during the feeding period (sheep 1, a - 14.3.66; b - 15.3.66; c - 16.3.66; d - 17.3.66).

group because of the small number of points; the group lay half way between the prefeeding line and the base line, indicating a decrease of only 50%.

The urine pH before feeding ranged from pH 6.98 to 8.13, with two-thirds of the values lying between pH 7.30 and 7.80. During feeding, the pH dropped in 40 out of 40 experiments with water provided, and in 8 out of 10 without water. The minimum pH before feeding, and during feeding under the different conditions, is shown in the distribution diagram (Fig 17). The lowest pH and HCO_3^- excretion was seen in the third 30 minute sample on about 50% of days, irrespective of water availability. On almost all the other days, the minimum occurred in either the preceding or following sample.

Acetazolamide, even in the lowest dose (5 mg/kg), prevented acidification if given before feeding, or reversed it promptly if feeding had already begun. The highest pH occurred 1½-2 hours after the injection in most cases. HCO_3^- excretion was variable.

In 2 sheep, on 7 days on which they did not receive acetazolamide, the urine failed to become acid (Fig 12b). A considerable reduction in HCO_3^- excretion occurred on 3 of these occasions, although not to the very low levels normally seen in feeding sheep. These effects were not related to the availability of water.

When feed was offered at different times of the day, urine acidification took place during feeding, indicating that, like the antidiuresis, urine acidification was not a diurnal rhythm (Fig 18).

Urine Na^+ excretion

When a prefeeding diuresis occurred, the rate of Na^+ excretion

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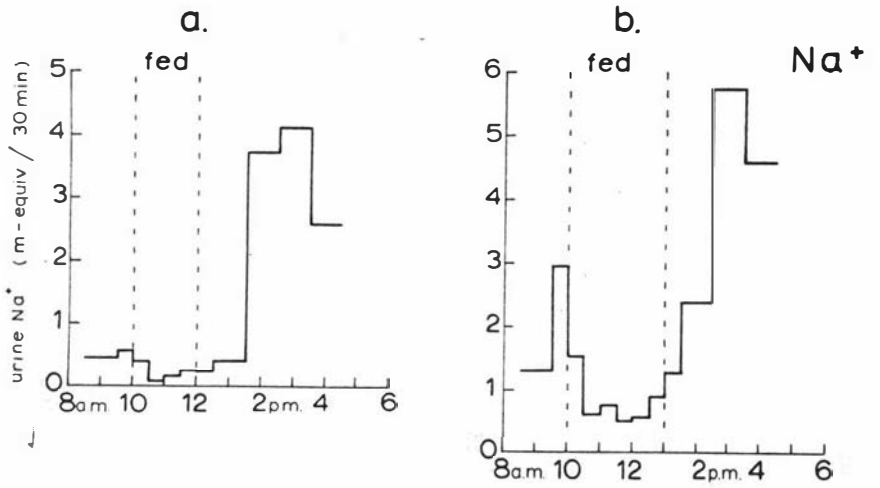

Fig 19. Na^+ excretion relative to a once-daily 2 or 3 hour feed; 5 gm NaCl on feed, a - water ad libitum, b - no water all day. Note high prefeeding Na^+ excretion on both days, followed by a marked reduction during feeding; in b. Na^+ excretion increased markedly during the prefeeding diuresis (a - sheep 2, 20.10.65; b - sheep 2, 2.2.66).

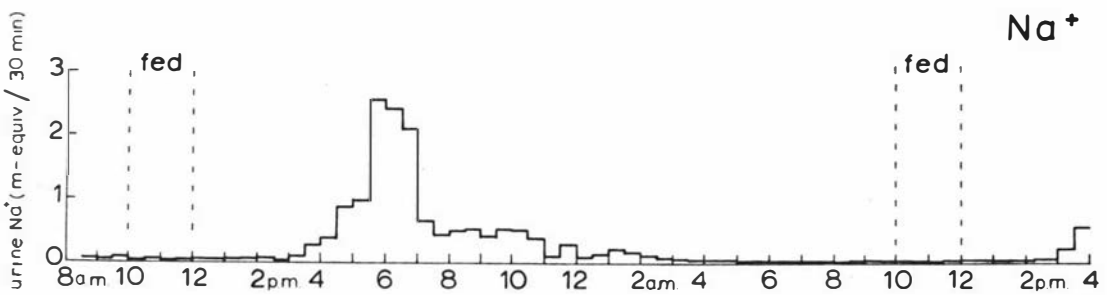

Fig 20. Na^+ excretion relative to a once-daily 2 hour feed; salt lick, water ad libitum. Note the very low prefeeding Na^+ excretion with resulting lesser effect of feeding; significant Na^+ peak in the post-prandial period (sheep 3, 22.6.65 - 23.6.65).

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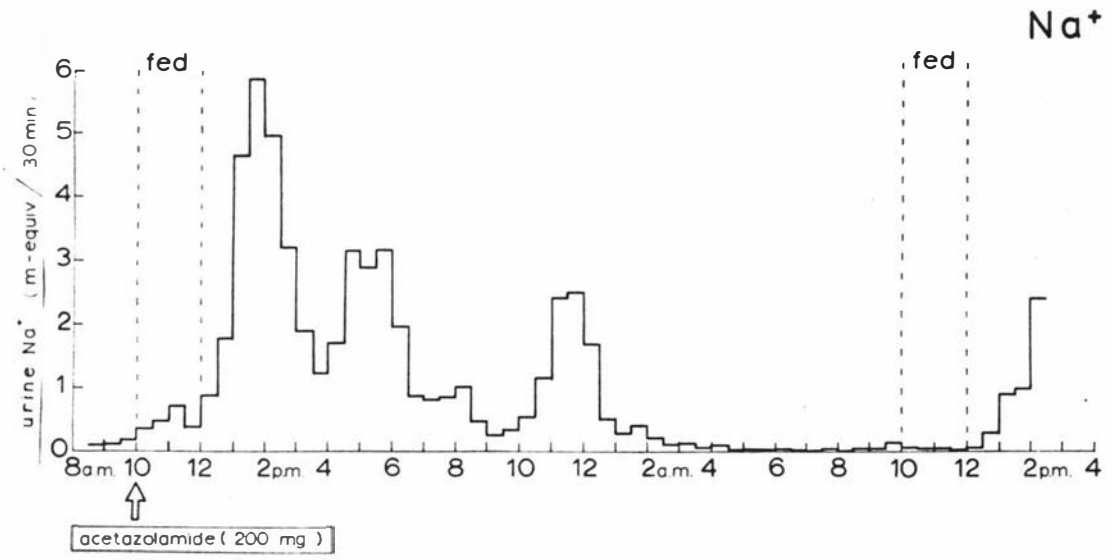
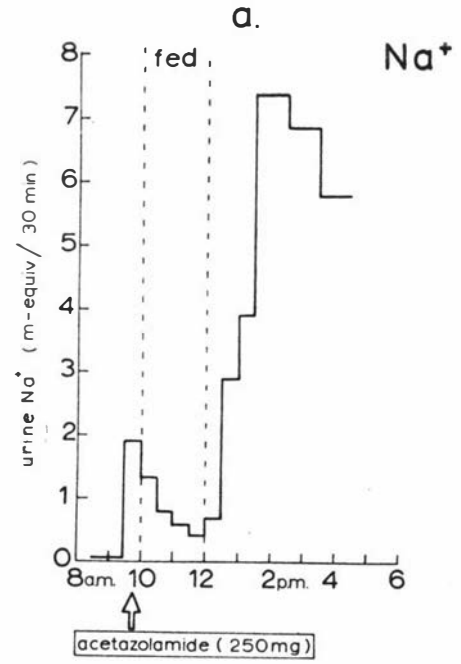


Fig 21. Na^+ excretion relative to a once-daily 2 hour feed; 5 gm NaCl on feed, water ad libitum, acetazolamide. Note increased Na^+ excretion during feeding following acetazolamide injection; low Na^+ before the second feed and no drop during feeding (sheep 3, 3.8.65 - 4.8.65).



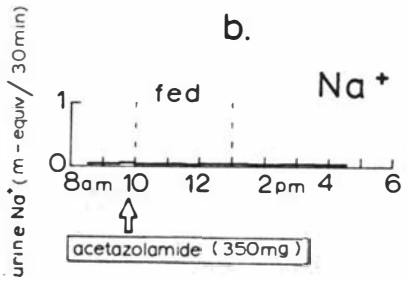


Fig 22. Na^+ excretion relative to a once-daily 2 or 3 hour feed; 5 gm NaCl on feed, acetazolamide, a - water ad libitum, b - no water all day. Note a - natriuresis after the acetazolamide, but decreased Na^+ excretion during feeding; b - failure of acetazolamide to raise Na^+ excretion, and no post-prandial Na^+ peak (a - sheep 2, 3.11.65; b - sheep 3, 8.6.66).

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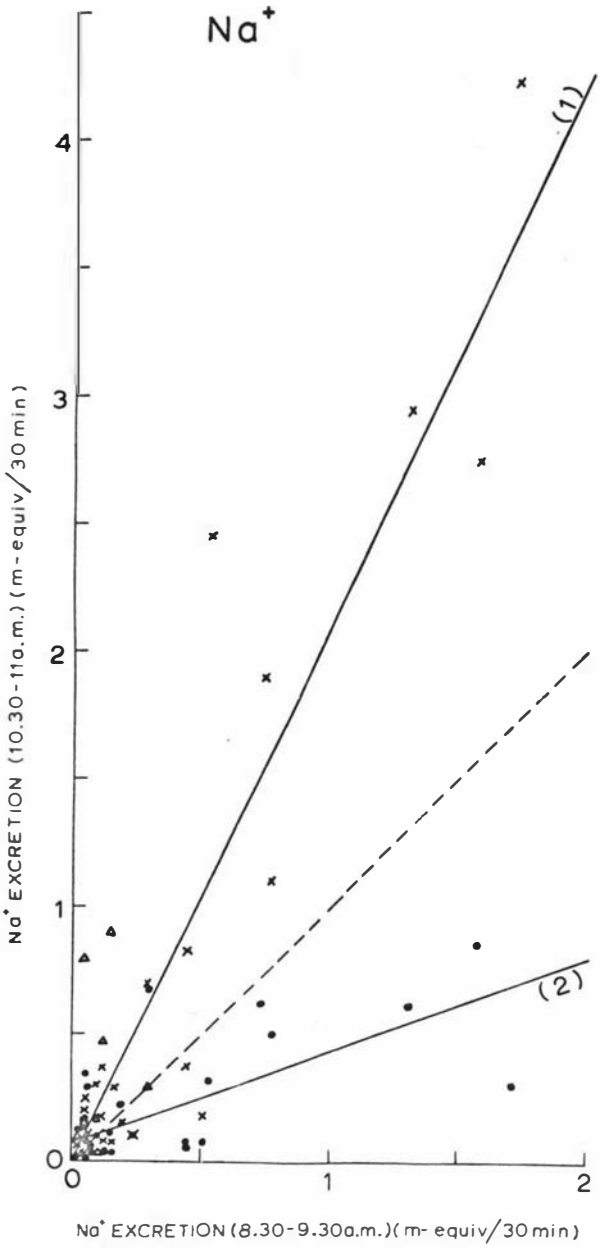

Fig 23. Effect of the prefeeding diuresis, feeding and acetazolamide on Na^+ excretion. Regression lines have been calculated for 3 groups of points (1)× - prefeeding 2 (9.30-10 a.m.) vs prefeeding 1 (8.30-9.30 a.m.); (2)● - feeding (10.30-11 a.m.) vs prefeeding 1, no acetazolamide; (3)Δ - feeding (10.30-11 a.m.) vs prefeeding 1, acetazolamide. Significant lines: (1) $y = -0.066 + 2.152x$ ($p < .001$), slope significantly different from 45° ($p < .001$); (2) $y = 0.066 + 0.363x$ ($p < .001$), slope significantly different from 45° ($p < .001$).

usually increased also. For this reason, the initial rate of Na^+ excretion (8.30-9.30 a.m.) has been taken as the prefeeding excretion. This prefeeding Na^+ excretion was frequently low. On 32 days it was less than 0.1 m-equiv/30 min, and on 17 others ranged from 0.14 to 2.37 m-equiv/30 min, including 3 values between 0.1 and 0.2 m-equiv/30 min. Low Na^+ excretion occurred even when salt lick was continuously available (Fig 20).

The effect of feeding was related to the rate of Na^+ excretion prior to feeding. Na^+ excretion decreased on 11 of 14 days where it exceeded 0.1 m-equiv/30 min prior to feeding (Fig 19), but in only 7 of 23 where it was below this level (Fig 20, two feeds; 21, second feed). The lowest Na^+ excretion was twice as common in the first hour of feeding as later. The response was modified by the administration of acetazolamide, but not by the withholding of water. In individual experiments, the response to the diuretic was not uniform. If it was injected before feeding, usually there was a large increase in Na^+ excretion, which then decreased when feeding began (Fig 22a). When acetazolamide was given as feeding started, often Na^+ excretion increased during feeding (Fig 21). However, on other days little change was evident (Fig 22b).

The effect of the prefeeding diuresis, of feeding and of acetazolamide can be seen from Fig 23, constructed in a similar way to Fig 16 for HCO_3^- excretion. The regression line of prefeeding 2 (9.30-10 a.m.) on prefeeding 1 (8.30-9.30 a.m.) lay to the left of the 45° line (significantly different, $p < .001$), indicating Na^+ excretion increased during the prefeeding diuresis, on average to twice the initial rate. Na^+ excretion during a feeding period (10.30-11 a.m.) was plotted against that in the first prefeeding period for the untreated group, and also for the group

Table 8. Maximum post-feeding Na^+ excretion under different experimental conditions.

Experimental conditions	Prefeeding Na^+ excretion / 30 min	
	< 0.1 m-equiv	> 0.1 m-equiv
Water ad lib.	2.3 m-equiv (mean of 17 obs.)	4.7 m-equiv (mean of 10 obs.)
No water	1.8 (6 obs.)	4.4 (4 obs.)
Water ad lib. + acetazolamide	6.2 (7 obs.)	-
No water + acetazolamide	0.8 * (2 obs.)	6.1 (3 obs.)

* 1 of these very low excretion, other 1.6 m-equiv/30 min.

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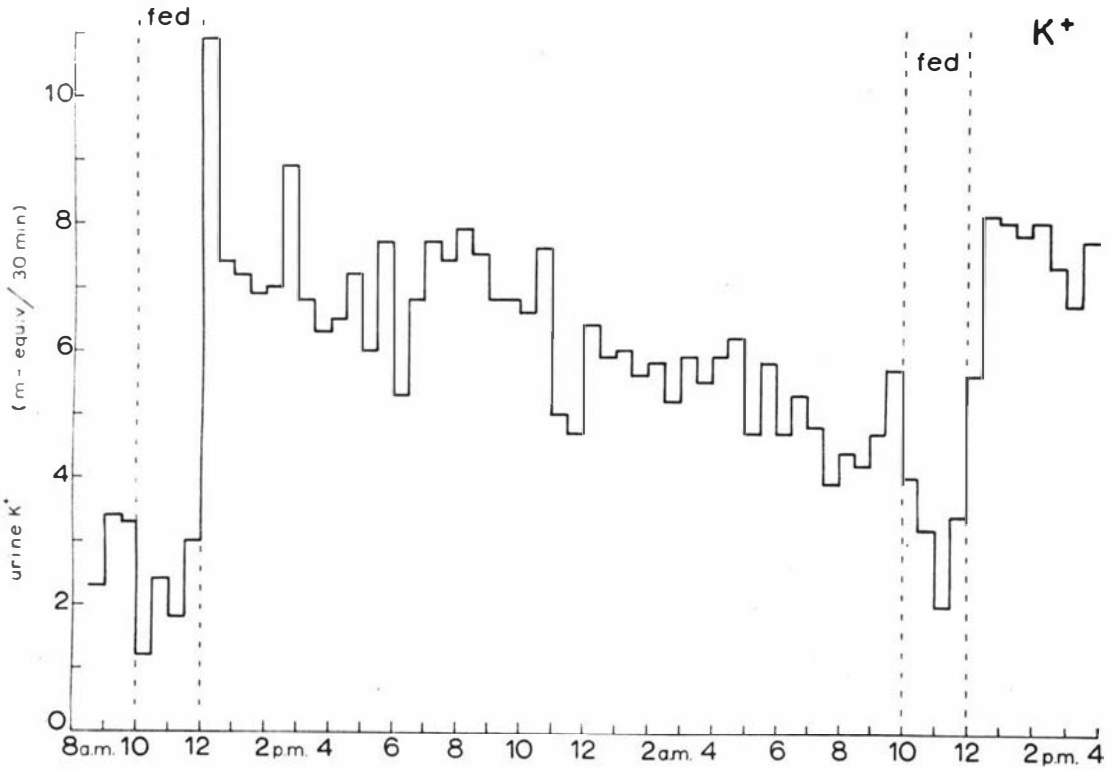

Fig 24. K^+ excretion relative to a once-daily 2 hour feed; water ad libitum. Note reduced K^+ excretion during feeding, maximal excretion immediately after feeding, decreasing to the next feed (sheep 3, 22.6.65 - 23.6.65).

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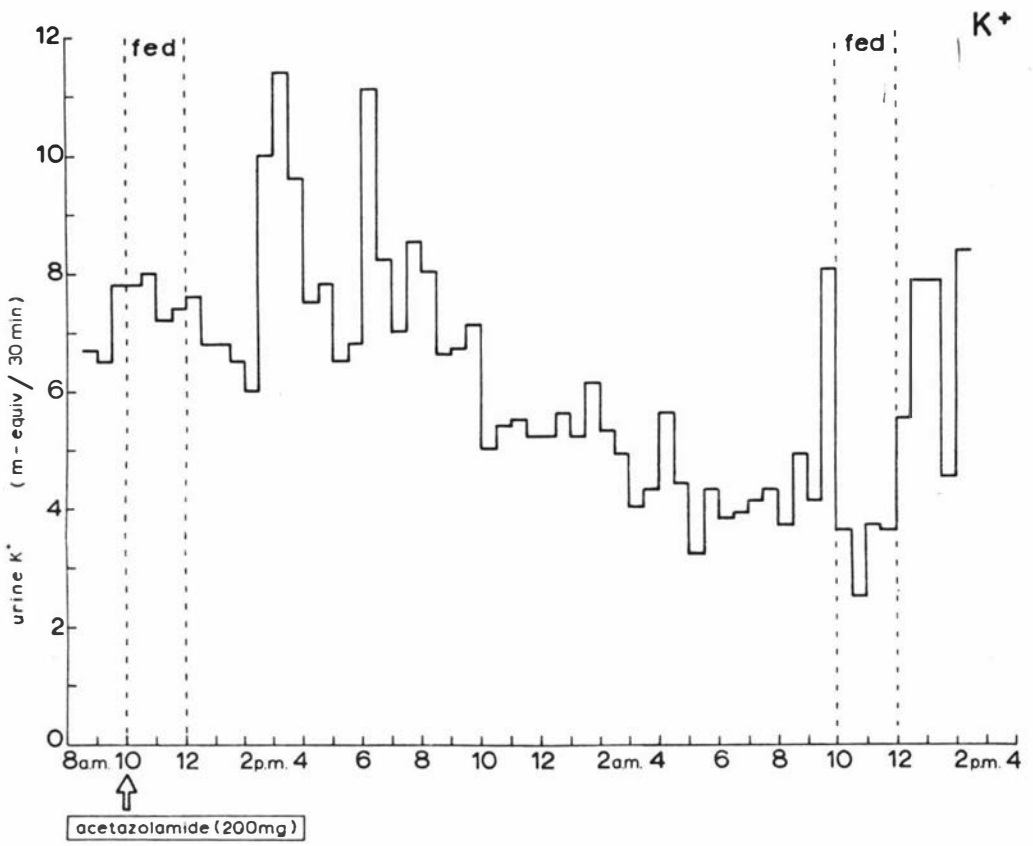

Fig 25. K^+ excretion relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note prevention by administration of acetazolamide of K^+ retention during feeding, compensatory lowered excretion immediately afterwards (sheep 3, 3.8.65 - 4.8.65).

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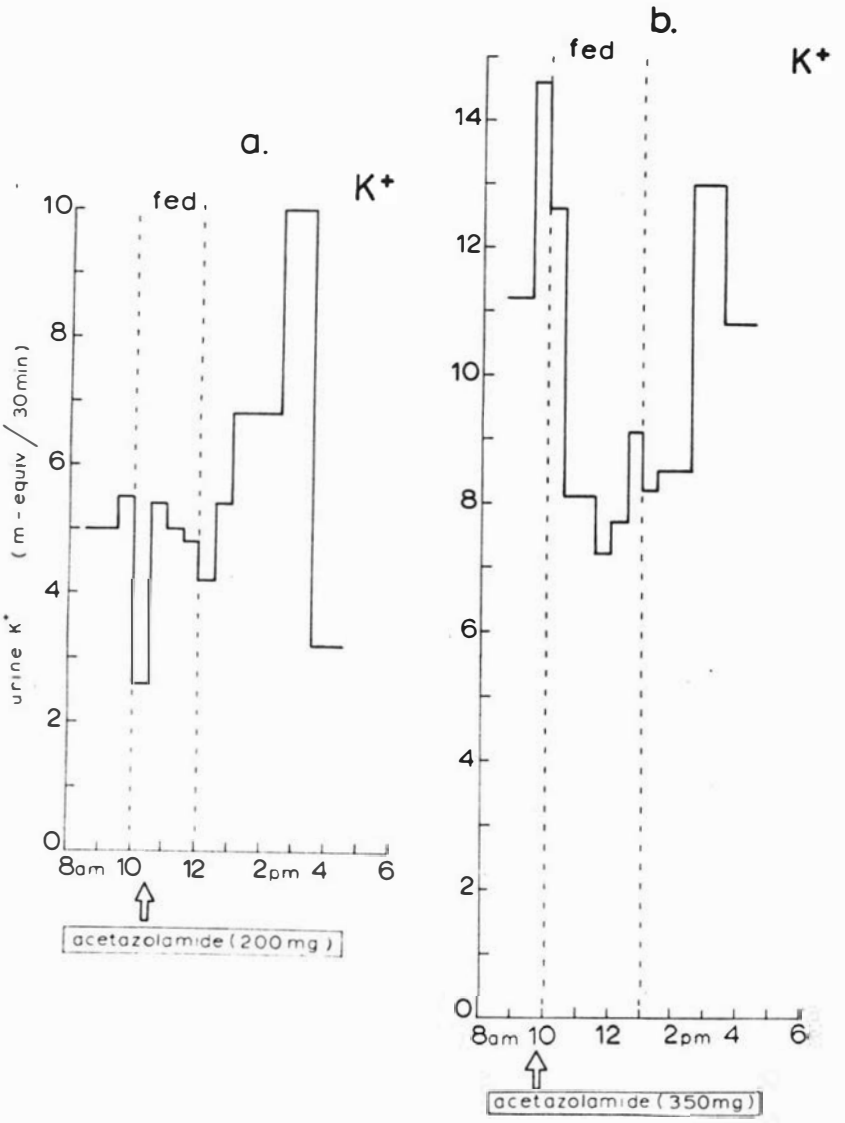

Fig 26. K^+ excretion relative to a once-daily 2 or 3 hour feed; acetazolamide, a - water ad libitum, b - no water all day. a - reversal of the K^+ retention of feeding by the administration of acetazolamide; b - decreased K^+ excretion during feeding in the presence of acetazolamide when the prefeeding K^+ excretion was very high (sheep 3, a - 2.9.65; b - 8.6.66).

receiving acetazolamide. From the slope of the line for the first group, it can be seen that Na^+ excretion during feeding was reduced to an average of 40% of the initial rate. In the group receiving acetazolamide, Na^+ excretion during feeding was greater, approximately that in the first prefeeding period, since the points lay close to the 45° line. No line could be drawn through these points, suggesting that the latter Na^+ excretion was not proportional to that before feeding.

After feeding ended, single or multiple peaks of Na^+ excretion, or a continuing elevation, were seen in 47 of 49 experiments (Fig 19-22). The time of maximum Na^+ excretion, usually between 1.30 and 4.30 p.m., showed no apparent dependence on prefeeding Na^+ excretion, although the magnitude was greater in sheep with high prefeeding excretion, and was increased by acetazolamide (Table 8). In 32 hour experiments (Fig 20, 21) and on 2 other days, later Na^+ excretion peaks were seen at a time after the experiments had normally been terminated.

Urine K^+ excretion

Feeding depressed K^+ excretion. This decrease was marked in 26 of 28 experiments where water was provided, and in all 10 where it was not (Fig 24, 2 feeds; 25, second feed; 37, 38). The lowest K^+ excretion occurred in the first 3 feeding samples with equal frequency when water was provided, but in the second half hour in 7 of 10 with no water.

Acetazolamide administration, in contrast to restricting water, had a pronounced effect. After injection of the diuretic, K^+ excretion was close to, or above, that before feeding on 9 days (Fig 25, first feed; 26a) but decreased on 3 days when prefeeding K^+ excretion was particularly high

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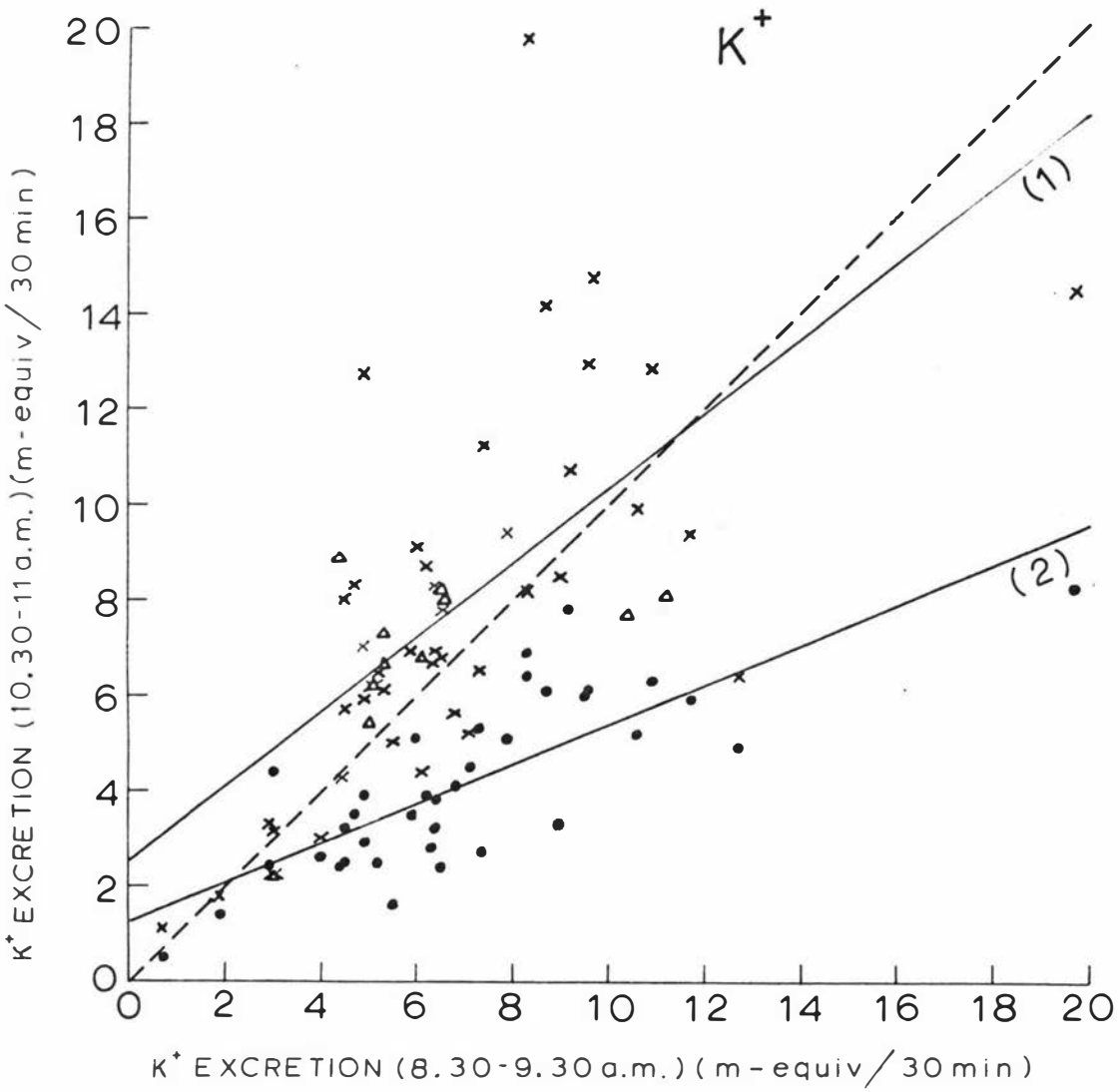

Fig 27. Effect of the prefeeding diuresis, feeding and acetazolamide on K^+ excretion. Regression lines have been calculated for 3 groups of points (1)× - prefeeding 2 (9.30-10 a.m.) vs prefeeding 1 (8.30-9.30 a.m.); (2)● - feeding (10.30-11 a.m.) vs prefeeding 1, no acetazolamide; (3)Δ - feeding (10.30-11 a.m.) vs prefeeding 1, acetazolamide. Significant lines: (1) $y = 2.531 + 0.778x$ ($p < .001$), slope not significantly different from 45° ($p < .10$); (2) $y = 1.240 + 0.414x$ ($p < .001$), slope significantly different from 45° ($p < .001$).

(Fig 26b, 39). When acetazolamide was given after feeding had started, the depressed K^+ excretion was promptly reversed (Fig 26a).

The effect of the prefeeding diuresis, of feeding, and of acetazolamide administration can be seen from Fig 27, constructed similarly to Fig 16 and 23. The slope of the regression line of prefeeding 2 (9.30-10 a.m.) on prefeeding 1 (8.30-9.30 a.m.) shows almost constant K^+ excretion in spite of increased urine flow (i.e. not significantly different from 45° slope). K^+ excretion during feeding (10.30-11 a.m.) was plotted against that in the first prefeeding period, for the untreated group and for those receiving acetazolamide. Feeding depressed K^+ excretion to an average of 40% of the initial rate. By contrast, the administration of acetazolamide maintained excretion near the prefeeding rate. The latter points lay close to the 45° line, however, a significant regression line could not be fitted to them.

Immediately after feeding, where the sheep had access to water, K^+ excretion increased sharply, usually to well above the prefeeding level, in all experiments but one (Fig 24; 25, second feed; 37). When no water was available, K^+ excretion was lower (Fig 38): in only 4 of 10 was it greater than before feeding, and in one it remained very low. On days when, following the injection of acetazolamide, K^+ excretion had increased during feeding, it usually was depressed for 2-3 hours after feeding ended (Fig 25-26, 39).

In the 32 hour experiment (Fig 24), K^+ excretion was maximal immediately after feeding, but decreased steadily to the next feed. In a comparable experiment, but where acetazolamide was administered (Fig 25), a similar excretory pattern occurred, with the exception that K^+ excretion was greater during feeding, and was followed by depression for a few hours.

Table 9. Change of Na^+, K^+, $[H^+]$ excretion during feeding under different experimental conditions.

Experimental conditions	Date	Sheep	ΔNa^+ (m-equiv.)	ΔK^+ (m-equiv.)	$\Delta[H^+]$ (m-equiv.x10^{-5})
Water ad lib.	16. 7.65	3	+0.01	-10.7	+774.9
	20. 7.65	7	-0.12	-10.7	+ 56.7
	31. 8.65	3	+0.04	- 5.5	+ 95.5
	19.10.65	2	-0.65	-14.5	+514.8
	20.10.65	2	-1.07	-12.8	+1328.1
	4.11.65	2	-1.32	-15.2	+1172.4
	10. 3.66	2	-0.11	+ 1.8	+ 0.3
	17. 5.66	3	-0.10	-15.0	+197.1
	24. 5.66	1	-0.08	- 9.4	+ 84.9
	7.12.66	5	+0.73	- 6.5	+ 3.7
Water ad lib. + acetazolamide	3.11.65	2	+2.96	+ 2.5	- 6.2
	2. 9.65	3	+0.25	- 2.8	- 0.8
	13.10.65	1	+0.30	+16.2	- 4.7
	19. 8.65	4	-0.15	+ 5.2	- 5.4
	3. 8.65	3	+1.38	+ 2.4	- 7.3
	21. 7.65	7	+0.82	+11.4	+ 3.0
	14. 7.65	3	+0.10	+ 5.1	- 1.4
	15. 7.65	3	+0.07	+ 0.6	+ 4.6
No water + acetazolamide	8. 6.66	3	-0.01	- 8.8	- 0.7
	15. 6.66	2	+4.24	- 9.2	- 5.6
	22. 6.66	1	+0.10	+ 8.2	- 5.3
No water	9. 3.66	2	-2.88	-38.6	- 1.2
	10. 2.66	1	+0.11	- 2.3	+1101.3
	1. 3.66	3	-2.27	- 8.7	+ 25.5
	8. 2.66	1	-0.07	-18.7	+1530.4
	4. 2.66	2	-1.33	-11.4	+ 24.5
	2. 2.66	2	-4.09	-16.9	+259.1
	7. 6.66	3	-0.05	-15.8	+ 91.3
	20. 6.66	1	-0.03	- 2.8	+ 52.2
	13. 6.66	2	-0.41	- 7.3	- 2.3
	10.11.65	1	+0.01	- 9.0	+602.5

Table 10. Significant multiple regression equations between ΔNa^+, ΔK^+, $\Delta[H^+]$ for 7 groups.

Experimental conditions	Multiple regression equations	p.
Water ad lib.	$\Delta Na^+ = 0.0107\,\Delta K^+ - 0.0009$ $\Delta[H^+] + 0.2187$ (n = 10)	$<.025$
No water		
Water ad lib. + acetazolamide		
All water ad lib.	$\Delta Na^+ = 0.0206\,\Delta K^+ - 0.0010$ $\Delta[H^+] + 0.4788$	$<.05$
	$\Delta K^+ = 2.033\,\Delta Na^+ - 0.0111$ $\Delta[H^+] - 0.9214$ (n = 18)	$<.05$
All no water		
All acetazol-amide		
All groups	$\Delta Na^+ = 0.0651\,\Delta K^+ - 0.00007$ $\Delta[H^+] + 0.3184$	$<.05$
	$\Delta K^+ = 3.109\,\Delta Na^+ - 0.0061$ $\Delta[H^+] - 4.476$ (n = 31)	$<.01$

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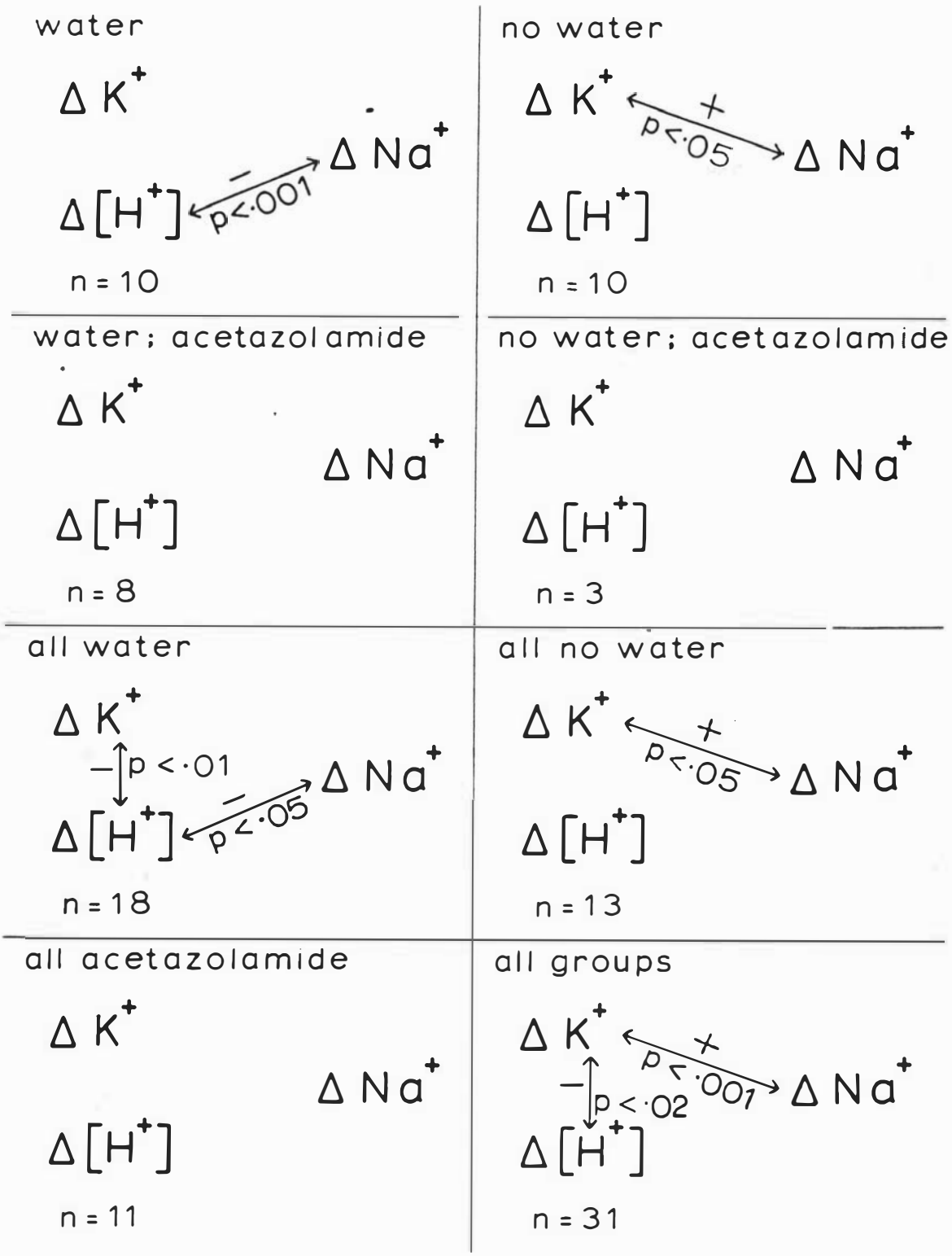

Fig 28. Significant correlations between ΔK^+, ΔNa^+ and Δ free $[H^+]$ during feeding under 7 experimental conditions and for all groups combined. The number of observations in each roup (n) and the level of significance of each relationship (p) are shown.

Interrelationship of urine Na^+, K^+, pH.

Relationships between the changes in urine pH, and Na^+ and K^+ excretion during feeding have been examined using the calculated values ΔNa^+, ΔK^+, Δfree $[H^+]$ for each experiment. The four experimental groups in Table 9 were further grouped to give 7 in all (Table 10, Fig 28). The values ΔNa^+, ΔK^+, Δfree $[H^+]$ were obtained in the following manner:

(a) Free $[H^+]$ for each half-hourly urine sample was obtained from the antilogarithm of the pH; Na^+ and K^+ values were already known;

(b) The average prefeeding rates of excretion (Na^+_b, K^+_b, $[H^+]_b$) were calculated;

(c) Expected total excretions (E) for the first 2 hours of feeding, assuming no change in rate, were obtained from $4 \times Na^+_b$; $4 \times K^+_b$; $4 \times [H^+]_b$;

(d) Actual total excretion (A) for this period was calculated;

(e) ΔNa^+, ΔK^+, $\Delta [H^+]$ were obtained as E - A, and shown in Table 9;

(f) Multiple regression equations using (i) $y=\Delta Na^+$, $x_1=\Delta K^+$, $x_2=\Delta[H^+]$, and (ii) $y=\Delta K^+$, $x_1=\Delta Na^+$, $x_2=\Delta[H^+]$ were calculated; By eliminating one variable at a time, the simple relationships between pairs of values of ΔNa^+, ΔK^+, $\Delta[H^+]$ have been examined.

The significant multiple regression equations obtained are shown in Table 10. It will be seen that these are confined to experiments with water available, and to the total group. The interactions between pairs of cations in the various groupings are shown diagrammatically in Fig 28. Where there is a significant relationship, Na^+ was negatively related to $\Delta[H^+]$ and positively to ΔK^+, while ΔK^+ and $\Delta[H^+]$ were negatively related. Signific-

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Table 11. Effect of feeding on Cl^- excretion under the different experimental conditions.

Experimental conditions	Change in Cl^- excretion (no. of obs.)			
	Increased	Decreased then increased	Unchanged	Decreased
Water ad lib.	11	12	1	2
No water	4	5	0	1
Total without acetazolamide	15	17	1	3
Acetazolamide	0	0	0	13

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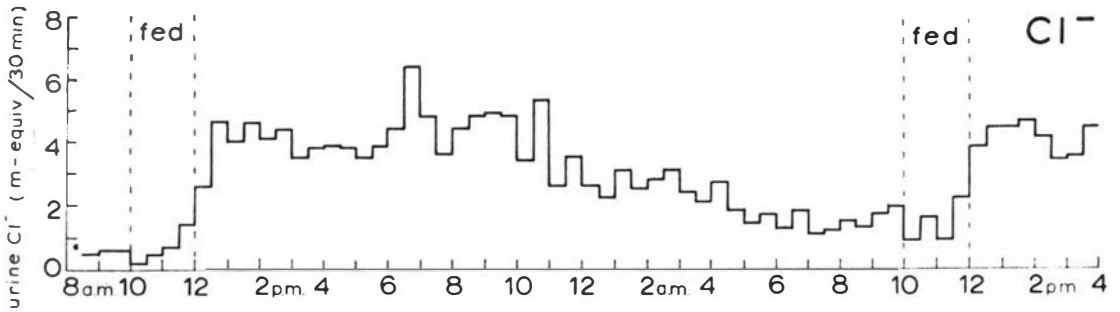
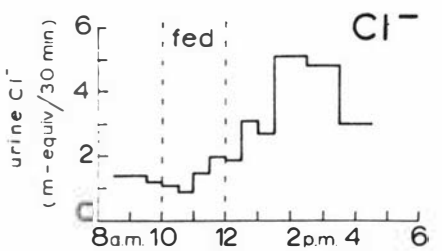


Fig 29. Cl^- excretion relative to a once-daily 2 hour feed; water ad libitum. Initial decline in excretion at onset of feeding followed by a rapid increase to a post-prandial peak (sheep 3, 22.6.65 - 23.6.65).

Fig 30. Cl^- excretion relative to a once-daily 2 hour feed; water ad libitum. (sheep 3, 31.8.65).



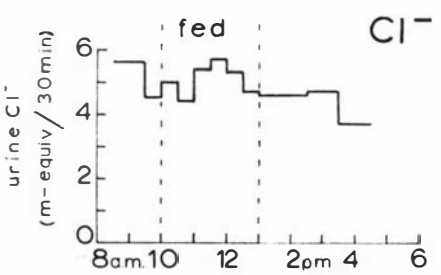


Fig 31. Cl^- excretion relative to a once-daily 3 hour feed; no water all day. Note Cl^- unchanged during feeding, and the unusual observation of no post-prandial Cl^- peak (sheep 1, 20.6.66).

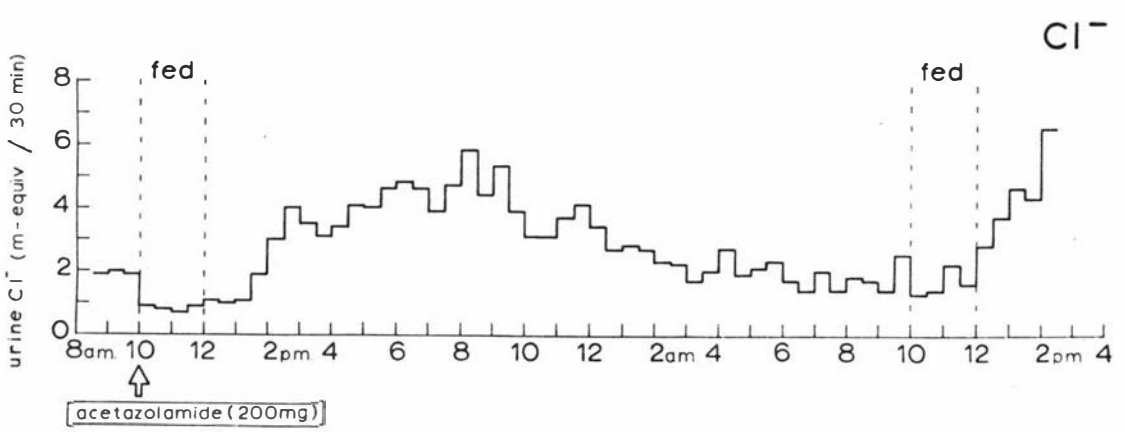


Fig 32. Cl^- excretion relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note depression of Cl^- excretion by acetazolamide (sheep 3, 3.8.65 - 4.8.65).

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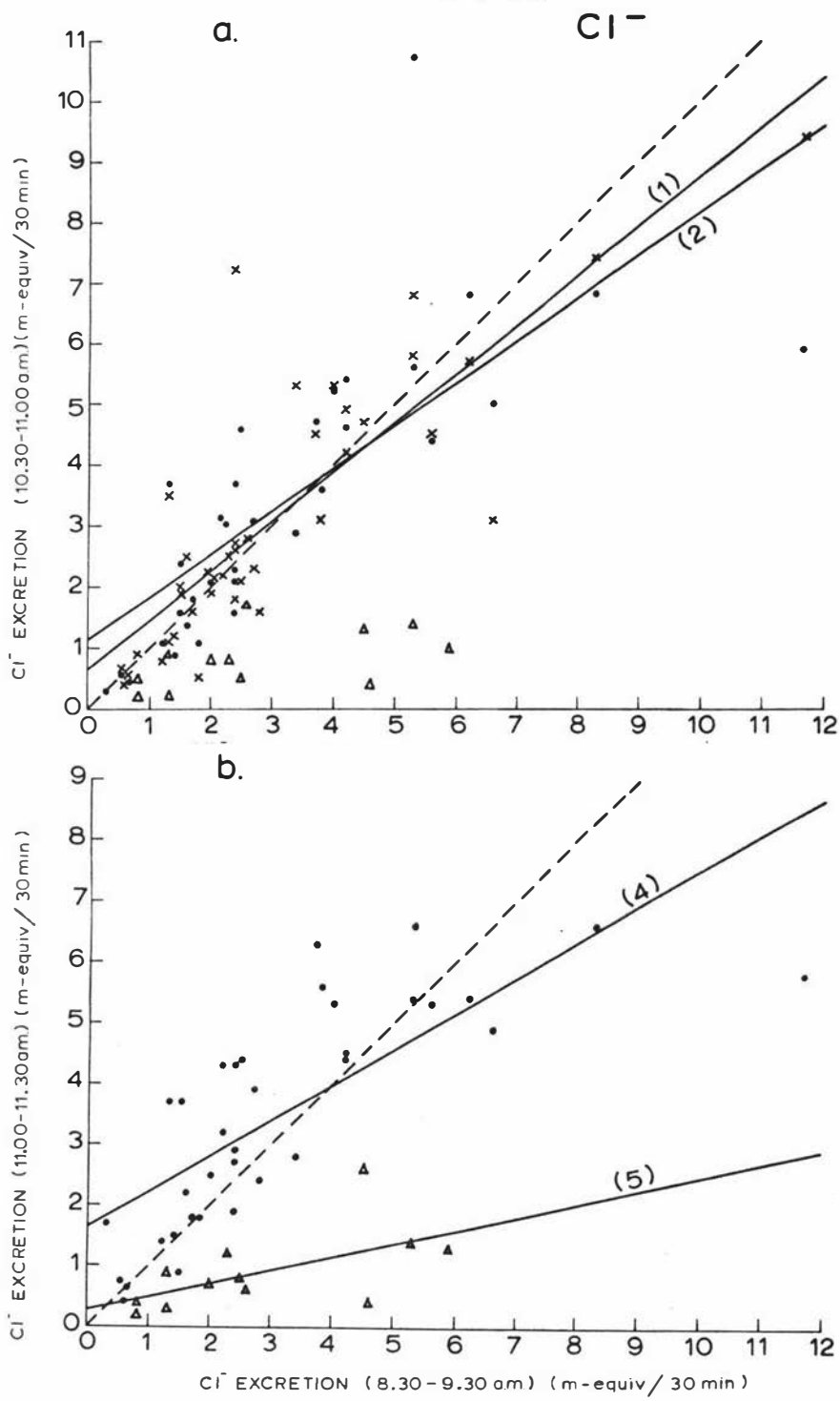

Fig 33. Effect of prefeeding diuresis, feeding and acetazolamide on Cl^- excretion. Regression lines have been calculated for 5 groups of points a - (1)× - prefeeding 2 (9.30-10 a.m.) vs prefeeding 1 (8.30-9.30 a.m.); (2)● - feeding (10.30-11 a.m.) vs prefeeding 1, no acetazolamide; (3)△ - feeding (10.30-11 a.m.) vs prefeeding 1, acetazolamide; b - (4)● - feeding (11-11.30 a.m.) vs prefeeding 1, no acetazolamide; (5)△ - feeding (11-11.30 a.m.) vs prefeeding 1, acetazolamide. Significant lines: (1) $y = 0.602 + 0.817x$ ($p < .001$); (2) $y = 1.133 + 0.703x$ ($p < .001$); (4) $y = 1.629 + 0.589x$ ($p < .001$); (5) $y = 0.273 + 0.222x$ ($p < .05$).

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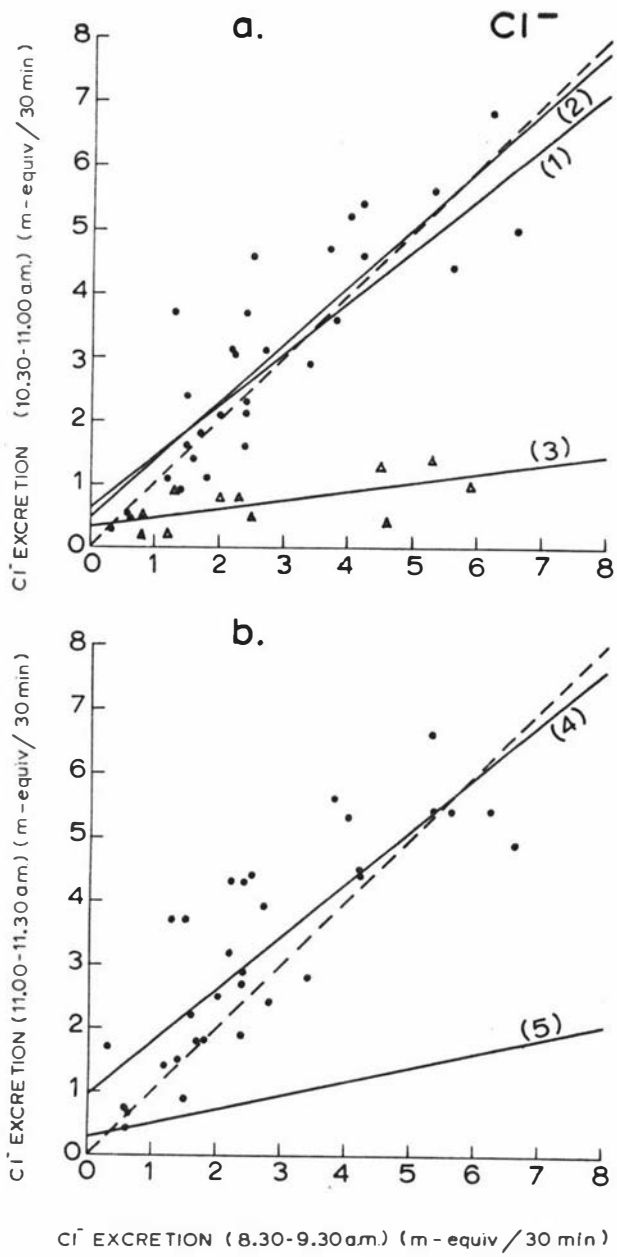

Fig 34. Effect of prefeeding diuresis, feeding and acetazolamide on Cl^- excretion. Points are identical with those in Fig 33, but those providing the greatest deviation from the line of best fit were identified and omitted from groups (2), (3) and (4) (see p. 74). Significant lines after recalculation: (1) $y = 0.602 + 0.817x$ ($p < .001$), slope significantly different from 45° ($p < .05$); (2) $y = 0.478 + 0.916x$ ($p < .001$), slope not significantly different from 45° ($p > .30$); (3) $y = 0.332 + 0.139x$ ($p < .05$), slope significantly different from 45° ($p < .001$); (4) $y = 0.983 + 0.829x$ ($p < .001$), slope not significantly different from 45° ($p > .10$); (5) $y = 0.273 + 0.222x$ ($p < .05$), slope significantly different from 45° ($p < .001$).

ant interactions were obtained in all groups except the acetazolamide ones (Fig 28).

Urine Cl^- excretion

Feeding had a variable effect on Cl^- excretion (Table 11). Most commonly, there was either an increase from the start of feeding, or following a 30-60 minute period of decrease (Fig 29-31; 32, second feed). The availability of water did not affect this response. When acetazolamide was administered, on all 13 days Cl^- excretion was depressed during feeding, and usually for some hours afterwards (Fig 32, 40).

The effect of the prefeeding diuresis, of feeding, and of acetazolamide on Cl^- excretion over all the experiments is shown in Fig 33a,b, constructed in a similar manner to Fig 16, 23, 27. The prefeeding line was calculated as before; two feeding periods, not one, were used, because of the variable effect of feeding on Cl^- excretion. Significant regression lines fitted 4 of the 5 groups of data, but for two of them the line did not fit the points near the origin. These points would make a smaller relative contribution to the overall calculation than would those of higher value. Hence, points providing the greatest deviation from the line of best fit were identified and omitted: (i) 3 of 33 for feeding 10.30-11 a.m. vs. prefeeding 1, (ii) 3 of 35 for feeding 11-11.30 a.m. vs. prefeeding 1, (iii) 1 of 12 for feeding 10.30-11 a.m. vs. prefeeding 1 after acetazolamide. These recalculated lines showed better fit of the points near the origin, although a curve may well have fitted the data in (i) and (ii). A second figure (Fig 34a,b) was constructed incorporating these new lines.

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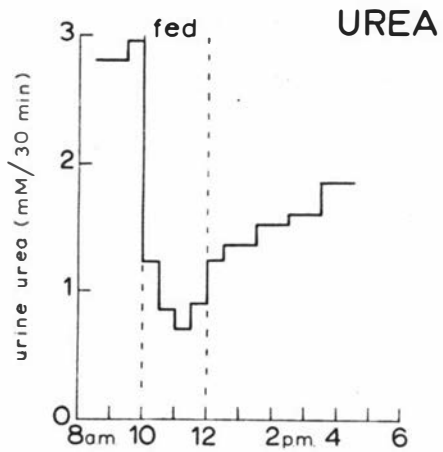

Fig 35. Urea excretion relative to a once-daily 2 hour feed; water ad libitum. Note decreased urea excretion during feeding and a post-prandial rise (sheep 3, 16.7.65).

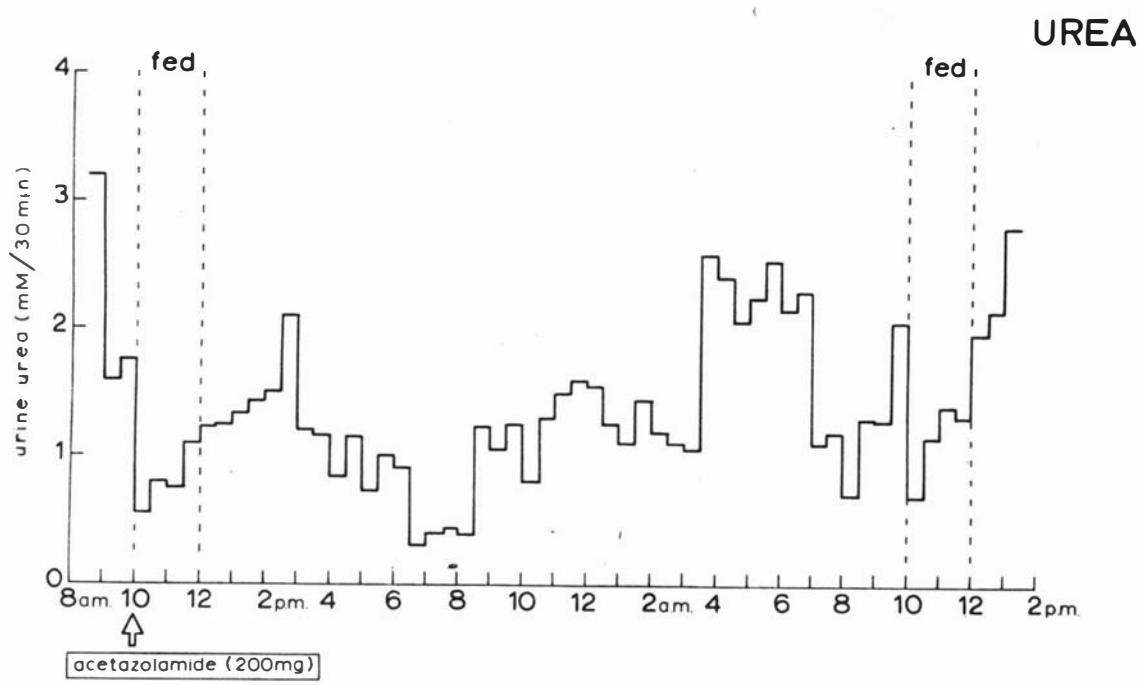

Fig 36. Urea excretion relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note decreased excretion during first feed, no significant change during the second, post-prandial peaks of excretion (sheep 3, 3.8.65 - 4.8.65).

It will be seen from Fig 34 that the average effect of the prefeeding diuresis was to lower Cl^- excretion to approximately 80% of that in the preceding hour. In the second and third half hours of feeding, the Cl^- excretion did not appear different from that between 8.30 and 9.30 a.m. The most marked effect was shown by acetazolamide, which decreased Cl^- excretion to 15% in the second half hour, and to 22% in the next.

On all but 2 days (both no water, Fig 31), Cl^- excretion increased considerably in the post-feeding period. In a 32 hour experiment (Fig 29), Cl^- excretion increased markedly over 1-2 hours just after feeding to a high rate which was maintained for 10 hours before decreasing up to the next feed. In a similar experiment but where acetazolamide was administered (Fig 32), the depressed Cl^- excretion rose after feeding to a peak rate over the period of 8-10 hours where there was a plateau on the control day, after which there was the same declining excretion to the next feeding period.

Urea excretion

The predominant pattern of urea excretion, seen on 21 of 25 days, was either a decrease or no change during feeding, followed by a considerable increase in the post-feeding period (Fig 35, 36). The lowest excretion usually occurred late in the feeding period. No effect of acetazolamide administration or removal of drinking water was apparent. On 3 days when the prefeeding level of excretion was high, urea excretion decreased all day, and on one day it increased all day. These unusual days did not appear to correlate with a particular treatment.

In two 32 hour experiments (Fig 36), lowest urea excretion occurred 6-9 hours after feeding, followed by an increase over 12 hours, but fell in

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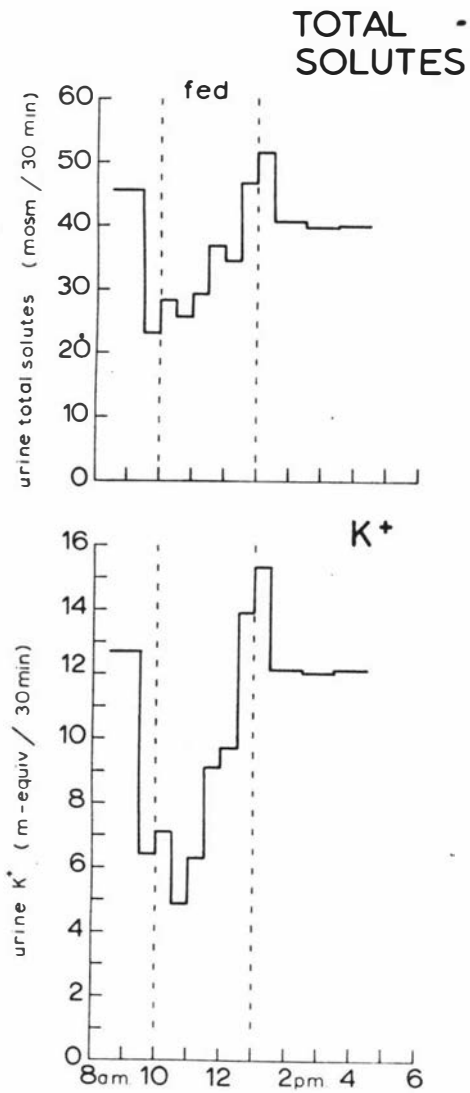

Fig 37. Total solute and K^+ excretion relative to a once-daily 3 hour feed; water ad libitum. Note no decrease in total solutes during feeding; frequent observation of parallel changes in excretion of total solutes and K^+ except during first 1½-2 hours of feeding (sheep 3, 17.5.66).

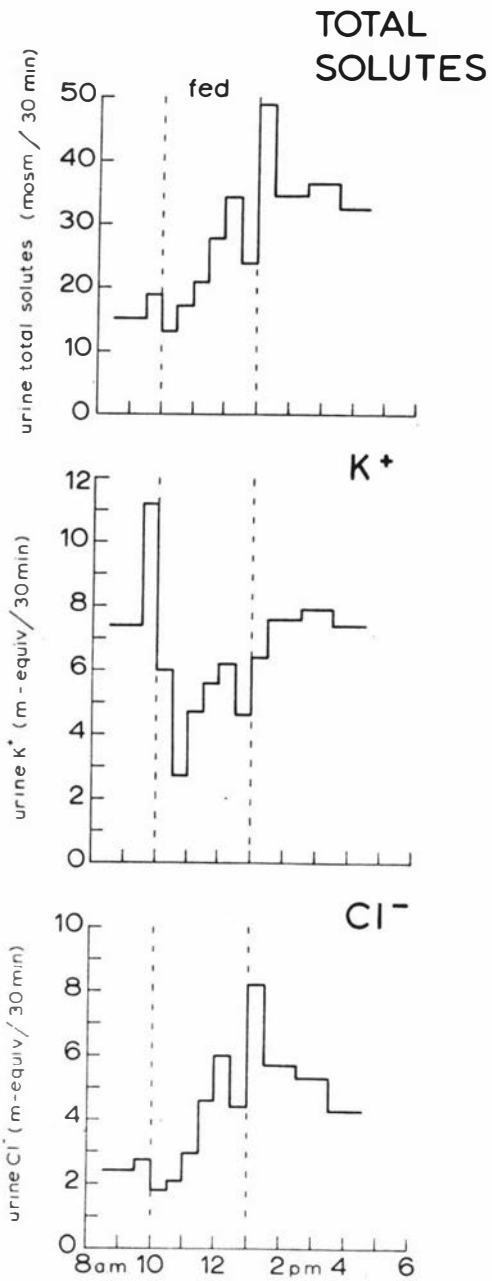

Fig 38. Total solute, K^+ and Cl^- excretion relative to a once-daily 3 hour feed; no water all day. Note only transient fall in total solutes during feeding; total solute changes generally parallel to those in K^+ and Cl^-, but the post-prandial rise in K^+ relatively less than in total solutes (sheep 3, 7.6.66).

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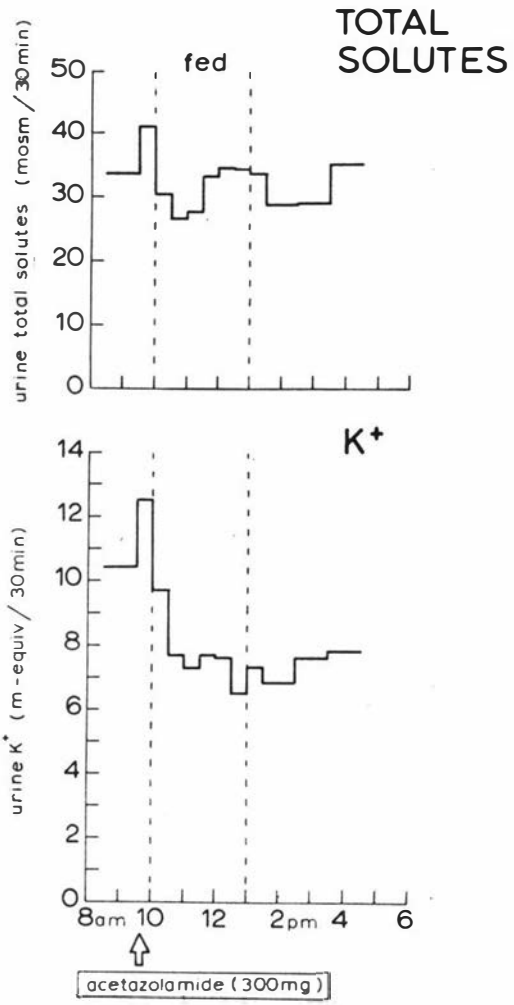

Fig 39. Total solute and K^+ excretion relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note fall in total solutes during feeding; the fall in K^+ excretion during feeding after acetazolamide when the prefeeding K^+ excretion was very high; changes in total solutes and K^+ were not parallel (sheep 2, 15.6.66).

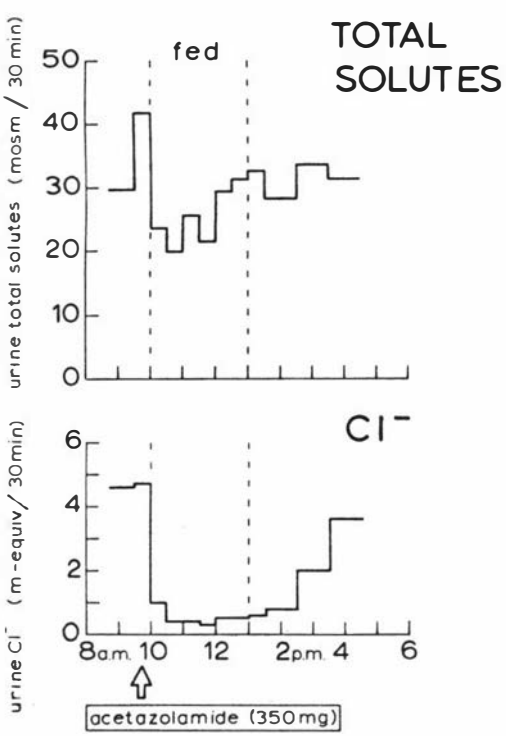

Fig 40. Total solute and Cl^- excretion relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note reduced total solute excretion during feeding; changes in total solutes and Cl^- not parallel (sheep 3, 8.6.66).

the 3-4 hours before the next feed.

Urine Total Solute excretion

Total solute excretion was measured on only 10 occasions: 4 water ad lib., 3 no water all day, and 3 no water all day + acetazolamide injection. Without the diuretic, irrespective of whether or not water was provided, it was usual (5 of 7) for only a transient decrease in total solutes to occur upon feeding (Fig 38). On the other 2 days there was no drop at all (Fig 37). When acetazolamide was injected before feeding, total solute excretion was reduced both during and after the feeding period on 2 days (Fig 39,40), while on one day total solutes were almost unchanged. Irrespective of the experimental conditions, during the earlier part of feeding total solute excretion lay most commonly between 20 and 30 mosm/30 minutes.

Apparent parallel changes in total solute and K^+ excretion were seen in experiments where water was provided, except during the first $1\frac{1}{2}$-2 hours of feeding (Fig 37). This was also true for the group with no water all day, except that after feeding total solute excretion exceeded the prefeeding rate, whereas K^+ excretion remained near the prefeeding level (Fig 38). When acetazolamide was injected, the two were not parallel (Fig 39).

Parallel excretion of total solutes and Cl^- was seen in 3 of 7 experiments when the diuretic was not given (Fig 38), but was less evident on other occasions. As with K^+, after acetazolamide, changes in Cl^- excretion were not parallel with total solute changes (Fig 40).

On many days, the total solute excretion early in feeding showed

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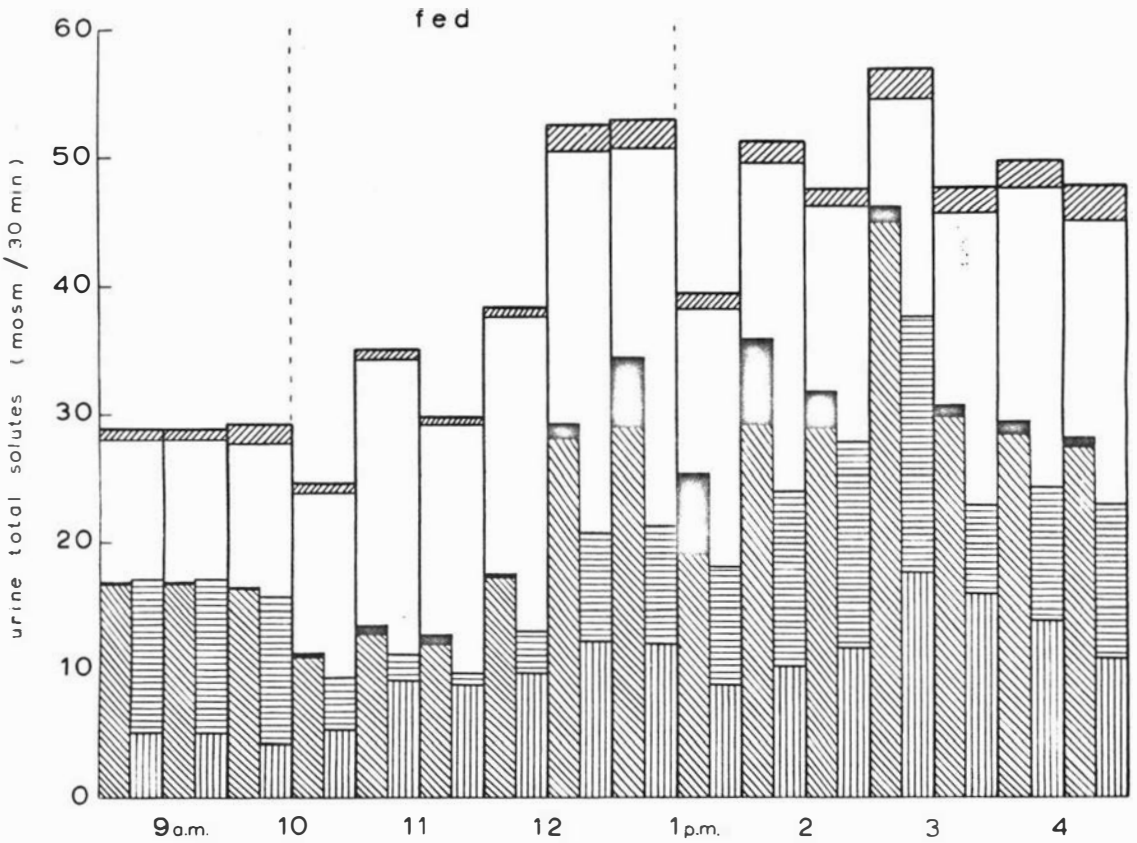

Fig 41. Urinary total and individual solute excretion each half hour relative to a once-daily 3 hour feed; water ad libitum. Solute excretion: urea; Na^+; K^+; HCO_3^-; Cl^-; not estimated (sheep 5, 7.12.66).

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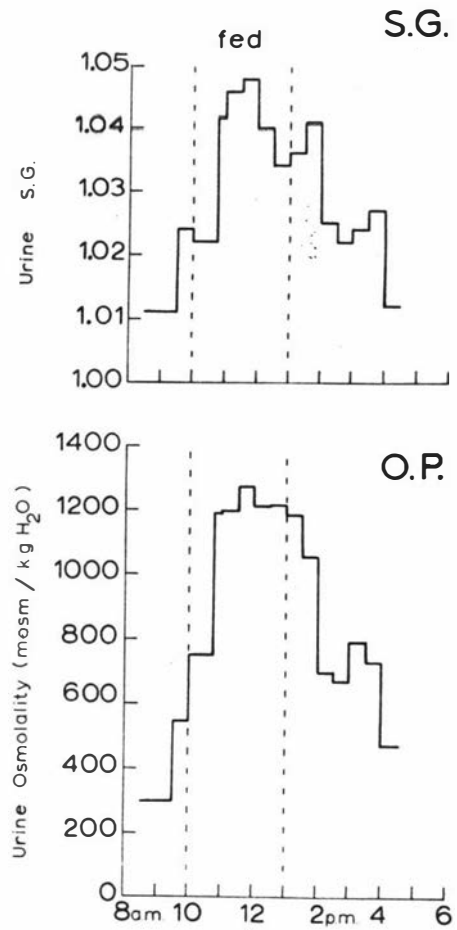

Fig 42. Urine osmolality and specific gravity relative to a once-daily 3 hour feed; water ad libitum. Note similarity of overall changes, but that specific gravity was not an accurate estimate of osmolality (sheep 5, 7.12.66).

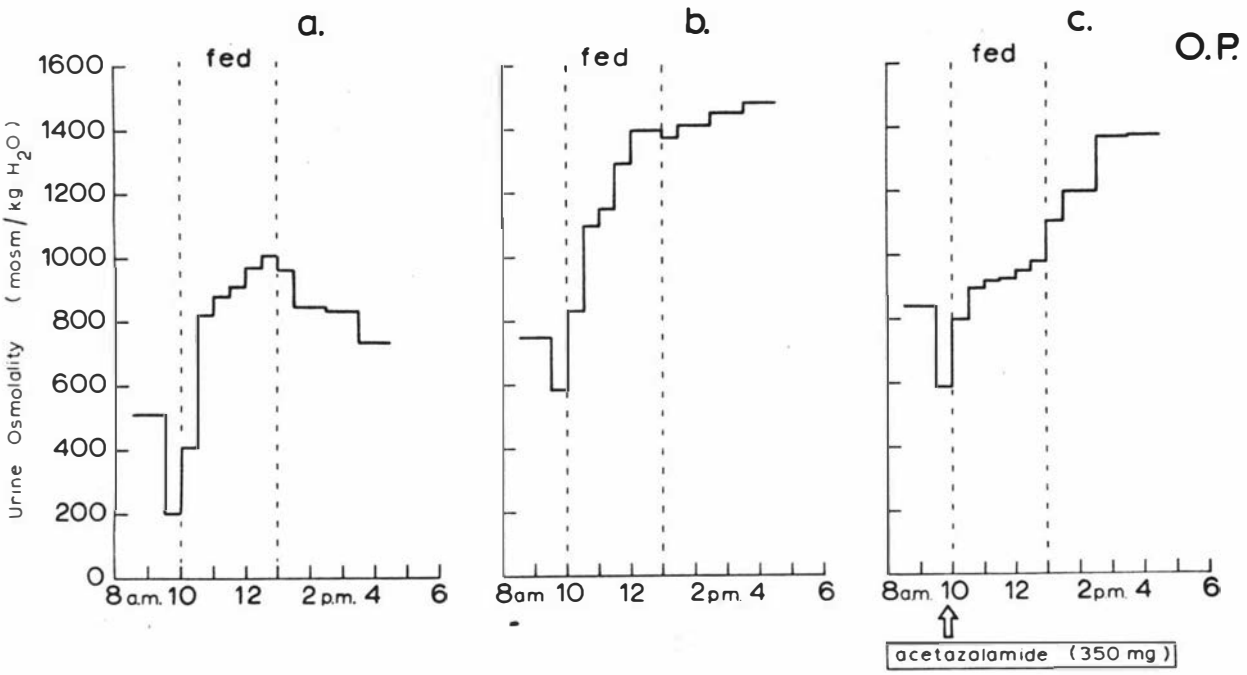

Fig 43. Comparison of changes in urine osmolality in a sheep relative to a once-daily 3 hour feed under 3 different experimental conditions: a - water ad libitum; b - no water all day; c - no water all day, acetazolamide. Note low prefeeding O.P.; steep rise during feeding, less when acetazolamide was given; postprandial further increase with no water (sheep 1, a - 24.5.66; b - 20.6.66; c - 22.6.66).

less of a decrease than might be expected from the individual changes in Na^+, K^+, Cl^-, HCO_3^- and urea excretion. Total solutes and individual solutes excreted in each urine sample were compared; one such day, when water was provided ad lib., is shown in Fig 41. Typically, when water was provided, 30-40% of the total solutes was not accounted for by the measured solutes before feeding; this increased during feeding to as much as 50%, then reverted to 30-40%. When no water was available all day, the picture differed in that after the feeding period the solutes not estimated represented a greater fraction (40-60%). Lastly, when acetazolamide was injected as well as no water being available, the unaccounted fraction was smaller in general, being only 20-30% during feeding, and 30-40% after feeding, both lower than on days with no water but no diuretic.

Urine Osmolality and Specific Gravity

Before feeding, urine osmolality was usually between 300 and 600 mosm/kg H_2O. Osmolality increased sharply over the first hour of feeding, then reached a plateau, or showed small stepwise increments (Fig 42, 43). Even when the prefeeding osmolality was as high as 1400-1600 mosm/kg H_2O, it rose still further during feeding to near 1800 mosm/kg. In 3 sheep, a comparison of urine osmolality changes during feeding were made on 3 days, one when water was available, one with no water all day, and one no water all day and administration of acetazolamide. One sheep showed the typical changes described above under all 3 experimental conditions, but in the other 2 sheep there was little effect of restricting water, but a slowed rise in osmolality after acetazolamide (Fig 43).

In the post-feeding period, the effect of restricting water was

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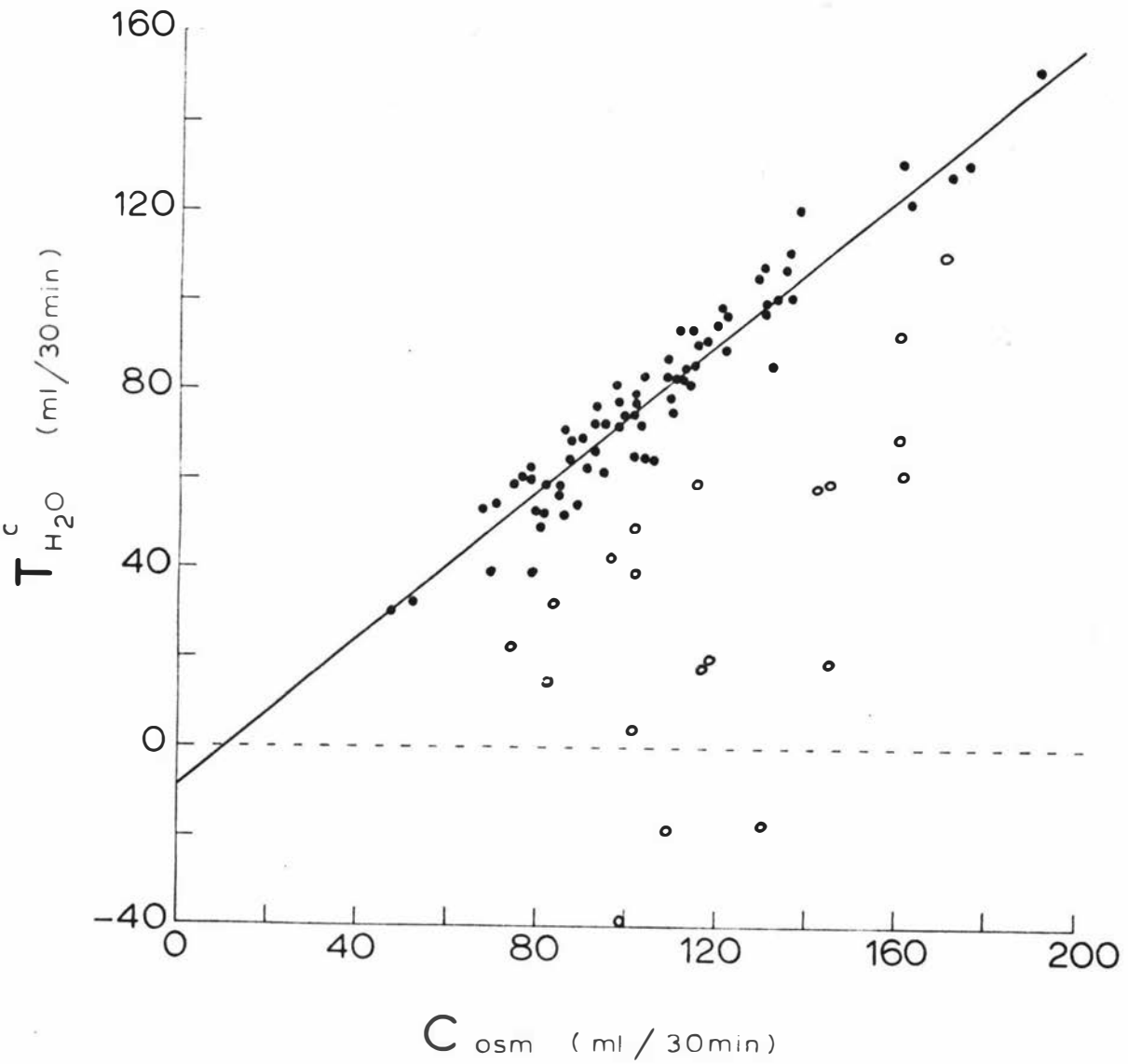

Fig 44. Graph of solute-free water reabsorption ($T^c_{H_2O}$) against osmolar clearance (C_{osm}) for all urine samples with a midpoint blood sample, irrespective of the experimental conditions or the relationship to feeding. ● - urine volume less than 50 ml/ 30 min; o - urine volume greater than 50 ml/ 30 min; the former points closely fitted a straight line $y = 0.836x - 9.222$ ($p < .001$; $n = 78$).

apparent. On 6 days when no water was provided (with or without acetazolamide), urine osmolality continued increasing, and on one day the maximum feeding value was maintained (Fig 43). When water was available ad libitum, on 2 days the feeding concentration was maintained, but on 2 days osmolality began decreasing after feeding ended (Fig 42, 43).

Urine specific gravity was not an accurate estimate of osmolality, although a large change in osmolality was reflected in a large change in specific gravity (Fig 42).

Osmolar clearance and solute-free water reabsorption

For each urine sample with a midpoint blood sample, osmolar clearance (C_{osm}) and solute-free water reabsorption ($T^c_{H_2O}$) were calculated, and $T^c_{H_2O}$ was plotted against C_{osm}. Where the urine volume exceeded 50 ml/30 min, the points were randomly distributed, but for smaller volumes the points closely fitted a straight line (Fig 44) irrespective of the experimental treatment. Where the volume exceeded 50 ml, the points lay to the right of and below the line, indicating less than the maximum water was lost from the collecting ducts for that C_{osm}.

Blood pH

On all 13 days on which blood pH was measured, it decreased during feeding irrespective of whether or not water was offered or acetazolamide was injected. On 6 of these days the pH meter was not read at a fixed interval after introduction of the blood into the electrode assembly (as described under methods), so these were not included in quantitative comparisons.

The minimum pH was seen late in feeding on the 2 days when water

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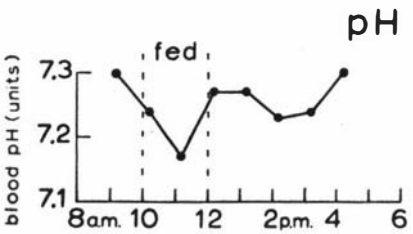

Fig 45. Blood pH (jugular) relative to a once-daily 2 hour feed; water ad libitum. Note fall in pH during feeding (sheep 2, 4.11.65).

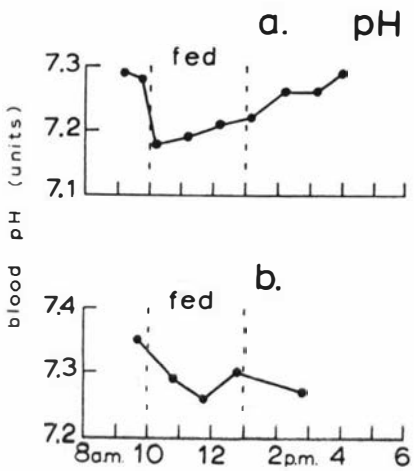

Fig 46. Blood pH (a - jugular, b - carotid) relative to a once-daily 3 hour feed; a - no water all day, b - no water during feed, water ad libitum after feed. (sheep 1, a - 8.2.66; b - 10.11.65).

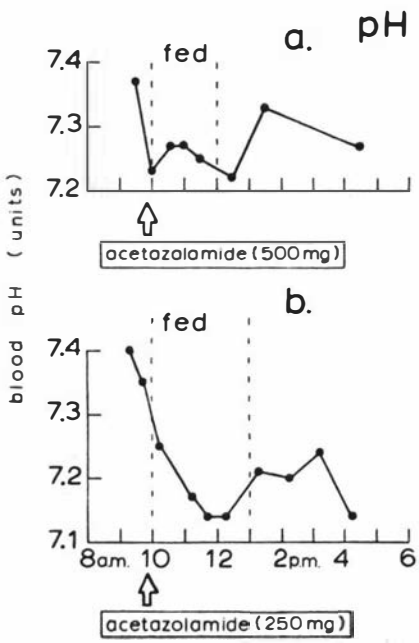

Fig 47. Blood pH (a - carotid, b - jugular) relative to a once-daily 2 or 3 hour feed; acetazolamide, a - water ad libitum, b - no water during feed, water ad libitum after feed. Note in both the drop in pH after injection of the diuretic before feeding began (a - sheep 1, 13.10.65; b - sheep 8, 11.11.65).

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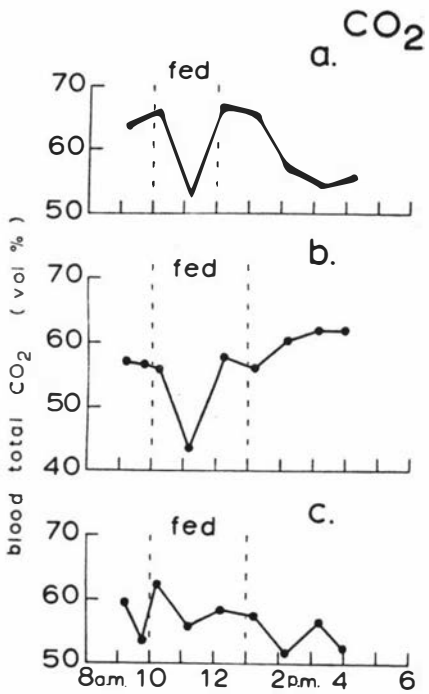

Fig 48. Blood total CO_2 content (jugular) relative to a once-daily 2 or 3 hour feed; a - water ad libitum, b,c - no water all day. Note the decrease during feeding in a,b but not in c; second decrease in the post-prandial period in a (a - sheep 3, 31.8.65; b - sheep 1, 10.2.66; c - sheep 2, 4.2.66).

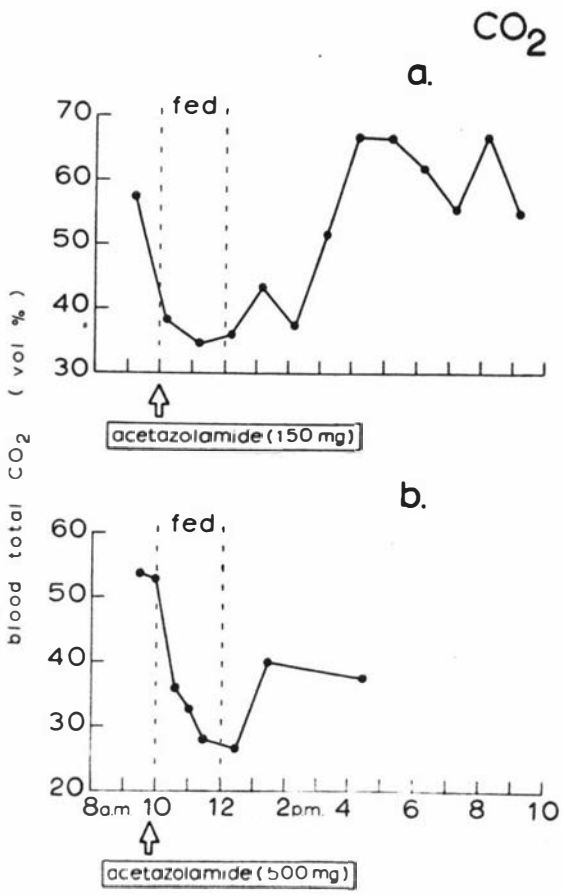

Fig 49. Blood total CO_2 content (a - jugular, b - carotid) relative to a once-daily 2 hour feed; water ad libitum, acetazolamide. Note large decrease during feeding (a - sheep 4, 19.8.65; b - sheep 1, 13.10.65).

was provided, representing a drop of 0.15 units. This occurred in both the arterial and the venous (Fig 45) samples. On the 3 days when water was not offered, the maximum decrease was a little less, 0.1 pH units (Fig 46a, b), and in 2 this occurred early in feeding (Fig 46a). Again there was no apparent difference in the magnitude of changes in arterial and venous blood.

After acetazolamide administration, on only 1 of 3 occasions did the decrease appear larger than on other days: 0.2 pH units in a series of jugular blood samples (Fig 47). The lowest pH occurred late in feeding, and the return to the prefeeding pH was more protracted than when the diuretic was not injected. On 3 days when acetazolamide was injected in the prefeeding period, within 15 minutes a drop in arterial or venous blood pH occurred, even before feeding began (Fig 47).

Blood total CO_2

Blood total CO_2 decreased by 5-10 vol % for only a short period in the middle of, or late in, feeding on 7 of the 9 occasions measured (Fig 48a, b). On 2 days no consistent change occurred (Fig 48c). There was no apparent effect of water restriction. In one series of arterial samples the decrease was more prolonged than usual, and on 3 days a second decrease was seen after feeding (Fig 48a).

After acetazolamide administration, blood total CO_2 invariably decreased during feeding, and by a greater amount than on other days. On 4 of 6 occasions a drop as large as 15-20 vol % was seen (Fig 49a, b). The effect of feeding was more prolonged; usually the low CO_2 persisted until feeding finished. Comparable changes were seen in arterial and venous blood.

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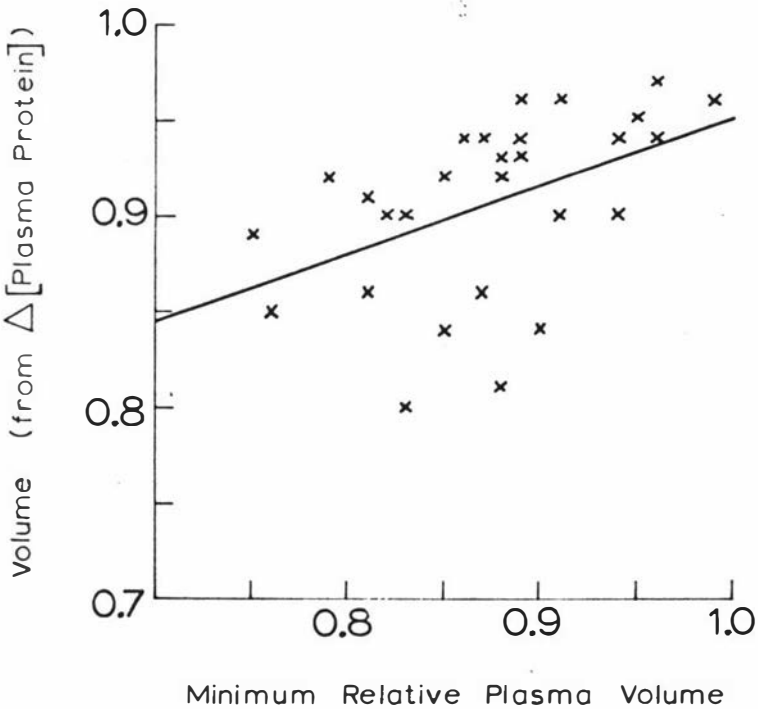

Fig 50. Graph of the plasma volume calculated from the change in [plasma protein] at the time of the minimum relative plasma volume against the minimum relative plasma volume. The points fit the straight line $y = 0.592 + 0.358x$ ($p < .01$, $n = 29$); correlation coeff. (r) = 0.47 ($p < .01$, $n = 29$).

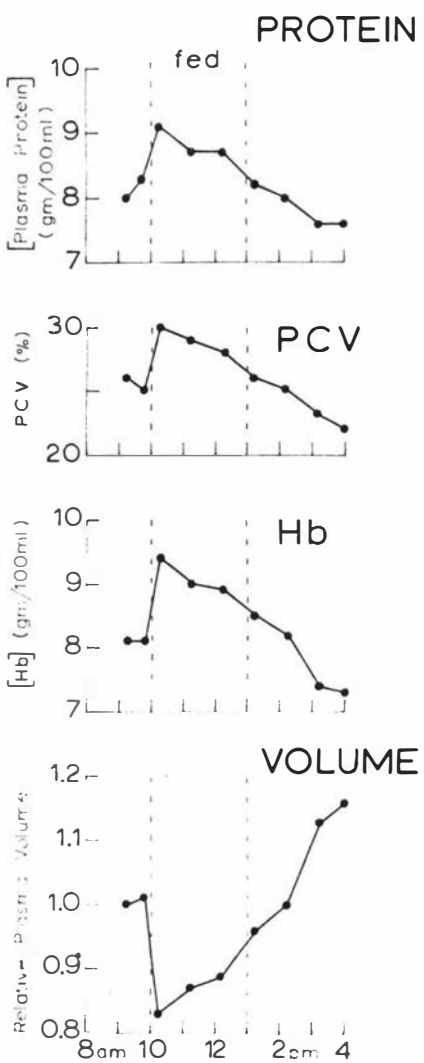

Fig 51. [Plasma protein], PCV, [Hb] and relative plasma volume relative to a once-daily 3 hour feed; water ad libitum. Note the maximum [protein], PCV and [Hb] and the minimum volume after 15 minutes feeding; the first three parameters were lower and the volume greater than before feeding in the post-prandial period (sheep 1, 24.5.66).

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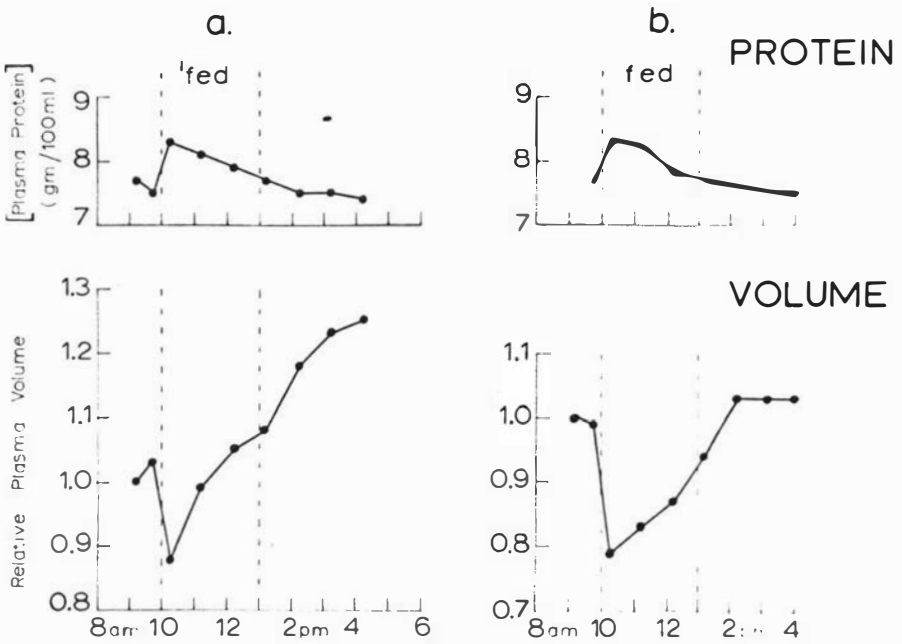

Fig 52. [Plasma protein] and relative plasma volume relative to a once-daily 3 hour feed; no water all day. Note maximum [protein] and minimum volume after 15 minutes feeding, a lower minimum volume in b, followed by lower volumes in the post-prandial period (a - sheep 2, 13.6.66; b - sheep 3, 7.6.66).

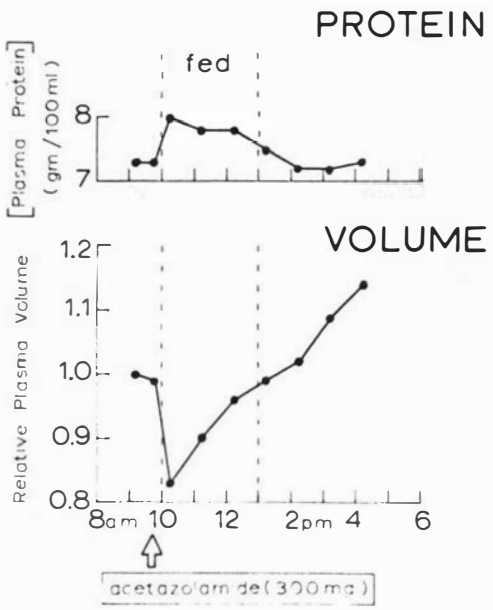

Fig 53. [Plasma protein] and relative plasma volume relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note changes similar to those in other experimental conditions (sheep 2, 15.6.66).

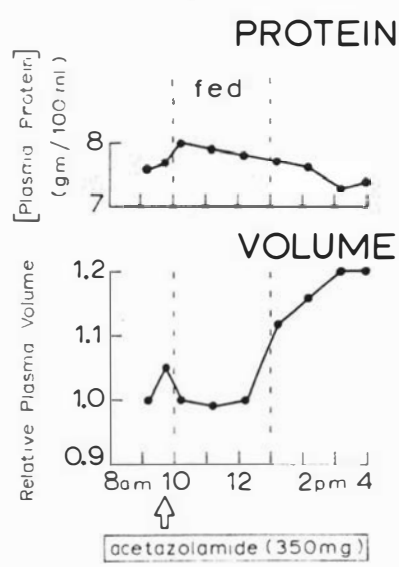

Fig 54. [Plasma protein] and relative plasma volume relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note unusual observation of unchanged volume during feeding, but the usual changes in [protein] (sheep 1, 22.6.66).

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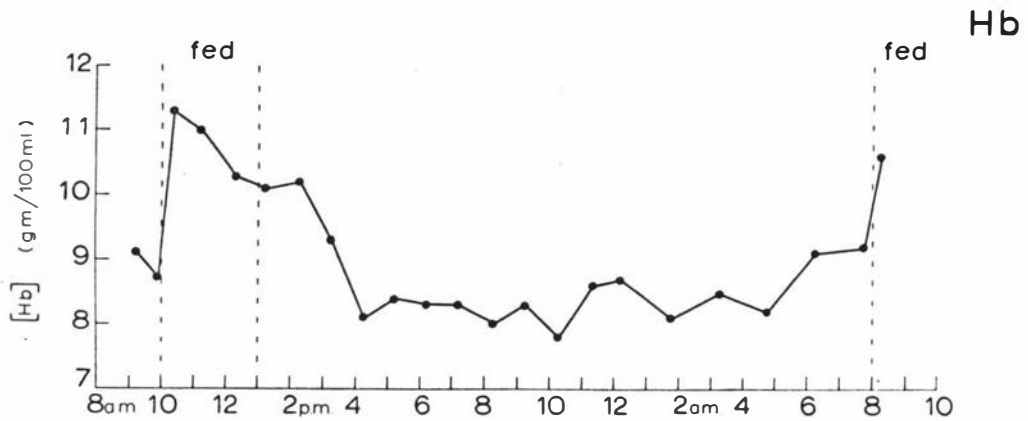

Fig 55. [Hb] relative to a once-daily 3 hour feed; water ad libitum. Note the maximum value after 15 minutes of feeding, the minimum about half way between feeds (sheep 3, 27.6.66).

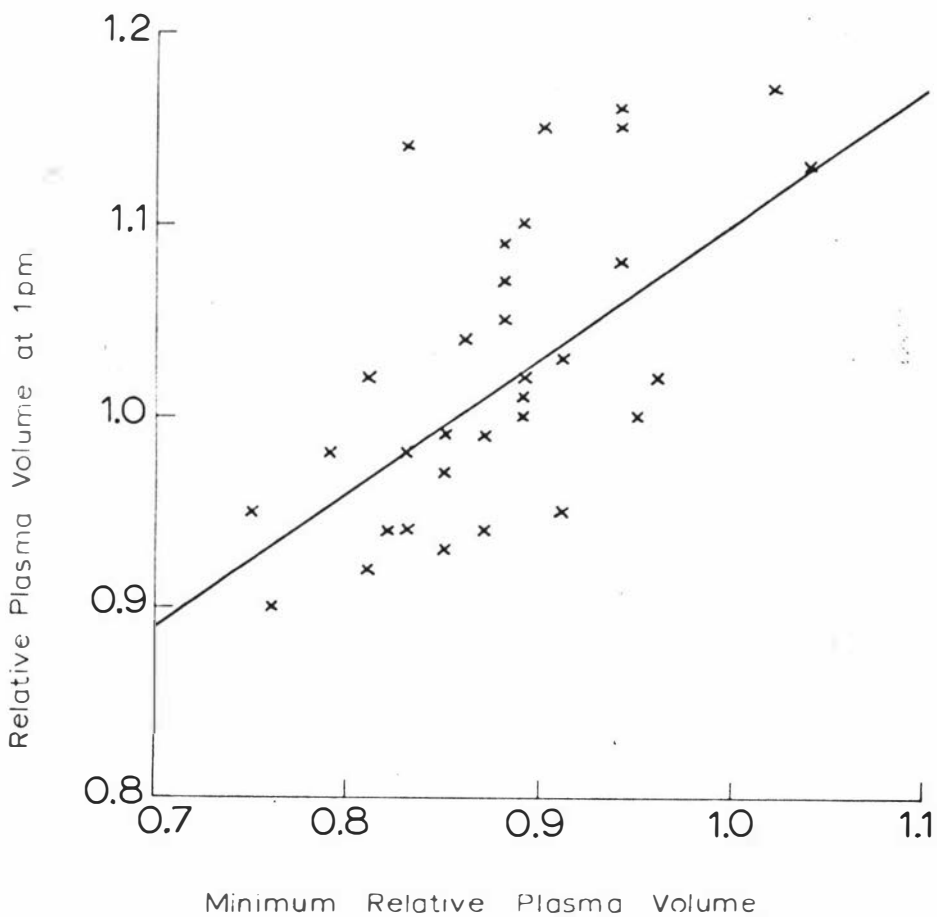

Fig 56. Graph of relative plasma volume at 1 p.m. against the minimum relative plasma volume during feeding. The points fit the straight line $y = 0.378 + 0.733x$ ($p < .001$, $n = 33$).

Plasma volume

Changes in plasma volume were inferred from two independent indices: plasma protein concentration, and a calculated value in terms of the 9.15 a.m. sample using [Hb] and PCV. Changes in [plasma protein] were smaller than changes in the relative volume calculated from [Hb] and PCV, but the two were correlated ($r = 0.47$, $p < 0.01$) (Fig 50). The calculated relative volumes were used for quantitative comparison of treatments in Tables 12 and 13.

Qualitatively similar changes in plasma volume were seen during and after feeding under the different experimental conditions, but on individual days the magnitude and rate of change varied (compare Fig 51-53). Similar changes occurred in arterial and venous blood. The plasma volume was lowest at 10.15 a.m. on 30 of 34 days, then gradually increased so that by 4.15 p.m. it was as much as 30% greater than before feeding. On 4 occasions the volume remained low for an hour before beginning to rise. Of the 4 unusual days, 1 had a slightly lower volume at 11.15 a.m., 1 had a constant calculated plasma volume in spite of normal changes in [plasma protein] (Fig 54), and on 2 days the volume never fell below that before feeding.

Similar variations in [Hb] were seen in 3 sheep observed for 24 hours (Fig 55). If this can be considered a close approximation of the inverse of plasma volume changes, then the minimum plasma volume occurred early in feeding, and the maximum volume half way between feeding periods or a little earlier.

It would appear that neither the removal of drinking water nor the administration of acetazolamide changed the average minimum plasma volume, or

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Table 12. Relative plasma volume * under different experimental conditions during a 3 hour feed.

Experimental conditions	No. of obs.	Av. min. volume during feeding	Range	Av. volume at 1 p.m.	Range
Water ad lib.	9	0.87	0.81-0.94	1.05	0.94-1.15
No water	12	0.89	0.79-1.04	1.03	0.94-1.16
Water ad lib. + acetazolamide	3	0.82	0.75-0.89	0.97	0.93-1.02
No water + acetazolamide	9	0.90	0.76-1.02	1.02	0.90-1.17
Combined water ad lib.	12	0.86	0.75-0.94	1.03	0.93-1.15
Combined no water	21	0.90	0.76-1.04	1.02	0.90-1.17
Total	33	0.88		1.03	

* Relative to 9.15 a.m.

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Table 13. Relative plasma volume 3½ hours after the end of a 3 hour feed (4.15 p.m.) under different experimental conditions.

Experimental conditions		Average volume at 4.15 p.m.	No. of obs.	Range
During feeding	After feeding			
Water ad lib.	water ad lib.	1.22	9	1.15–1.30
No water	no water	1.18	8	1.03–1.34
No water	water ad lib.	1.20	3	1.15–1.25
Water ad lib. + acetazolamide	water ad lib.	1.16	3	1.07–1.22
No water + acetazolamide	no water	1.14	5	1.05–1.22
No water + acetazolamide	water ad lib.	1.22	4	1.19–1.24
All water ad lib. after feeding		1.21	19	1.07–1.30
All no water after feeding		1.16	13	1.03–1.34

Table 14. Average minimum relative plasma volume during feeding, and average feed intake in the first 30 minutes in 3 sheep.

Sheep	Av. minimum volume & S.D.	No. of obs.	Av. 30 min feed intake & S.D. (gm)	No. of obs.
1	0.90 ± .07	10	230 ± 78	10
2	0.90 ± .05	11	255 ± 75	9
3	0.84 ± .06	10	315 ± 67	10

Table 15. Plasma volume from Evan's Blue dilution before feeding and 7 hours after feeding.

	Sheep		
	2	3	9
Body weight (kg)	34	38	29
PCV 10 a.m.	32%	28%	28%
8 p.m.	27%	24%	26%
Plasma volume (ml) 10 a.m.	1540	1610	1430
8 p.m.	1650	1730	1530
increase	110	120	100
	(7%)	(7%)	(7%)

that at 1 p.m. (Table 12). Only the group of 3 water ad lib. experiments where acetazolamide was injected appeared to be different. Comparison of the two groups fed without water showed no independent effect of acetazolamide. Similar comparison of the combined groups with, and without water, revealed little difference in the minimum plasma volume or that at 1 p.m. The reason for the apparent discrepancy in the small group of 3 becomes clearer after examining the relationship of the plasma volume at the end of feeding and the minimum volume (Fig 56). The lowest volumes at 1 p.m. were seen in sheep with the lowest minimal volumes. The sample of 3, by chance, all were days when large decreases in volume occurred, and consequently there were lower volumes at the end of feeding.

The magnitude of the decrease in plasma volume appeared to be related to the rapidity of feeding over the same period. Of 3 sheep for which most data are available, one usually ate more in the first 30 minutes of feeding and showed larger decreases in plasma volume than did the other 2 animals (Table 14). Since the two sets of measurements were made on different days, the two cannot be correlated individually.

The plasma volume at 4.15 p.m. in the 6 groups is shown in Table 13. Since from Table 12 it was seen that the average plasma volume at 1 p.m. was 1.03 irrespective of the conditions during feeding, the data has also been grouped according to whether or not drinking water was available after feeding. A slightly greater expansion of plasma volume was seen in the group with drinking water: 1.21 compared with 1.16. The 4.15 p.m. volume was only related to the minimum plasma volume at the 10% level ($r = .339$, $p < .10$, $n = 32$).

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Table 16. Plasma osmolality during feeding under the different experimental conditions.

Experimental conditions	Av. maximum O.P. during feeding (mosm/kg)	No. of obs.	Range (mosm/kg)
Water ad lib.	307	8	298-314
No water	306	3	300-312
No water + acetazolamide	305	4	298-309

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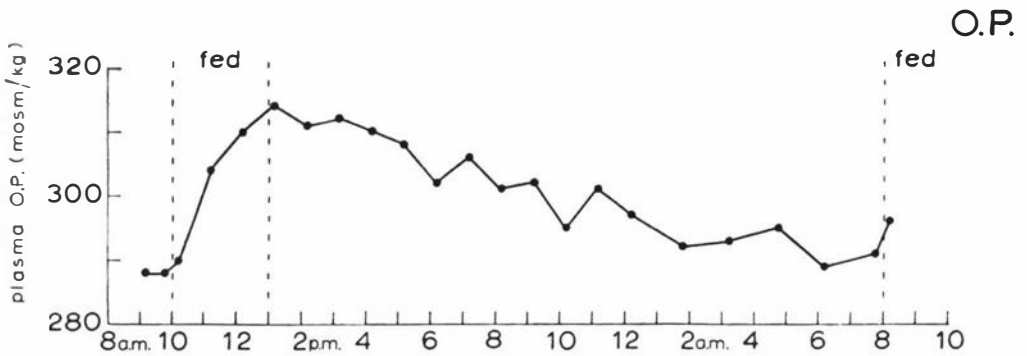


Fig 57. Plasma osmolality relative to a once-daily 3 hour feed; water ad libitum. Note the rapid rise in O.P. to a maximum late in feeding, followed by a slow decline to the next feed (sheep 3, 27.6.66).

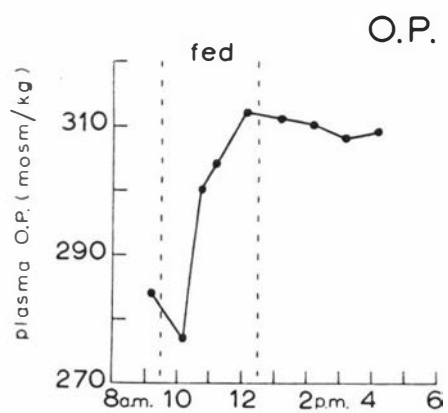
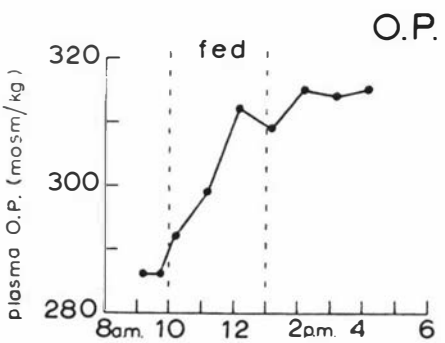


Fig 58. Plasma osmolality relative to a once-daily 3 hour feed; water ad libitum. Note the maximum O.P. late in feeding and a slow fall in the post-prandial period (sheep 3,19.5.66).

Fig 59. Plasma osmolality relative to a once-daily 3 hour feed; no water all day. Note the further increase in O.P. in the post-prandial period (sheep 2, 13.6.66).



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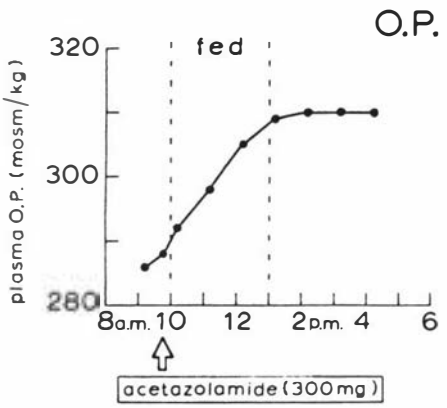

Fig 60. Plasma osmolality relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note the further small rise in O.P. after feeding (sheep 2, 15.6.66).

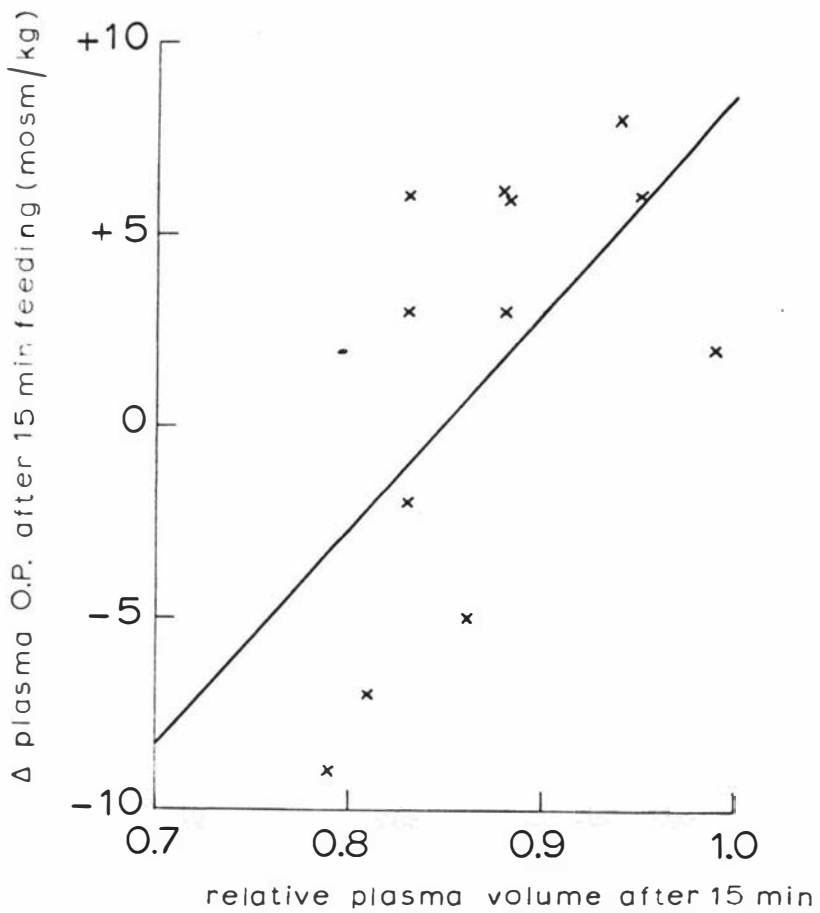

Fig 61. Graph of the change in plasma osmolality from 9.15 to 10.15 a.m. (45 minutes before to 15 minutes after the start of feeding) against the relative plasma volume at 10.15 a.m. The points fit a straight line $y = -47.68 + 56.28x$ (p .05, $n = 12$).

A third independent method, that of Evan's Blue dilution, was used to estimate plasma volume at 10 a.m. (before feeding), and at 8 p.m. when the plasma volume would be expected to be greatest and fairly constant (Table 15). In 3 sheep, the plasma volume at 8 p.m. was 100-120 ml greater than that before feeding.

Plasma Osmolality

Prefeeding plasma osmolality was in the range 280-290 mosm/kg H_2O. During feeding, plasma osmolality increased rapidly at first, then more slowly to maximal values late in the feeding period (Fig 57-60). Table 16 shows the average maximum osmolality during feeding under the different experimental conditions. No effect of either restricted water or acetazolamide administration was apparent during feeding, although the numbers were small.

The osmolality of the plasma 15 minutes after feeding began, when plasma volume was lowest, was compared with that 45 minutes before feeding (9.15 a.m.) on the 11 days when no diuretic was administered. There appeared to be no change in the average plasma osmolality: that before feeding was 286$\pm$ 1 mosm/kg, compared with 287$\pm$ 7 mosm/kg after feeding had begun. The change in osmolality from 9.15 to 10.15 a.m. (having regard to the direction of change) was plotted against the plasma volume at 10.15 a.m. (Fig 61); Δ O.P. was linearly related to the plasma volume. Although only a small number of paired observations (12) were made, they would appear to be representative, since for a plasma volume of 0.88, the overall mean, the ΔO.P. was +2 mosm/kg, the mean for the smaller group.

In the post-feeding period, the absence of drinking water caused a different pattern in plasma osmolality. When water was available, on 6 of 8

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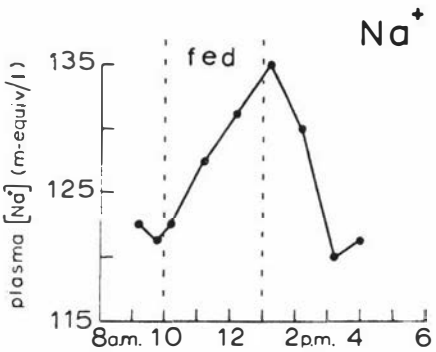
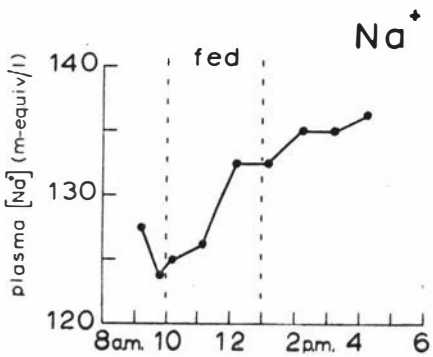


Fig 62. Plasma $[Na^+]$ relative to a once-daily 3 hour feed; water ad libitum (plasma separated under paraffin). Note the rise during feeding, a fall afterwards (sheep 1, 24.5.66).

Fig 63. Plasma $[Na^+]$ relative to a once-daily 3 hour feed; no water all day (plasma separated under paraffin). Note rise during feeding, further increase in the post-prandial period (sheep 2, 13.6.66).



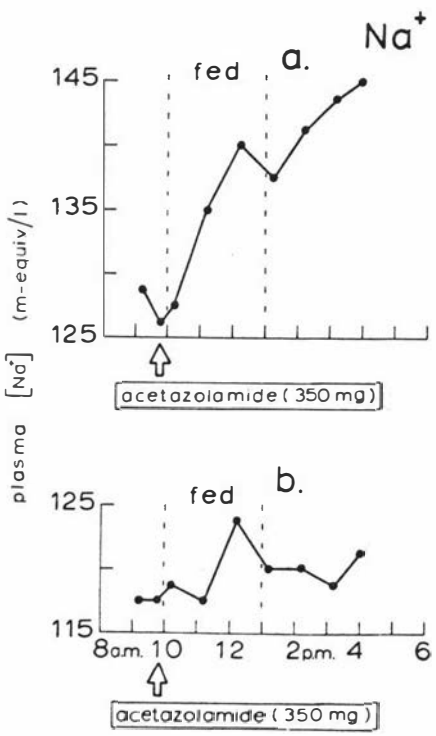


Fig 64. Plasma $[Na^+]$ relative to a once-daily 3 hour feed; no water all day, acetazolamide (plasma separated under paraffin). Note a - usual observation of rise during feeding and further increase after it; b - atypical day (a - sheep 3, 8.6.66; b - sheep 1, 22.6.66).

days plasma osmolality began decreasing at the end of feeding (Fig 57, 58), while on 6 of 7 with no water further increases occurred (Fig 59, 60). The pattern in sheep which had received acetazolamide appeared no different from that in the others denied water.

Observations were made for 24 hours in 3 sheep with water ad lib. Plasma osmolality changes were similar in all 3 (Fig 57): the osmolality was maximal at the end of feeding, and decreased steadily until the next feed.

Plasma $[Na^+]$

Before feeding, plasma $[Na^+]$ was 120-130 m-equiv/l on nearly every day. In 12 of 35 experiments the plasma was separated under paraffin. Compared with samples exposed to the atmosphere, anaerobically prepared samples consistently gave increments in plasma $[Na^+]$ during and after feeding of an order predictable from the concurrent change in osmolality, a closer pair of prefeeding values and smoother graphs.

In general, plasma $[Na^+]$ changes followed those in plasma osmolality. Plasma $[Na^+]$ increased during feeding on every day, irrespective of the experimental conditions (Fig 62-64). When no water was provided after feeding, the plasma $[Na^+]$ remained elevated more frequently than on days when drinking water was available, although some of the latter group also showed elevated $[Na^+]$. No independent effect of acetazolamide administration was apparent. Atypical days were seen occasionally (Fig 64b).

Plasma $[Cl^-]$

Prefeeding plasma $[Cl^-]$ ranged from 90-110 m-equiv/l. The

Table 17. Comparison of the plasma $[Cl^-]$ increase during feeding with the different experimental conditions and with two methods of plasma preparation.

Experimental conditions	Plasma separation method			
	Anaerobic		Open tube	
	Av. max $[Cl^-]$ increase (m-equiv/l)	No. of obs.	Av. max $[Cl^-]$ increase (m-equiv/l)	No. of obs.
Water ad lib.	4.4	5	9.4	7
No water	6.0	3	9.6	8
Water ad lib. + acetazolamide	-	-	13.9	7
No water + acetazolamide	6.0	4	14.0	4

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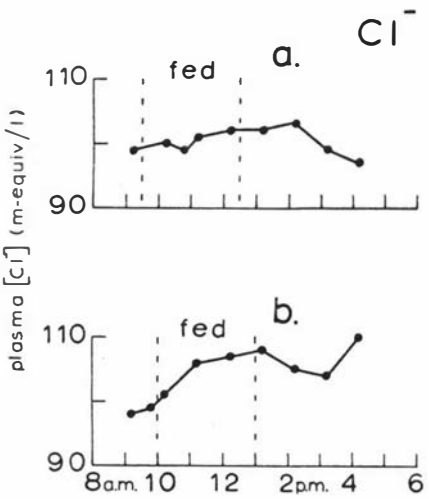
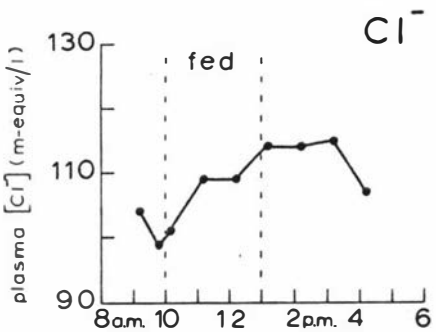


Fig 65. Plasma [Cl⁻] relative to a once-daily 3 hour feed; water ad libitum (plasma separated under paraffin). Note the increase during feeding, larger in b than a, followed by a fall after feeding (sheep 3, a - 19.5.66; b - 17.5.66).

Fig 66. Plasma [Cl⁻] relative to a once-daily 3 hour feed; no water all day (plasma separated under paraffin). Note rise during feeding; elevated [Cl⁻] until late in the post-prandial period (sheep 2, 13.6.66).



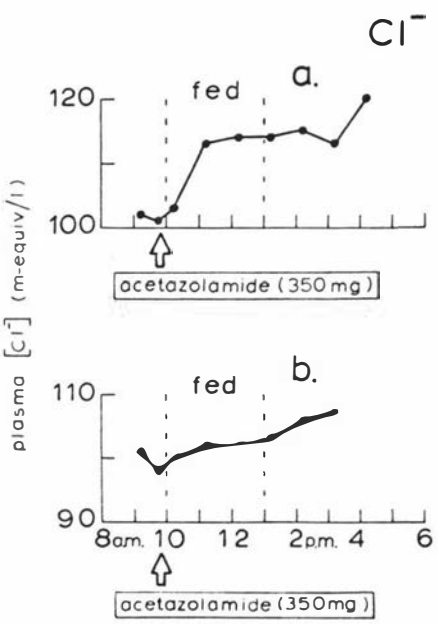


Fig 67. Plasma [Cl⁻] relative to a once-daily 3 hour feed; no water all day, acetazolamide (plasma separated under paraffin). Note the increased concentration during feeding, larger in a, remaining high after feeding and even increasing further (a - sheep 2, 15.6.66; b - sheep 1, 22.6.66).

second prefeeding sample was 1-7 m-equiv/l (usually 3-5 m-equiv/l) lower than the first in 23 of 27 experiments. The mean of the two samples was considered the prefeeding value.

On 38 days, under all experimental conditions, plasma $[Cl^-]$ increased during feeding, usually to a maximum late in feeding. The method of plasma preparation had a major effect on the magnitude of the changes and the effects of the treatments. Table 17 shows the average maximum increase with each experimental treatment, with 2 methods of plasma preparation: anaerobic and in open tubes. It can be seen that increases were twice as great in the samples exposed to the air. The method of preparation also influenced the apparent effect of the diuretic. On days when open tubes were used, there was no difference when water was not provided, but greater increases were seen after acetazolamide administration. On the other hand, in the 12 experiments where the plasma was separated under paraffin, the range of increments of $[Cl^-]$ was similar with all treatments (Fig 65-67); there was certainly no demonstrable effect of the diuretic.

After feeding, according to the availability of water to the animal, plasma $[Cl^-]$ either decreased again or remained elevated for some hours. In anaerobically prepared plasma, if water was available, all showed a decrease (Fig 65), whereas if water was not provided, only 1 of 7 decreased. In contrast, in the samples exposed to the air, half of the experiments showed a decrease irrespective of the availability of water.

Plasma $[K^+]$

As with Na^+ and Cl^-, the method of separation of plasma had a marked

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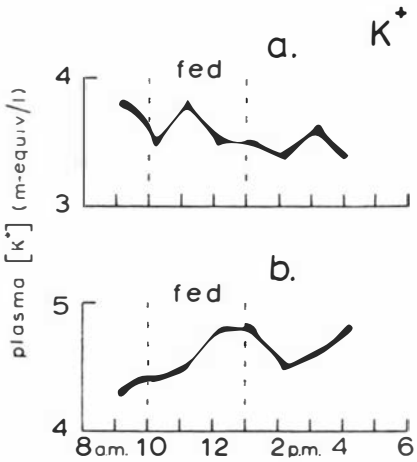
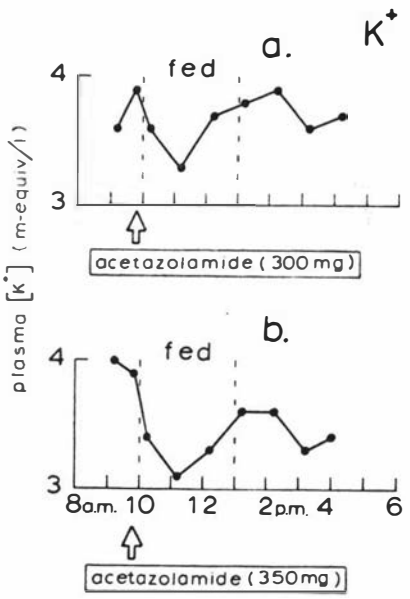


Fig 70. Plasma $[K^+]$ relative to a once-daily 3 hour feed; no water all day (a - plasma separated under paraffin, b - open tubes). Note in a (anaerobic) a transient drop over the first 15 minutes of feeding, and a tendency to decrease over the whole feed; in b (aerobic) a higher prefeeding $[K^+]$ and an increase during feeding (a - sheep 3, 7.6.66; b - sheep 2, 2.2.66).

Fig 71. Plasma $[K^+]$ relative to a once-daily 3 hour feed; no water all day, acetazolamide (plasma separated under paraffin). Note in both a and b a fall during feeding increasing again to a peak just after the end of feeding (a - sheep 2, 15.6.66; b - sheep 1, 22.6.66).



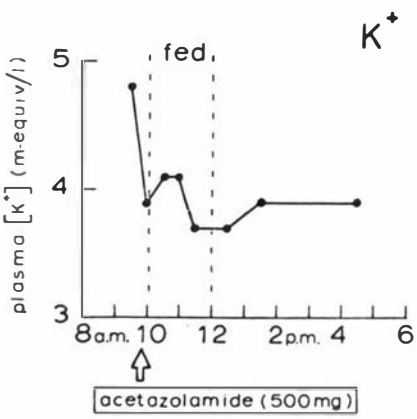


Fig 72. Plasma $[K^+]$ relative to a once-daily 2 hour feed; water ad libitum, acetazolamide (carotid blood, plasma separated in open tubes). Note decreased $[K^+]$ after acetazolamide, even before feeding began (sheep 1, 13.10.65).

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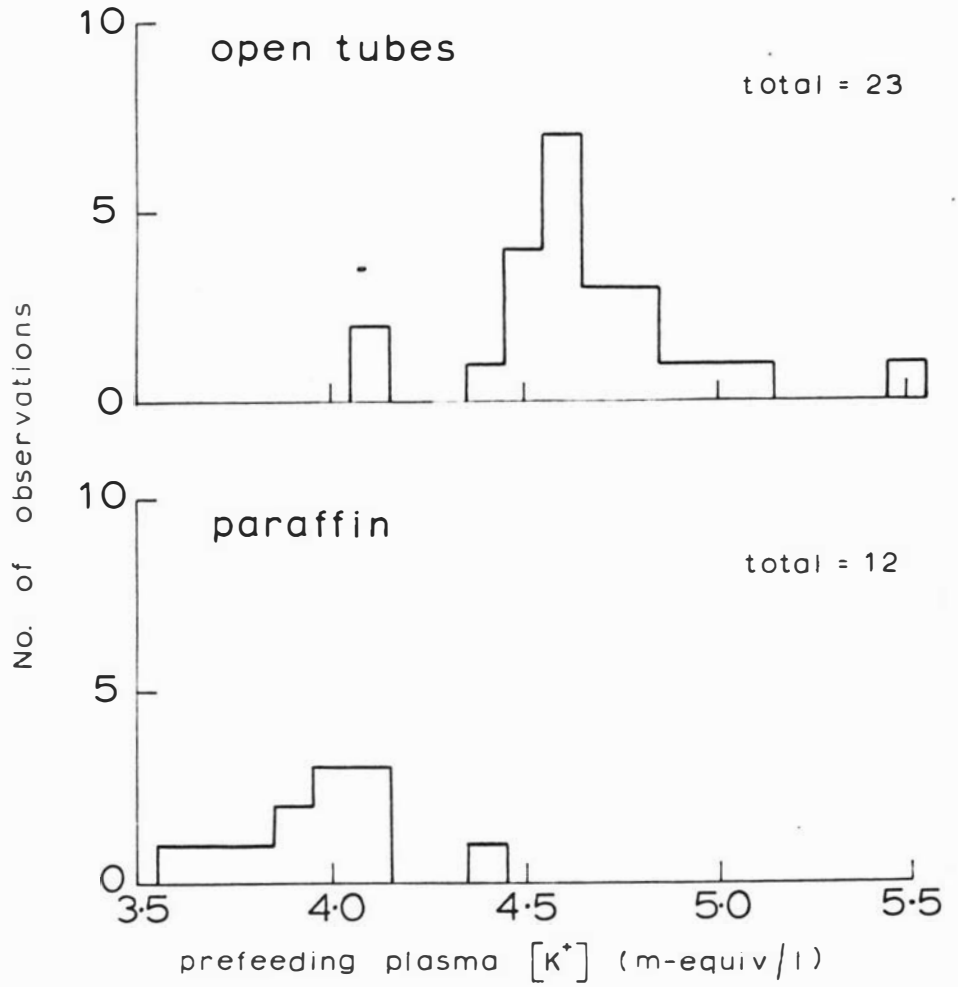

Fig 68. Distribution diagram of prefeeding [K+] with two methods of plasma separation, in open tubes and under paraffin. The concentration has been taken as the mean of the two prefeeding samples (open tubes - 23 observations; under paraffin - 12 observations).

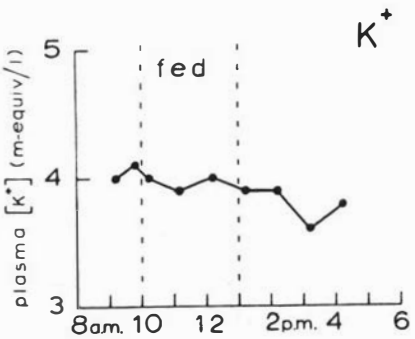

Fig 69. Plasma [K+] relative to a once-daily 3 hour feed; water ad libitum (plasma separated under paraffin). Note little change during feeding, a tendency to decrease after feeding (sheep 2, 9.5.66).

influence on the levels of K^+ found. This is shown by the distribution of prefeeding plasma $[K^+]$ with the two methods of separation (Fig 68): higher concentrations were seen in samples exposed to the air. In general, the magnitude of the plasma $[K^+]$ fluctuations was smaller in the anaerobically prepared series. Since the differences seen with the two methods of preparation may reflect changes occurring *in vitro*, results obtained in anaerobically prepared samples should be considered the more reliable.

In anaerobic samples, feeding had little effect on plasma $[K^+]$ in 5 sheep with water *ad lib.*, but $[K^+]$ decreased in 4 of 5 in the post-feeding period (Fig 69). In 3 sheep without water all day, plasma $[K^+]$ showed a transient drop of 0.2 m-equiv/l within 15 minutes of feed being offered in all; $[K^+]$ had returned to the prefeeding value by the next hour, but in 2 there was a slight tendency for $[K^+]$ to decrease over the whole feeding period (Fig 70a). In the post-feeding period that followed, none of the 3 showed the decreased plasma $[K^+]$ seen in sheep with water. Where open tubes were used for plasma preparation (13 experiments), larger plasma $[K^+]$ changes were seen during and after feeding. During feeding, in sheep with water, in 3 $[K^+]$ increased and in 2 it decreased, while in sheep fed without water, in 6 there was an increase and 2 were unchanged (Fig 70b).

When acetazolamide was administered and plasma was separated under paraffin, plasma $[K^+]$ decreased immediately after feeding began, the maximum drop being 1.0 m-equiv/l in one sheep, 0.5 m-equiv/l in 2, and only 0.1 m-equiv/l in the fourth. Following this minimum, $[K^+]$ increased again to a peak just after feeding, then decreased again (Fig 71a, b). The effects of the method of plasma preparation appeared less when acetazolamide was administered. The same pattern was seen in all 11 experiments where open

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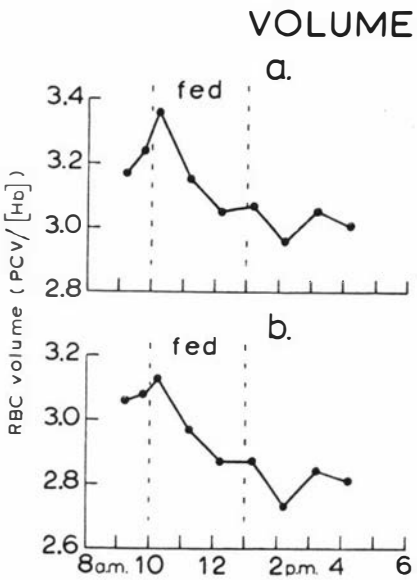


Fig 73. Erythrocyte volume relative to a once-daily 3 hour feed; a - water ad libitum, b - no water all day. Note the usual observation under all experimental conditions of an increase in volume over the first 15 minutes of feeding followed by erythrocyte shrinkage over the next 6 hours (sheep 2, a - 10.3.66, b - 4.2.66).

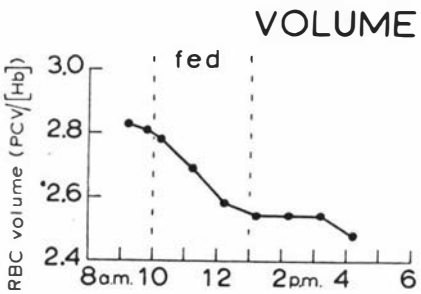
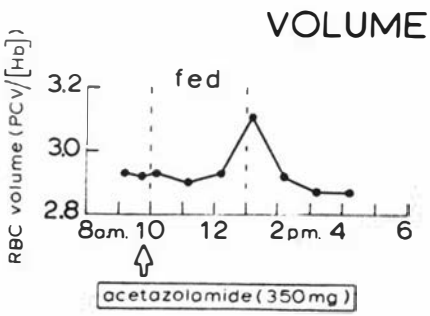


Fig 74. Erythrocyte volume relative to a once-daily 3 hour feed; no water all day. Note the occasional observation of failure of the cells to swell over the first 15 minutes of feeding (sheep 3, 7.6.66).

Fig 75. Erythrocyte volume relative to a once-daily 3 hour feed; no water all day, acetazolamide. Note atypical changes in erythrocyte volume (there were also unusual changes in plasma volume, O.P. and $[Na^+]$ on this day) (sheep 1, 22.6.66).



tubes were used, and the maximum drop was 0.5 m-equiv/l in most. The changes were similar in arterial and venous blood (Fig 72).

Erythrocyte volume

The red cells increased in volume within 15 minutes of the start of feeding. Thereafter they began to shrink, at first rapidly then more slowly, throughout the observation period which terminated 3 hours after the end of feeding. Within 1-2 hours, the volume was below that before feeding, and the minimum volume reached averaged 7% less than that in the prefeeding period (Fig 73a,b). These changes were seen on 26 of 32 occasions under all feeding conditions; there was no apparent effect either of injecting acetazolamide or removing the sheep's drinking water.

On 5 out of 32 days, the initial increase in volume did not occur (Fig 74). On 1 day of 32 a completely different pattern was shown, there being almost no change in erythrocyte volume (Fig 75). This was associated with a number of other unusual patterns - plasma O.P. and $[Na^+]$ increased less than usual, and the plasma volume did not decrease during feeding.

Erythrocyte Na^+ and K^+

The ratio of Na^+:K^+ in the erythrocytes of sheep fall into two main groups: those with a high Na^+ and low K^+ (Na^+:K^+ 7-8:1) and those with a relatively high K^+ content (Na^+:K^+ 1:2-3). In these experiments, only 1 sheep out of 9 examined was in the latter group.

There was no consistent pattern of changes in erythrocyte $[Na^+]$ or $[K^+]$ with any treatment (Fig 76-79), however, on individual days large increases and decreases frequently occurred. Both Na^+ and K^+ content tended

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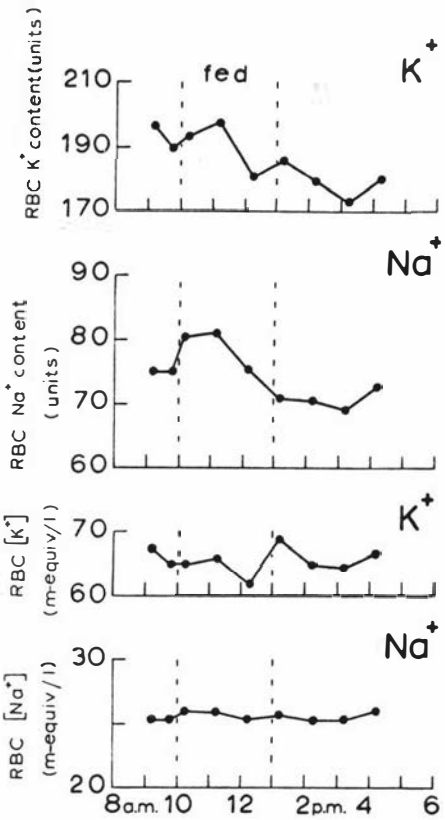

Fig 76. Erythrocyte $[K^+]$, $[Na^+]$, K^+ and Na^+ content relative to a once-daily 3 hour feed; water ad libitum. On this day, Na^+ and K^+ content both increased in the first 15 minutes of feeding but overall tended to decline (sheep 3, 17.5.66).

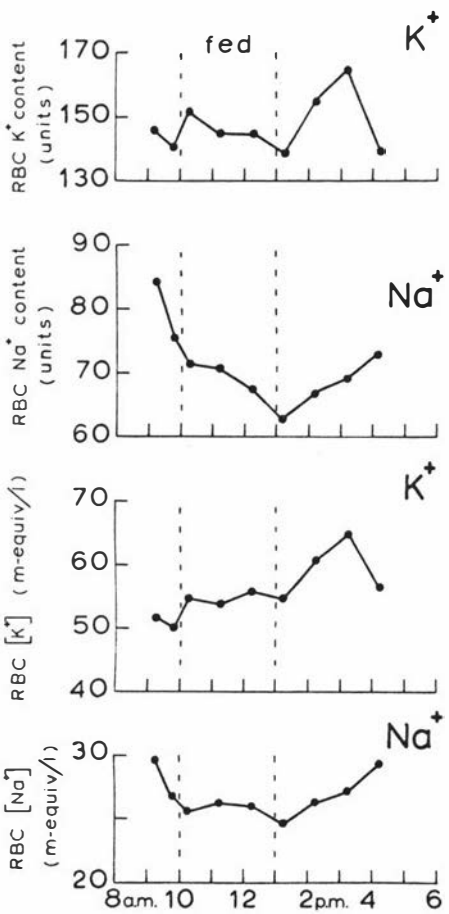

Fig 77. Erythrocyte $[K^+]$, $[Na^+]$, K^+ and Na^+ content relative to a once-daily 3 hour feed; no water all day. Overall, and in the first 15 minutes of feeding Na^+ content fell, but K^+ content increased both early in feeding and in the post-prandial period (sheep 3, 7.6.66).

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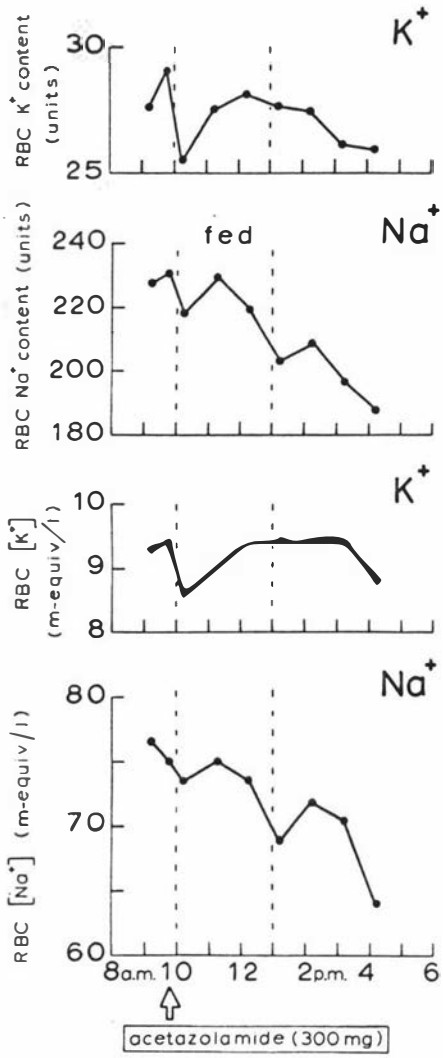

Fig 78. Erythrocyte $[K^+]$, $[Na^+]$, K^+ and Na^+ content relative to a once-daily 3 hour feed; no water all day, acetazolamide. Overall, and in the first 15 minutes of feeding, both Na^+ and K^+ content fell (sheep 2, 15.6.66).

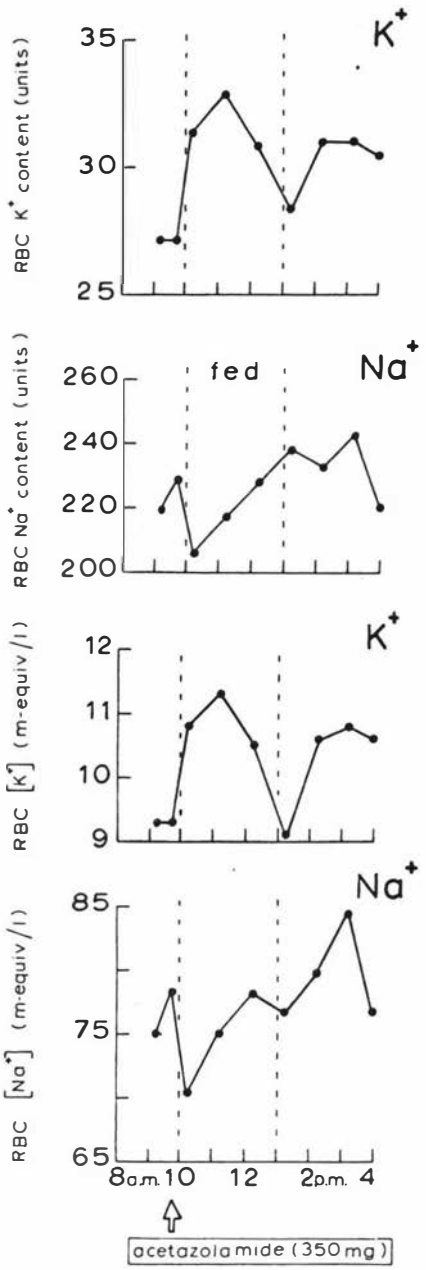

Fig 79. Erythrocyte $[K^+]$, $[Na^+]$, K^+ and Na^+ content relative to a once-daily 3 hour feed; no water all day, acetazolamide. Na^+ content fell early in feeding, thereafter increased, K^+ content rose, particularly early in feeding (sheep 1, 22.6.66).

to decline over the 7 hours of observation under all experimental conditions (Fig 76, 78) although exceptions to this were seen (Fig 77, 79). Transient fluctuations made recognition of trends difficult.

In contrast to the variability of the overall changes, there appeared to be an important relationship between changes in Na^+ and K^+ content in the first 15 minutes after feed had been given and the changes in red cell volume: unless the ($Na^+ + K^+$) content increased, the red cells failed to swell. This relationship was evident in each of the following situations:

(i) where both cations increased (4 of 5 days with water ad lib.), the red cell volume increased (Fig 76),

(ii) where both cations decreased (1 of 5 days with water ad lib., 1 of 4 after acetazolamide) the cells shrank (Fig 78),

(iii) where one cation increased and the other decreased such that there was an almost negligible change in the total cations (3 days with no drinking water, 1 of 4 after acetazolamide), the cell volume change was negligible (Fig 77); but where the net result was an increase (2 of 4 with acetazolamide) the cells increased a little in volume (Fig 79).

DISCUSSION

The principal aim of these experiments was to obtain information about the shifts of water and electrolyte between the body fluid compartments, i.e. between the ECF and the ICF, gut contents and urine, induced by once daily feeding. Such movements can be studied only indirectly by experiments such as these where changes only in urine excretion and in plasma volume and composition are determined. Sampling the ICF presents practical problems,

since the only accessible cells, without a tissue biopsy, are the blood cells. Lysed erythrocytes have been examined in these experiments, although they cannot be regarded as cells representative of the general cell population because of their highly specialized nature. It is this heterogeneity of cells which makes the concept of an ICF a theoretical one, since individual cell types may show differing water and electrolyte shifts in the one situation.

A confounding influence in experiments of the kind carried out here would be pain or fright. For this reason there must be a training period when the animals become accustomed to their cages, the feed, the experimental routine and to handling. The undesirable aspect of training is the development of conditioned responses, such as evidenced in the prefeeding diuresis, and in a few cases of reduced feed intake immediately after removal of the drinking water. The use of jugular cannulae for blood sampling, and the collapsed vaccine bags at the end of the clamped catheter for collection of urine samples, enabled the experiments to be performed without apparent pain or disturbance to the sheep.

In nearly all cases both urine and blood samples were collected anaerobically; always so for pH and HCO_3^- estimations. The importance of not exposing blood to the air before measuring pH and CO_2 content is generally accepted; however, possible effects on plasma electrolyte concentrations are frequently overlooked. When plasma samples were separated under paraffin, $[K^+]$, $[Na^+]$ and $[Cl^-]$ showed differences from samples in open tubes. Further, the difference was not consistent across the various experimental treatments. The shift of electrolytes into and out of the erythrocytes on exposure to air appeared to be different after acetazolamide administration,

and was perhaps related to the different plasma pCO_2 or to carbonic anhydrase inhibition.

It has been possible in these experiments to provide a better definition than hitherto published of the shifts of water and electrolytes associated with feeding in sheep. These observations confirm and extend those of Stacy and Brook (1964, 1965), Stacy and Warner (1966) and Tennouth (1967) that, during a single daily feed, sheep show urinary concentration and acidification and reduced Na^+ and K^+ excretion, a transient hyperproteinaemia, increased plasma osmolality and $[Na^+]$. However, the attempts made to obtain further insight into the mechanisms involved by variation of the experimental conditions have been disappointing. In particular, the effect of restriction of the drinking water on water metabolism was less than might have been expected. The principal effect of this treatment appeared to be a small reduction of feed intake (also reported in ruminants by Balch, Balch, Johnson and Turner (1953) and by Macfarlane et al. (1961)), resulting in slightly smaller changes in urine pH, blood pH and CO_2 content, and in a reduced K^+ excretion in the post-feeding period. When, in addition, water was not returned after feeding, smaller increases in urine flow and in plasma volume were seen, and the plasma electrolytes and urine O.P. remained elevated. Thus water deprivation for one day had a relatively small effect. It is probable that more marked effects would have been obtained if, as well, these experiments had been preceded by a period of water deprivation.

The use of acetazolamide has underlined two features: firstly, that usually during feeding there is increased secretion of H^+; and secondly, that forced kaliuresis at the prefeeding rate lowers plasma $[K^+]$.

suggesting K^+ retention during feeding is of regulatory origin. In general, the effects of acetazolamide were similar to those reported in the literature for other species (Berliner et al., 1951; Leaf, Schwartz and Relman, 1954; Maren et al., 1954; Counihan et al., 1954). Thus, in the cow, 5 mg/kg of acetazolamide, given intravenously, increased the urine flow, total electrolyte, Na^+, K^+ and HCO_3^- excretion, increased the urine pH, had little effect on the Cl^- excretion, and lowered plasma $[K^+]$ by up to 1 m-equiv/l (Anderson and Pickering, 1964). The maximum effect was at 30 minutes. In the sheep, acetazolamide had only a modest action as a diuretic. This may be related to the dose rate, or perhaps to its action in creating an osmotic diuresis through increased HCO_3^- excretion, which may be less effective where the urine is usually rich in HCO_3^-. In contrast to the usual observation, Cl^- excretion was very low. Weinstein (1968) produced low Cl^- excretion in the rat after acetazolamide, and concluded that the increased Cl^- reabsorption allowed some Na^+ to be reabsorbed along with Cl^- rather than in cation exchange. In these experiments, when the prefeeding Na^+ excretion was low, Na^+ excretion was not invariably raised by the diuretic. It has been noted in man and the dog that whether Na^+ or K^+ is the principal cation excreted with HCO_3^- depends on the previous dietary intake: in K^+ deficiency $NaHCO_3$ is excreted (Evans et al., 1954), and in Na^+ deficiency, $KHCO_3$ (Counihan et al., 1954). It is doubtful if the use of larger doses of acetazolamide would have provided additional useful information. The interpretation of the results would be even more difficult since, as well as the reduced GFR associated with intravenous acetazolamide (Berliner et al., 1951; Madsen, 1954; Maren et al., 1954; Anderson and Pickering, 1964) the larger doses would be more likely to have extrarenal actions in erythrocytes (Tumasherski, Chinn and Clark, 1954; Cranston, Sanderson and Stapleton, 1955),

gastric mucosa (Janowitz, Colcher and Hollander, 1952), the pancreas (Birnbaum and Hollander, 1953; Rawls, Wistrand and Maren, 1963), salivary glands, ciliary body and choroid plexus.

A prominent feature of this study was the high day to day variation in the prefeeding excretion pattern and the responses to feeding. This was such that it necessitated the analysis of the results not on a control-treatment basis, but on the basis of a comparison of the whole group of replicates for each experimental condition with the other groups. Undoubtedly, some part of this variation was due to variation in the feed and water intake, but the origin of the great part could not be defined. The physiological status was not constant, but was not easy to determine, as evidenced by the Na^+ status. The prefeeding Na^+ excretion levels fell into two groups - high and low- but was not clearly indicative of Na^+ deficiency and Na^+ repletion, since low excretion occurred in sheep with continuous access to salt lick, and in animals with feed supplemented with NaCl, and peaks of Na^+ excretion were seen in the post-feeding period. The prefeeding rate of Na^+ excretion, however, had a subsequent effect on the degree of Na^+ retention during the feeding period.

Although a constant amount of feed was offered each day for the same time, neither the pattern of intake, nor the total amount consumed was uniform. The water intake, being related to the feed intake, also varied in time course and amount. There were two basic feeding patterns, and the same two patterns in water intake plus a third which was the inverse of one of them. The association of these feed and water intake patterns is likely to be of significance in the sensation of thirst and satiation. Such variation in the feed and water consumption would be expected to be related

to some of the variation seen in the other responses. No attempt was made to correlate these in the present experiments since it was found that the removal of the feed tin for weighing caused great excitement. Feed and water intake patterns were therefore observed on days when urine and blood samples were not being collected. Water intake was almost entirely confined to the feeding period (Table 2). This was also noted by Hogan (1964) and Yernouth (1967). The feed and water intakes on each day were significantly correlated in 4 of 5 sheep (Table 2), the exception being the animal with the lowest water:feed ratio. The water:feed ratio ranged from 1.94 to 3.62 ml/gm, similar to that evident in the results of English (1966) and to 2.8 ml/gm for a high plane of nutrition reported by Riek et al. (1950), but considerably lower than the 9.1 ml/gm for sheep on a low plane of nutrition. When water was offered at 1 p.m., the sheep drank nearly as much as during a normal feeding period, most being consumed in 1-2 minutes. If the water was not offered until 4.30 p.m., the amount drunk was less. Thirst had apparently been partially satisfied by internal redistribution of fluid.

In the successive phases of the experiment - before, during and after the feeding period - the water loss in the urine appeared to be determined by different principal factors. Before feeding there was a profuse diuresis of apparent psychic origin; during feeding, the marked antidiuresis seemed to be related to ADH release and possibly reduced GFR; in the post-feeding period, under maximum antidiuretic conditions urine flow was probably determined by the solute load.

The prefeeding diuresis observed in these experiments would appear to be a consequence of recognition by the sheep that feeding was imminent. It could be prolonged by postponing feeding, and was less pronounced when an

experiment continued through the night and staff were always present. The prefeeding diuresis was also seen by Dewhurst and Harrison (1966), and the urine flows recorded by Stacy and Brook (1964) suggested it also occurred in their animals. In the present study, and that of Dewhurst and Harrison (1966), the prefeeding diuresis provided the largest change in urine flow over the 24 hours.

Feeding produced a marked reduction in the urine flow, and an increase in osmolality, both of which appeared more prominent if a prefeeding diuresis was established and large volumes of dilute urine (300-600 mosm/kg) were being excreted. The volume was minimal at the 30 or 60 minute sample after feed was given, and from the observations in 32 hour experiments would appear to be comparable to those in the 8-12 hours before the prefeeding diuresis. The onset of this antidiuresis was rapid, reaching an almost minimal flow rate in 20 minutes (Fig 9, and Stacy and Brook, 1964).

Stacy and Brook (1965) reported that release of ADH was responsible for the antidiuresis of feeding, since they could detect antidiuretic activity in the urine of fed, but not unfed, sheep. Their assay appeared to have low sensitivity, since they were unable to detect activity even in concentrated urine before feeding. In their experiments, and in these, it would be very likely that the transition from dilute to concentrated urine would involve the release of ADH. However, ADH is unlikely to be the sole determinant of urine flow on every day. On 25% of all occasions, the prefeeding urine volume did not exceed 50 ml/ 30 minutes. In such cases the $T^{c}_{H_2O}$: C_{osm} relationship was the same as in samples collected during feeding (Fig 44), suggesting that ADH secretion was adequate to allow equilibration of collecting duct fluid with the medullary interstitium. It would seem unlikely that the reduced

urine flow early in feeding on these days was the result only of ADH release.

An additional factor may well be a haemodynamic change, probably reduced GFR. Stacy and Brook (1964), in their observations in 5 sheep, claimed that no change in GFR took place, however, a reduced GFR, at least during the first 30-60 minutes, would not be excluded by their results. Inspection of their results showed that, although there was great variability from one sample to the next, in all cases the inulin clearance decreased from the last prefeeding period of 15-30 minutes to the first feeding period. As they point out, clearance measurements during declining urine flow tend to underestimate the true value. It was for this reason, as well as that of the undesirability of an infusion during this period, that no inulin or PAH clearance measurements were undertaken in the present experiments. Other observations during feeding also suggest that a change in GFR cannot be ruled out. A reduced GFR could well occur as a consequence of the rapid decrease (averaging 12%) in plasma volume within 15 minutes of beginning to feed. As well, [plasma protein] increased over this period (Fig 51, and Stacy and Brook (1964)).

Whatever other factors are involved, increased ADH secretion would appear to be associated with the feeding antidiuresis on most days. The commonly recognised stimuli for vasopressin release are increased ECF osmolality (Verney, 1947; Leaf and Mamby, 1952a) and reduced ECF volume (Leaf and Mamby, 1952b; Lemaire et al., 1959; Dyball, 1966). In the present experiments, it would seem that both may be involved in the initial stimulus to ADH hypersecretion, but that its maintenance was due to the rising plasma osmolality. If they are the stimuli, the relative contributions

of reduced volume and raised tonicity to the initial ADH release would vary from day to day. Fifteen minutes after feeding commenced, the greatest decreases in plasma volume were associated with lower osmolality, but only small drops in volume with raised O.P. (Fig 61).

Changes in plasma volume and tonicity were not inverse, indicating independant movement of water and solute. The extent of the initial plasma volume contraction (and probably therefore ECF), which appeared to be related to the amount of feed consumed when it was first offered (Table 14), influenced plasma volume for the rest of the feeding period: the greater the initial drop, the lower the volume at 1 p.m. In the immediate post-feeding period the increase in plasma volume above the prefeeding value became dissociated to a large extent from the early feeding shifts of water. The plasma volume increased steadily over the 6 hours of observation after feed was offered. Over the first 3 hours of this re-expansion, the gain of solute exceeded water gain in the ECF, while in the next 3 hours the concentration began falling, i.e. water gain exceeded solute.

The plasma volume estimations used here depend upon the validity of two basic assumptions: the total quantity of circulating marker (red cells or plasma protein) remains constant, and the blood sample is a representative one. A factor which could have effected the estimations is splenic contraction; this can be produced by disturbance of the animals and would increase the total erythrocyte mass (Turner and Hodgetts, 1959). Every effort was made to avoid this. It might be considered that the increased PCV and $[Hb]$ 15 minutes after feed was offered could be attributed to this phenomenon and not to decreased plasma volume. This would not appear to be the case since the $[$plasma protein$]$ also increased, and since the minimum plasma

volume using the two markers was correlated (Fig 50). Leakage of plasma protein into the interstitial fluid or change in the total amount could not be controlled; movement of plasma protein between the plasma and interstitial fluid probably explains the smaller increases and decreases in $[$plasma protein$]$ than in PCV and $[Hb]$. Evan's Blue dilution confirmed the expansion of the plasma volume after feeding. It was attempted to collect representative blood samples by the use of long jugular vein cannulae reaching almost to the heart.

Over the first 15 minutes of feeding there was usually an increase in erythrocyte volume ($^{PCV}/_{[Hb]}$). This appeared to be secondary to solute movement, principally Na^+; when the ($Na^+ + K^+$) content of the red cells did not increase, the increase in volume was small or absent. After this initial increase in volume, the erythrocytes showed osmometric behaviour in shrinking for some hours as the plasma O.P. rose. However, as pointed out earlier, other cells may or may not show the same electrolyte and water shifts.

The reason for the movement of Na^+ into the erythrocytes in the first 15 minutes of feeding was not examined, but it is possibly hormonal in origin. Should this shift also involve other cells, particularly muscle tissue, a substantial part of the Na^+ lost by the ECF in the early period may be gained by the ICF and not enter the gut in digestive secretions. Over the remainder of the 8 hours studied, in general the erythrocytes lost Na^+ and shrank. This may be similar to the net Na^+ movement with water movement from isolated retina as the external osmolality increased (Ames et al., 1965), but on the other hand it may be a diurnal trend since similar shifts occurred in sheep fed ad libitum (as described in the next chapter; Fig 86-88).

The mechanism of the urinary Na^+ retention during feeding has not been satisfactorily established. Stacy and Brook (1964) felt that it was not due to adrenal steroid secretion, hyperproteinaemia nor reduced GFR. The rapid onset of the reduced Na^+ excretion would certainly seem to eliminate the participation of adrenal steroids in view of the 30–60 minute time lag observed in the experiments on dogs of Barger et al. (1957). Hyperproteinaemia could affect Na^+ excretion through the filtration fraction or through the tubular reabsorption, however, the effect of an increase in [plasma protein] cannot be distinguished in these experiments from the simultaneous effect of reduced plasma volume. Its effect on the proximal tubule water and Na^+ reabsorption has not been positively established, since reversal of the normal plasma protein gradient alters reabsorption little (Whittembury et al., 1959; Giebisch et al., 1964). The reduced plasma volume could have two actions: via reduced GFR, or increased fractional reabsorption of Na^+. As pointed out in Chapter 1, a decrease of 1% in GFR could produce a significant effect on Na^+ excretion (Pitts, 1963); reduced GFR was neither demonstrated nor excluded by the experiments of Stacy and Brook (1964). Variation in tubular reabsorption can be produced through the activity of "volume receptors" believed stimulated by saline loading (Cortney et al., 1965; Dirks et al., 1965) and in the opposite direction by haemorrhage and quiet standing (Epstein, 1956; Carpenter, Davis, Holman, Ayers and Bahn, 1961). The rapid shrinkage of the plasma volume at the onset of feeding may provide a similar stimulus to that of a mild haemorrhage, resulting in antidiuresis and Na^+ retention.

The Na^+ excretory rate before feeding had a marked effect on Na^+ excretion later in the day. Animals with a high prefeeding rate (over 0.1

m-equiv/30 min) almost invariably showed decreased excretion during feeding, whereas sheep in the "low" group did so only on one-third of the occasions studied, and, in addition, the peaks of Na^+ excretion seen after feeding were smaller in magnitude in this group. It should be pointed out that the figure of 60% for the average depression of Na^+ excretion during feeding (see Fig 23) is heavily weighted towards the high excretory group. The sheep of Stacy and Brook (1964) appeared to be excreting more Na^+ than the sheep used here, nevertheless, in their animals also there was a wide variation in the prefeeding urine Na^+ excretion, so that the fall in Na^+ excretion was marked in some animals, but less in others.

In contrast to the latter situation, the prefeeding urine K^+ excretion rate, which was always high, had no prominent effect on subsequent changes. After feeding, excretion rose to a peak in the immediate post-prandial period, then declined steadily to the next feed, appearing to reflect the absorption of dietary K^+. However, during the early part of feeding, the K^+ excretion decreased by an average of 60%. The most obvious cause of this K^+ retention would be competition with H^+ for secretion in the face of reduced Na^+ excretion. This was accepted by Stacy and Brook (1964). However, three observations in the present experiments would indicate that this is not the sole explanation. First, the lowest K^+ excretion preceded the lowest urine pH. Secondly, the plasma $[K^+]$ did not increase during feeding; if the rate of K^+ secretion were depressed, not in accordance with the body K^+ status, but by increased acid excretion, this would be expected, particularly as the plasma volume decreased. Thirdly, plasma $[K^+]$ fell when K^+ excretion was maintained close to the prefeeding rate by acetazolamide, probably because of the failure to conserve K^+ during feeding, rather than

from a shift of K^+ into cells. In the slight plasma acidosis, net K^+ movement would more likely be from the cells into the ECF (Abrams et al., 1951; Darrow et al., 1953; Swan and Pitts, 1955). This is further supported by the observation that the plasma $[K^+]$ fell only an insignificant amount on a day when, although acetazolamide had been administered, K^+ excretion fell during feeding (Fig 26b). It would thus appear that the K^+ retention may be primary, not secondary.

In general, total solute excretion paralleled that of K^+ except during feeding. About 30-40% of the total urine solutes were not accounted for by Na^+, K^+, HCO_3^-, Cl^- and urea, but this fraction increased during feeding when total solutes did not decrease as much as predicted from the changes in individual solutes. The identity of the remaining solutes is not known, but they are unlikely to be strong electrolytes, since phosphate is low in the urine of sheep (Tomas, Moir and Somers, 1967), as are Ca^{++} and Mg^{++}, both of which show a transient increase during the urinary acidification (Stacy, 1967). This unestimated fraction does not remain constant under the different experimental conditions: when no water was available after feeding, it increased to 60%; and after acetazolamide it was less than 30-40%.

A prominent feature of the response to feeding was the marked fall in urine pH from the alkaline prefeeding state. On a few days, reduced HCO_3^- excretion was not accompanied by an acid pH, suggesting urinary pH alone is not an adequate index of acid excretion in all cases. 1-1½ hours after the start of feeding, HCO_3^- excretion was only about 10% of the prefeeding rate and the pH was close to 5.0. This urinary acidification during feeding appeared adequate to prevent large changes in blood acid-base balance; only a small drop in pH - arterial and venous - and blood total CO_2 occurred

late in feeding. Whereas the absence of drinking water appeared to have only a small quantitative effect on acid-base balance, acetazolamide had a marked effect on both urine and blood composition. Acidification of the urine was prevented, and HCO_3^- excretion fell by 50%, not 90%, during feeding. As a result of the suppressed acid excretion, in both carotid arterial and jugular blood a greater and more prolonged fall in blood total CO_2 occurred, although the change in blood pH was no greater because of the buffer activity. Thus acetazolamide administration proved useful in elucidating the mechanism of raised acid excretion.

Acidification and almost complete HCO_3^- reabsorption could be brought about either by increased H^+ secretion or reduced filtered load of HCO_3^-, or possibly both. Increased H^+ secretion is very likely involved, since the acidification was prevented by acetazolamide administration. There may also be reduced filtered HCO_3^- since blood total CO_2 (plasma $[HCO_3^-]$) was often reduced late in feeding, and GFR may decrease early in feeding.

Observations on urine and plasma Cl^- changes in general showed a degree of variability expected from its role in balancing changes in total cations, and shifts with pH. During 1-1½ hours of feeding, on individual days Cl^- excretion increased or decreased, but overall was unchanged. In contrast to this diversity, a consistent pattern was seen in two situations. During the prefeeding diuresis, although HCO_3^- excretion was unchanged, there was a 20% decrease in Cl^- excretion on average, while at the same time plasma $[Cl^-]$ fell in most cases 3-5 m-equiv/l. The basis of these changes in Cl^- is not clear. The second consistent pattern was the marked depression of Cl^- excretion after acetazolamide, which may reflect a greater loss of HCO_3^- than of the cations Na^+ and K^+.

Since H^+ excretion is believed to be influenced by both K^+ and Na^+ excretion (Pitts and Alexander, 1945; Berliner et al., 1951 and others), $\Delta[H^+]$, ΔNa^+, ΔK^+ over the first 2 hours of feeding were related for the different experimental groups, using a multiple regression calculation and elimination of variables (Table 10, Fig 28). The estimates of the overall change in excretion of Na^+ and of K^+ as made here would be expected to be reasonably accurate. However, the change in H^+ secretion is difficult to calculate. Secreted H^+ can have three fates: it may react with HCO_3^- which is later reabsorbed as CO_2 and water; it may be excreted as titratable acidity; or, it may be excreted with ammonia as NH_4^+. Thus, the best measurement of H^+ secretion would be the sum of HCO_3^- reabsorbed, ammonia excreted and titratable acidity. However, in practice, measurement of the latter poses some problems, especially in the selection of the end-point at blood pH of 7.4 since the urine pH varies from 5.0 to 8.5. The pH of the sample would be expected to reflect the acid content, but how precisely is uncertain. The results of Pitts et al. (1948) suggest that $[H^+]$ (or pH) might be correlated with H^+ secretion, since they found little titratable acidity or ammonia was excreted at high urine pH, and nearly all HCO_3^- was reabsorbed before urine pH began falling. It is possible that in the present calculations the use of $\Delta[H^+]$ rather than Δsecreted H^+ resulted in greater variation in the estimation, and hence failed to reveal significant relationships with ΔNa^+ and ΔK^+.

Where significant relationships between the pairs of ions were seen, they were consistent: ΔNa^+ and ΔK^+ were positively related, and $\Delta[H^+]$ was negatively related to both ΔNa^+ and to ΔK^+. In any particular case, ΔNa^+ was related to either ΔK^+ or $\Delta[H^+]$, not to both. Further, when

drinking water was provided, ΔNa^+ and $\Delta[H^+]$ were correlated, but when no water was available, the correlation was between ΔNa^+ and ΔK^+. The failure to find a significant relationship in such small groups may not indicate that the treatment has disrupted such a relationship, but may be caused by the small numbers.

The calculated relationships in overall excretion may reflect purely renal interactions, and so would be likely to be indicative of similar correlations in the distal tubule, since this latter segment is involved in regulation of the composition of final urine. Application of these observations to the distal tubule, therefore, depends on the validity of final urine being a reflection of events in that segment. On the other hand, the correlations of change of overall excretion of the three ions may stem from the association of net changes in the ECF content of these ions as a result of movements into the gut, and not primarily be associated with renal events.

The negative correlation of $\Delta[H^+]$ and ΔK^+ is not unexpected, since the transport of these two ions is inversely related across cell membranes in general, and those of the distal tubule in particular. If the correlation is of renal origin, it could be the result of either competition for a carrier system, or a negative correlation of renal cell $[H^+]$ and $[K^+]$ where the excretion of each ion is dependent on its concentration in renal cells. In contrast, the correlation of ΔNa^+ with ΔK^+ and $\Delta[H^+]$ appears to be of extrarenal origin rather than equally likely resulting from a renal or extrarenal interaction. If the correlation were of distal tubule origin, then, according to both the ion exchange and electrical coupling hypotheses, ΔNa^+ should be negatively related to both $\Delta[H^+]$ and ΔK^+ if Na^+ excretion is regulated by distal tubule reabsorption, and positively related to both if

the amount of Na^+ reaching the distal tubule is altered more proximally, such as by GFR. The negative correlation of ΔNa^+ with one and positive with the other probably reflects their association in digestive secretions.

Changes in urinary excretion and blood composition associated with feeding would reflect redistribution of water and electrolytes between ECF and ICF on the one hand, and ECF and gut contents on the other. While, as pointed out above, the extent of the first of these, that between ECF and ICF cannot at present be gauged, the second, that between the ECF and the gut, is a well recognised occurrence of major dimensions. Feeding for only a limited time of the day would be expected to accentuate these shifts. After a 24 hour fast, the net effect of feeding on the rate of digestive secretions, and on passage through the digestive tract, exposing the contents to regions of differing absorptive properties and rates of transfer directly across the gut wall, would be seen firstly in a predominantly secretory phase, and later in an absorptive phase. Individual aspects of this whole process have been examined by different groups of workers, however, all parameters have not been measured in the one experiment, so that the relative importance of one to another has not been examined directly.

While the observed changes in blood and urine composition will be the sum of the transfers between the gut and ECF, it would appear that of all the glandular secretions there is a dominating influence of the increased salivary secretion. This would seem to be so because, not only is the time sequence appropriate, but also the volume and composition are such that losses in saliva apparently outweigh losses in the other glandular secretions - gastric juice, pancreatic juice or bile. In contrast to the situation in the monogastric, from the ECF acidosis during feeding, gastric secretion would

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appear to be of less significance than is salivary secretion. However, gastric secretion does increase during a short daily period of feeding, the rise becoming evident within 15 minutes of starting to eat, and reaching a maximum 1-1½ hours later (Hill, 1960; McLeay, 1967). It would seem unlikely that bile and pancreatic juice would contribute significantly to the changes seen since both are secreted continuously in the sheep fed once-daily, and although the composition alters during feeding, little change in volume occurs (Taylor, 1962; Harrison, 1962).

Feeding evokes profuse secretion of saliva, particularly from the parotid and submaxillary glands. Parotid and mixed saliva are similar in composition, having $[Na^+]$ of 160-180 m-equiv/l, $[K^+]$ of 7-10 m-equiv/l, and $[HCO_3^-]$ of 100-140 m-equiv/l (Denton, 1957; Somers, 1957; Kay, 1960; Bailey and Balch, 1961a,b). During rapid secretion, the concentrations of Na^+ and HCO_3^- rise, and those of $PO_4^{=}$, K^+ and Cl^- fall. With a limited feed intake, the rate of salivation is high in the first hour and declines by half over the second hour (Wilson, 1963), and is low immediately after the feeding period (Bailey and Balch, 1961b; Wilson, 1963). The high rate of salivation during feeding would result in Na^+, HCO_3^-, K^+ and water loss from the ECF, and would explain the ECF acidosis, decrease in volume and increase in tonicity, and in the urinary retention of water, Na^+, HCO_3^- and K^+, and acidification.

The second form of transfer across the gut wall, that directly across the epithelium, is of unknown magnitude. The amount of direct water and electrolyte transfer would depend not only on the gradients established, but also on the degree of permeability of the mucosa. Since this varies in different regions, the rate of passage of contents down the gut will affect

the overall net transfer as the contents will be exposed to areas of differing absorptive capacity. The increased outflow from the reticulo-rumen (Balch, 1958) and abomasum (Phillipson, 1952) during feeding would increase the amount of digesta presented to the omasum and small intestine for absorption. This effect is lessened in more distal areas of the gut, and, according to Goodall and Kay (1965a), the feeding rhythm has been lost by the time the contents have reached the large intestine.

The reticulo-rumen contents represent a large proportion of the total gut contents. Transfers between the reticulo-rumen and the ECF are potentially important, but the magnitude is at present unknown under physiological conditions. It is known that Na^+ is actively taken up from rumen contents, and that in certain experimental situations K^+, HCO_3^-, Cl^- and water distribute themselves according to the existing gradients. The changes in water and electrolytes in rumen liquor have been followed in sheep and cattle under restricted feeding conditions. During feeding, $[Na^+]$ falls and $[K^+]$ rises in rumen liquor (Reid, 1965; Warner and Stacy, 1965; Ternouth, 1967), although the total amount of both increases (Reid, 1965). In the early post-feeding period, $[K^+]$ begins to fall before $[Na^+]$ rises (Warner and Stacy, 1965; Ternouth, 1967). Stacy and Warner (1966) inferred the osmotic stimulation of Na^+ absorption after feeding from their calculations, while Scott (1967) demonstrated increased Na^+ uptake from the rumen resulting from raised rumen $[K^+]$ more directly. An osmotic gradient is created at the onset of feeding as the rumen contents become hypertonic (Reid, 1965; Warner and Stacy, 1965; Ternouth, 1967), which may draw water into the rumen. Ternouth (1967) found that the rumen volume increased 2 hours after feeding commenced by 5-6 litres in sheep. A proportionately

similar increase was seen in a cow (Reid, 1965). A second peak of rumen volume was observed 5-6 hours after feeding by Ternouth (1967), but may be an artefact (Warner and Stacy, 1968a).

Water gained by the rumen, other than that ingested, enters in the saliva, and across the rumen wall to an unknown extent. Warner and Stacy (1965) and Ternouth (1967) have attempted to partition ECF fluid transfer during restricted feeding into transruminal flow and salivary flow. Ternouth (1967) concluded that osmotic water movement during feeding was significant, while Warner and Stacy (1965) concluded it was not. However, in both cases the assumptions used in making their calculations were not entirely justified.

The present experiments suggest that transruminal flow may not be large from a comparison of the estimated ECF fluid loss and predicted salivary loss. The relative importance of the two fluid contributions may vary from day to day even with the same experimental conditions. In Fig 61, Δ plasma O.P. was linearly related to the minimum plasma volume. For small decreases in volume the plasma O.P. increased; for large decreases in volume the O.P. fell. Such a relationship would require the simultaneous loss in varying proportions of at least one hypertonic and one hypotonic fluid; isotonic transfers would have no effect. These three would correspond respectively with saliva, fluid entering the rumen across the wall, and fluid plus solute entering the red cells (and perhaps other cells). The essential parameter is not the absolute volume, but the amount of solute involved in the deviation from isotonicity. At slower feeding rates, when the loss from the ECF was smaller, the hypotonic loss made a relatively smaller contribution than the hypertonic loss. This would suggest that water entry across the rumen wall was of greater importance at lower rates of feeding

than at higher ones.

Experiments such as these which attempt to evaluate water and electrolyte shifts between body fluid compartments by examination of changes in the plasma, urine and erythrocytes are limited by their indirect nature. In particular, events in the gut are detected only by their systemic effects. However, there appears to be still a place for indirect experiments because of the difficulty in following directly net movements between the ECF and rumen contents under physiological conditions. The pitfalls can be seen from such experiments of Stacy and Warner (1966) and Ternouth (1967) where the assumptions used nullify the conclusions reached. The major defect in indirect studies is the unknown movements across the cell membrane, since the ICF makes up over half of the total body water.

The effects of feeding have been exaggerated in these experiments by feeding for only a short period each day. Possible confounding influences are diurnal and environmental factors, which, if prominent, would either reinforce or reduce the feeding effects. An attempt has been made to gain some indication of the importance of diurnal factors in a short series of preliminary experiments in which sheep had continuous access to feed, described in Chapter 3.

CHAPTER 3

CHANGES IN URINE AND BLOOD COMPOSITION DURING *AD LIBITUM* FEEDING

In the previous chapter, profound changes in urine excretion and in blood composition were shown to occur in sheep fed for one 2-3 hour period a day. This feeding pattern is not the usual one; it must be imposed on the sheep by training. A single, short daily feed would be expected to accentuate the changes associated with feeding, compressing them into a short period, and probably increasing their magnitude. By contrast, the changes would be expected to be less marked the shorter the intervals between feeds and the more constant the environment. This is confirmed by the finding of Minson and Couper (1966), that variation in faecal dry matter, urine volume, S.G. and nitrogen excretion was almost eliminated when sheep kept in a constant environment were fed $^{1}/_{24}$ of their daily ration every hour, each meal being entirely consumed before the next was given. An intermediate situation between these extremes is likely to exist in the animal with continuous *ad libitum* access to feed.

Urine excretion and blood composition are also affected by Circadian rhythms and environmental stimuli as well as by feeding. Partitioning of the several factors is not easy. Lindan, Baker, Greenway, King, Piazza and Reswick (1965) evaluated the contributions of the Circadian rhythm, of eating and of environmental stimuli (alteration of body position) to the daily excretory pattern in the quadriplegic human. Their study involved long term observations of the excretory pattern under random feeding and turning schedules to determine the Circadian rhythm, and eating at regular 19 hour

intervals, as well as "nibbling" and "gobbling", to determine the effect of a meal. Such a time-consuming investigation in sheep was not feasible here. Instead, a short series of experiments was carried out in animals given continuous ad libitum access to feed to establish the pattern of changes occurring under these feeding conditions. Some evidence was obtained for the existence of diurnal changes as well as feeding components.

MATERIALS AND METHODS

Experimental design

Urine and blood was examined in 4 sheep, 3 Romney ewes and 1 Romney-Cheviot cross ewe, during ad libitum feeding. Observations were made on 6 days. The sheep had been accustomed to this feeding regime and had not at any time been trained for the restricted feeding regime used in Chapter 2.

Other than the different feeding regime, the sheep were housed, handled and sampled in an identical manner to those in the once-daily feeding experiments. The occurrence of feeding and rumination was determined from records of jaw movements (see below).

Feed and feeding

The lucerne fed in these experiments was from a different batch, and was offered in the form of hay as it was unsuitable for chaffing. On non-experimental days, a quantity of hay in excess of their daily intake was offered to the sheep at 10 a.m. On the 6 experimental days, at 6 hourly intervals extra feed was added to that given at 10 a.m. to ensure palatable feed was always available. Water was freely available at all times. A

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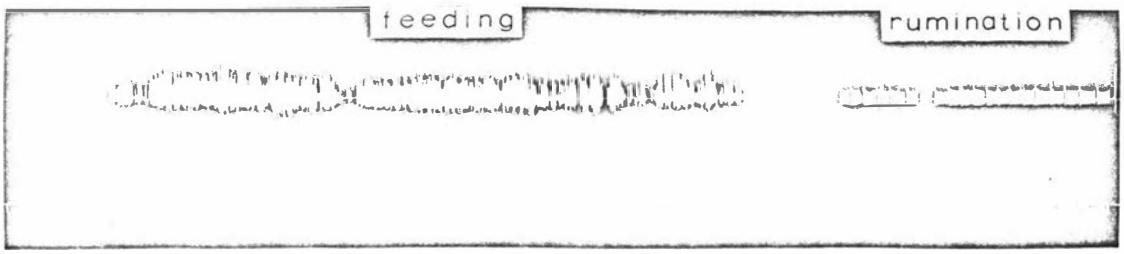

Fig 80. Kymograph tracing of jaw movements showing the characteristic patterns of feeding and rumination. A one-minute time marker tracing is below.

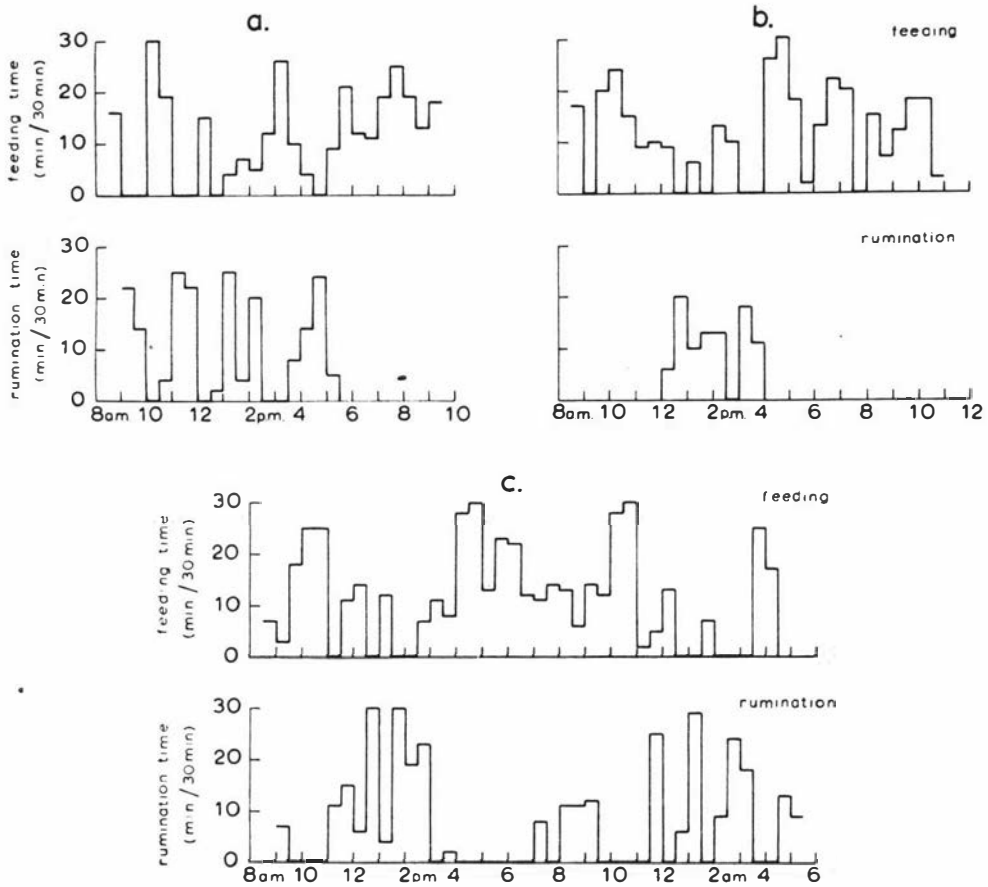

Fig 81. Feeding and ruminating activity in 3 sheep with ad libitum access to feed and water. (a - sheep 12, 6.2.67; b - sheep 11, 29.11.66; c - sheep 5, 25.10.66).

supplement of 5 gm of NaCl was given with the feed at 10 a.m. on all non-experimental days and on 3 of the 6 experimental days.

Sampling and methods

Sampling and analytical procedures were identical with those in the previous feeding experiments, except PCV estimations. Urine was collected in 30 minute samples (60 minute samples on one day), and blood each hour. All blood samples were centrifuged anaerobically.

PCV estimation: Blood was drawn up into capillary tubes which were heat sealed at one end, then centrifuged for 10 minutes in an International Microcapillary centrifuge, Model M6. The PCV was determined with the aid of an International circular capillary reader.

Jaw movements: The apparatus used to record jaw movements consisted of a balloon held under the sheep's lower jaw by a head halter and connected by tubing to a tambour and writing lever. Feeding and rumination were identified from the characteristic patterns traced out on a smoked paper on a kymograph (Fig 80). The time spent ruminating, feeding and resting each half hour was measured.

RESULTS

Pattern of feeding and rumination

The feeding and ruminating time for each half hour on 3 days is shown in Fig 81. The pattern of activity was not regular. Of the 4 sheep, the behaviour of sheep 11 appeared different in having only one period of rumination for 2-4 hours between noon and 4 p.m.; the other 3 alternated

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Table 18. Average percentage of time spent feeding, ruminating and resting on 6 days under ad libitum feeding conditions.

Sheep	Duration of observation (hours)	Feeding (%)	Ruminating (%)	Resting (%)
5	21	37.0	25.7	37.3
5	15	44.3	27.0	28.7
11	15	37.3	10.0	52.3
11	15	37.3	4.7	58.0
12	13	37.7	24.3	38.0
13	13	41.3	24.0	35.0

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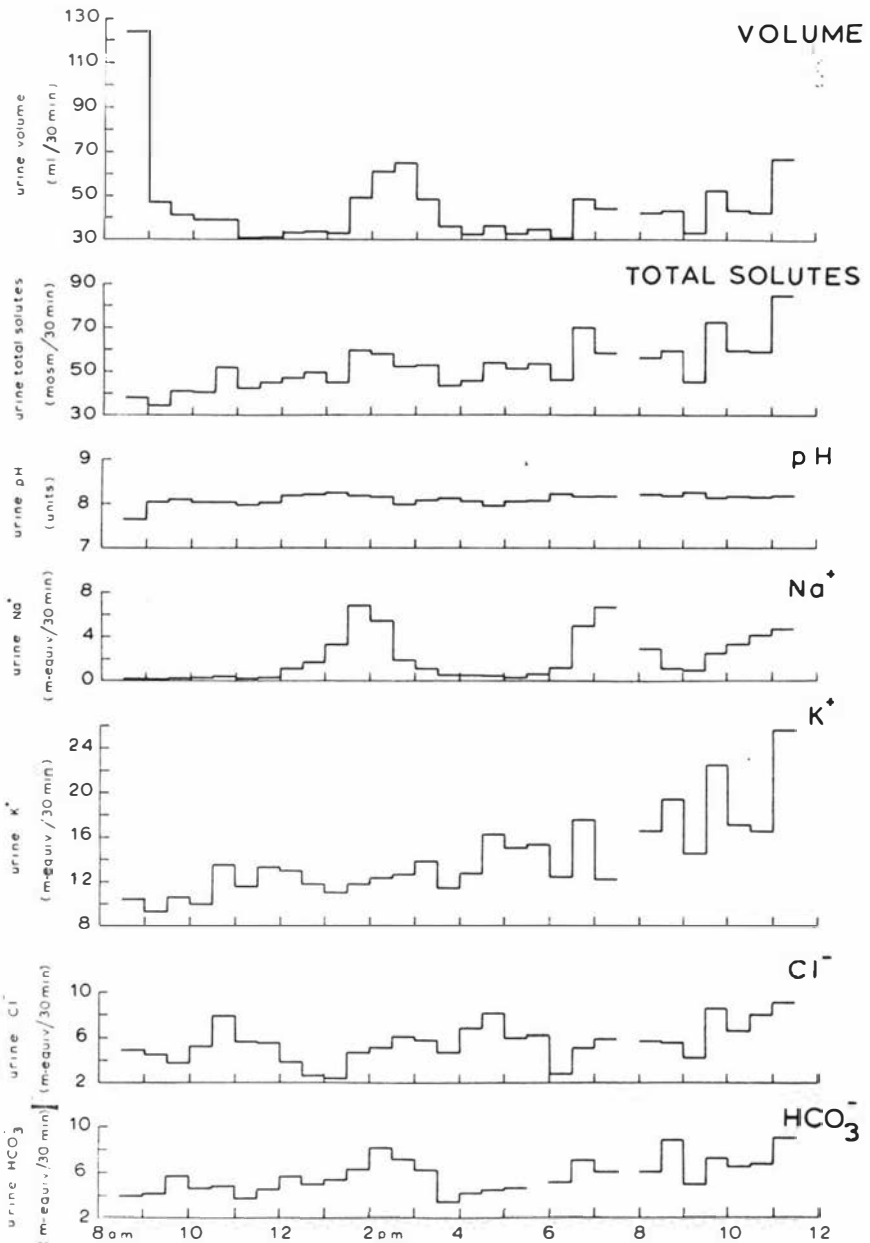

Fig 82. Urine volume, total solute, pH, Na^+, K^+, Cl^- and HCO_3^- excretion in a sheep with *ad libitum* access to feed and water. (Sheep 11, 8.11.66).

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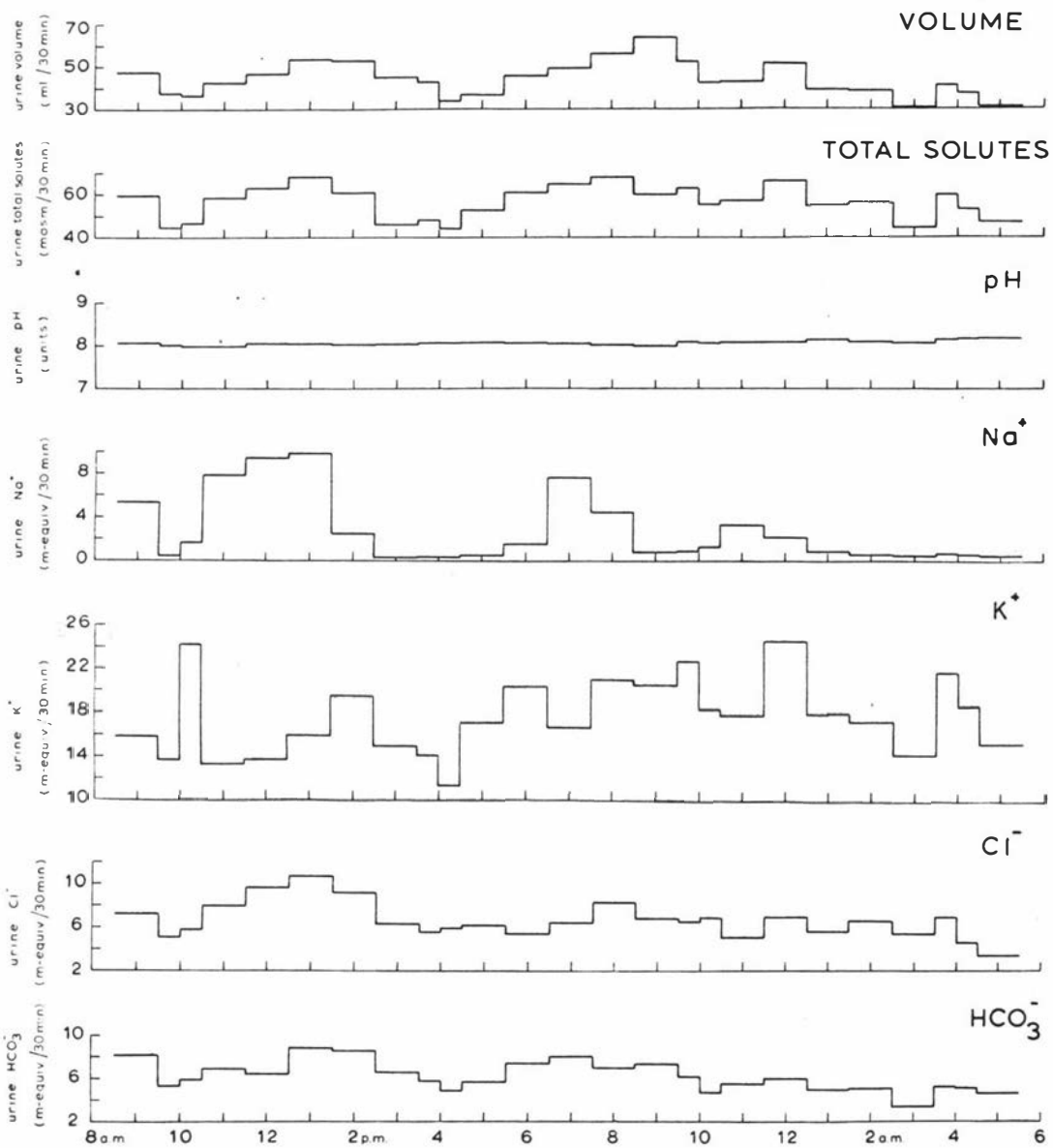

Fig 83. Urine volume, total solute, pH, Na^+, K^+, Cl^- and HCO_3^- excretion in a sheep with *ad libitum* access to feed and water. (Sheep 5, 25.10.66).

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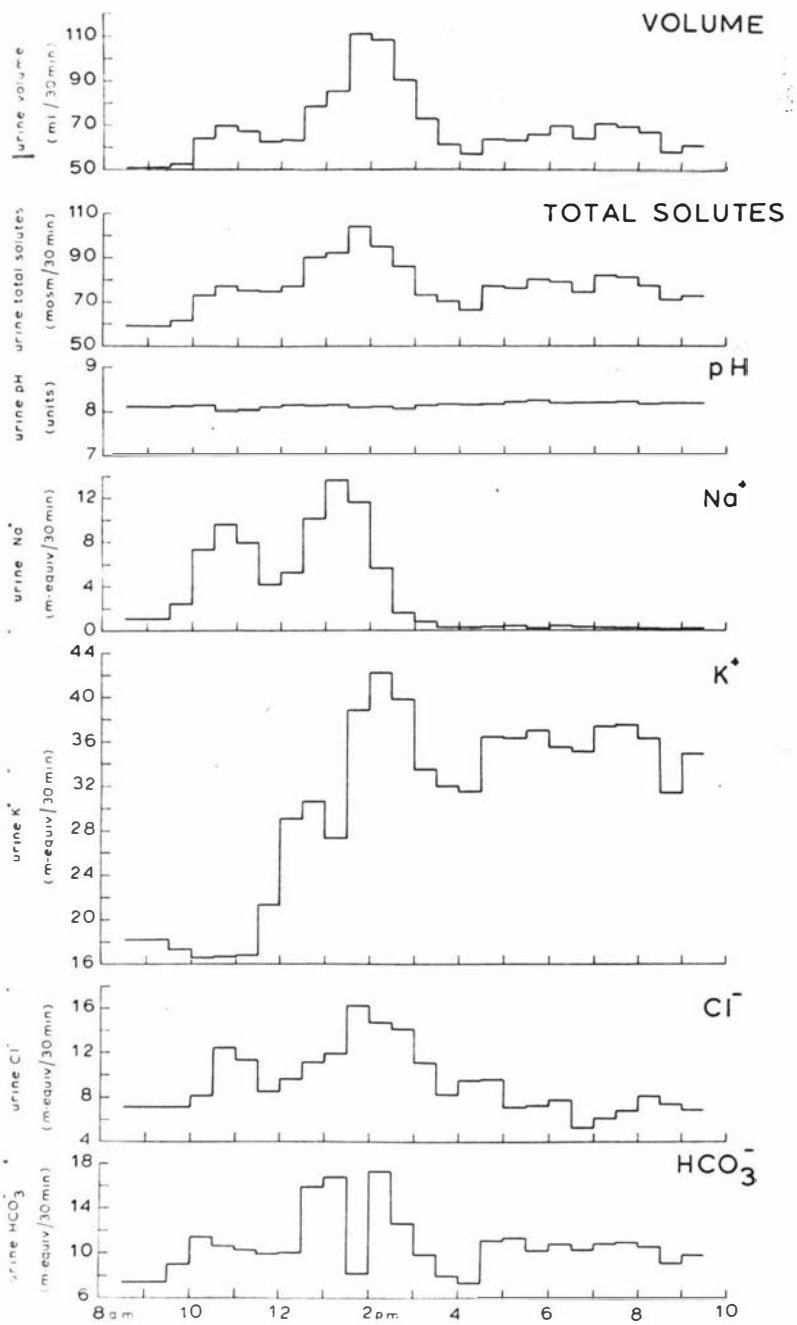

Fig 84. Urine volume, total solute, pH, Na^+, K^+, Cl^- and HCO_3^- excretion in a sheep with *ad libitum* access to feed and water. (Sheep 13, 8.2.67).

between feeding and ruminating through the period of observation. The proportion of time spent feeding, ruminating and resting by the 4 sheep on the 6 days is shown in Table 18. It will be seen that sheep 11 spent rather less time ruminating during the experimental period than did the other three.

The addition of fresh feed to that in the tin usually, but not invariably, was followed by a period of increased intake or resumption of feeding. The total intake in these animals was 1.5 - 2.0 times that in the once-daily fed sheep.

Urine excretion

(i) Constancy of excretion. Urine excretion was not constant from hour to hour, and except for pH, showed quite large fluctuations (Fig 82-84). At no time was the urine pH below 7.5.

(ii) Diurnal variation. Patterns suggestive of a diurnal rhythm were observed. These were:

(a) a decrease in pH around 10 a.m. (5 out of 6 days); an increase over the following 12 hours (all days) (Fig 82-84);

(b) an increase in K^+ excretion from noon to midnight (5 days); on 3 of these it was a steady increase (Fig 82), on 2 the increase was rapid around noon, but slower thereafter (Fig 84);

(c) the occurrence of a large peak of Na^+ excretion around 1 p.m. (all days); earlier and later peaks were also seen;

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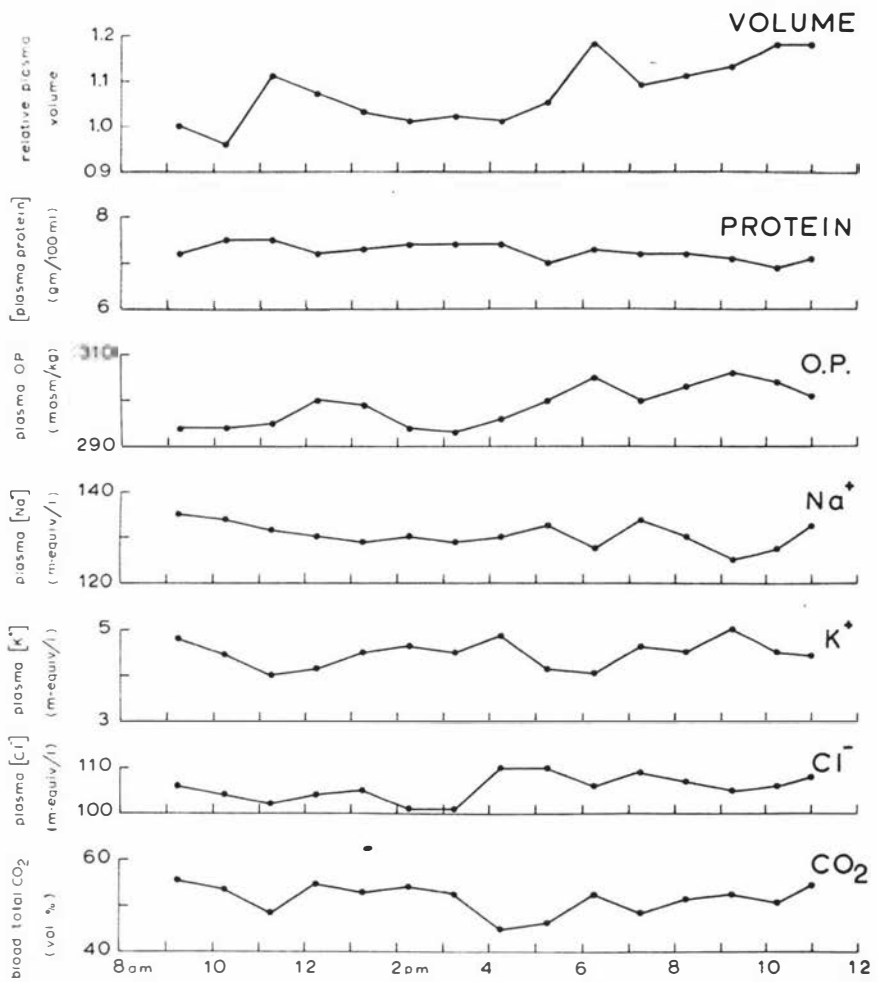

Fig 85. Relative plasma volume, [plasma protein], O.P., [Na^+], [K^+] and [Cl^-] and blood total CO_2 content in a sheep with ad libitum access to feed and water. (Sheep 11, 8.11.66).

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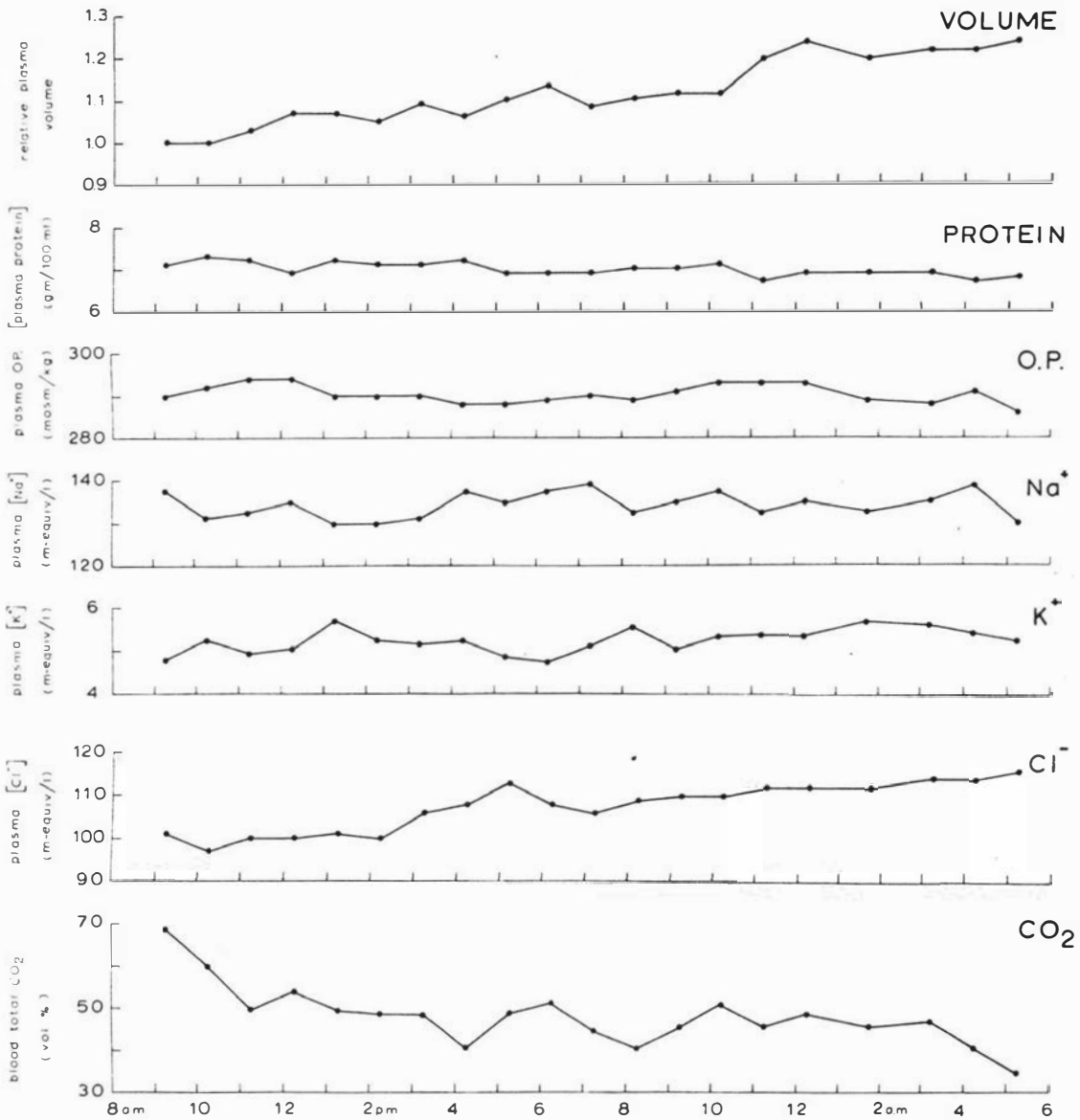

Fig 86. Relative plasma volume, [plasma protein], plasma O.P., [Na^+], [K^+] and [Cl^-] and blood total CO_2 content in a sheep with ad libitum access to feed and water. (Sheep 5, 25.10.66).

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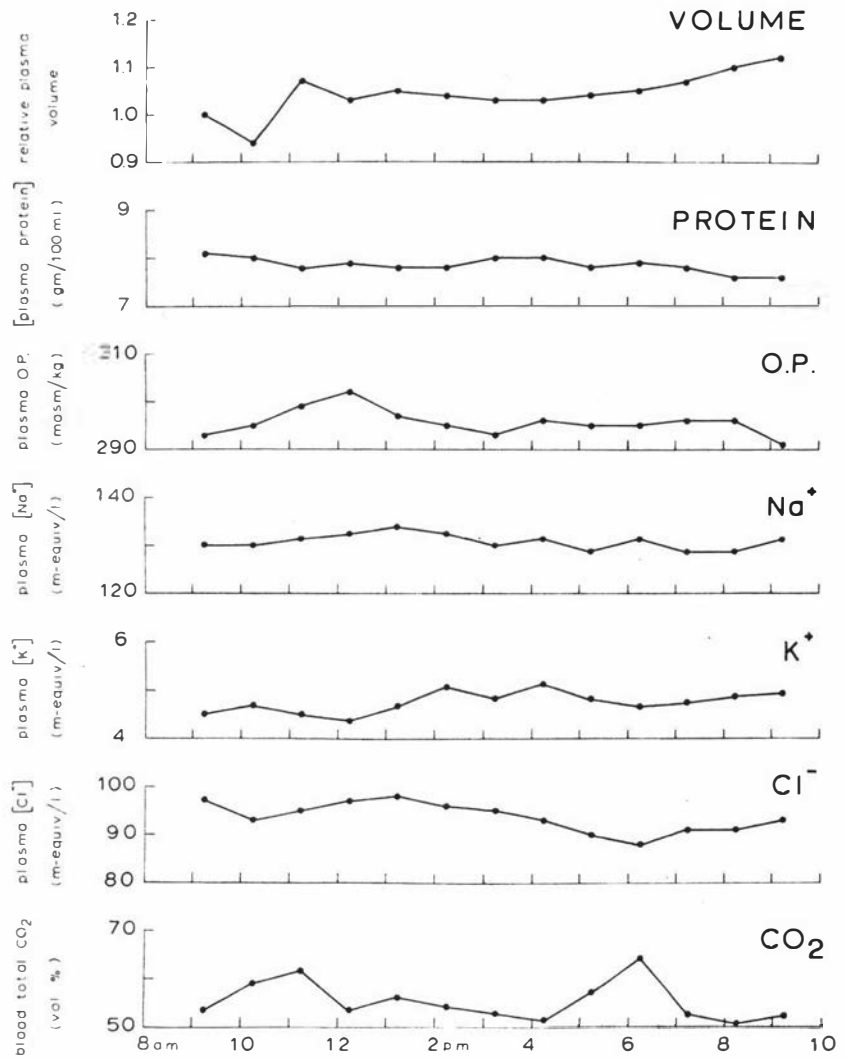

Fig 87. Relative plasma volume, [plasma protein], plasma O.P., [Na^+], [K^+] and [Cl^-] and blood total CO_2 content in a sheep with ad libitum access to feed and water. (Sheep 13, 8.2.67).

(d) the occurrence of a peak of Cl^- excretion at 1.30 p.m. (5 days).

(iii) Effect of feeding. There appeared to be a 6 hour cycle in the excretory pattern of urine volume, total solutes, HCO_3^- and to a lesser extent K^+, the lower excretion rates in most cases occurring at the onset of a feeding period (Fig 82-84). The individual excretory patterns for urine volume, total solutes and HCO_3^- were commonly similar.

There was no apparent correlation between the occurrence of peaks of Na^+ excretion and whether or not NaCl was administered on the experimental day.

Plasma electrolytes

Compared with sheep on the 3 hour feeding schedule, in sheep fed ad libitum plasma O.P., $[Na^+]$ and $[K^+]$ were higher at 9-10 a.m. by approximately 10 mosm/kg, 5 m-equiv/l and 0.5 m-equiv/l respectively.

The plasma volume, O.P., electrolytes and blood total CO_2 during 3 of the 6 days are shown in Fig 85-87. The magnitude of the fluctuations in electrolyte concentrations was in general within the limits of the normal range seen in most mammals (Spector, 1956). A 6 hour cyclic pattern was apparent on many days for most of the electrolytes.

Erythrocyte electrolytes

Red cell changes were suggestive of a diurnal variation (Fig 88-90). The general trend was for Na^+ and K^+ content to decrease from 9 a.m. to a minimum at 6-8 p.m., then to increase again. Superimposed on this, transient

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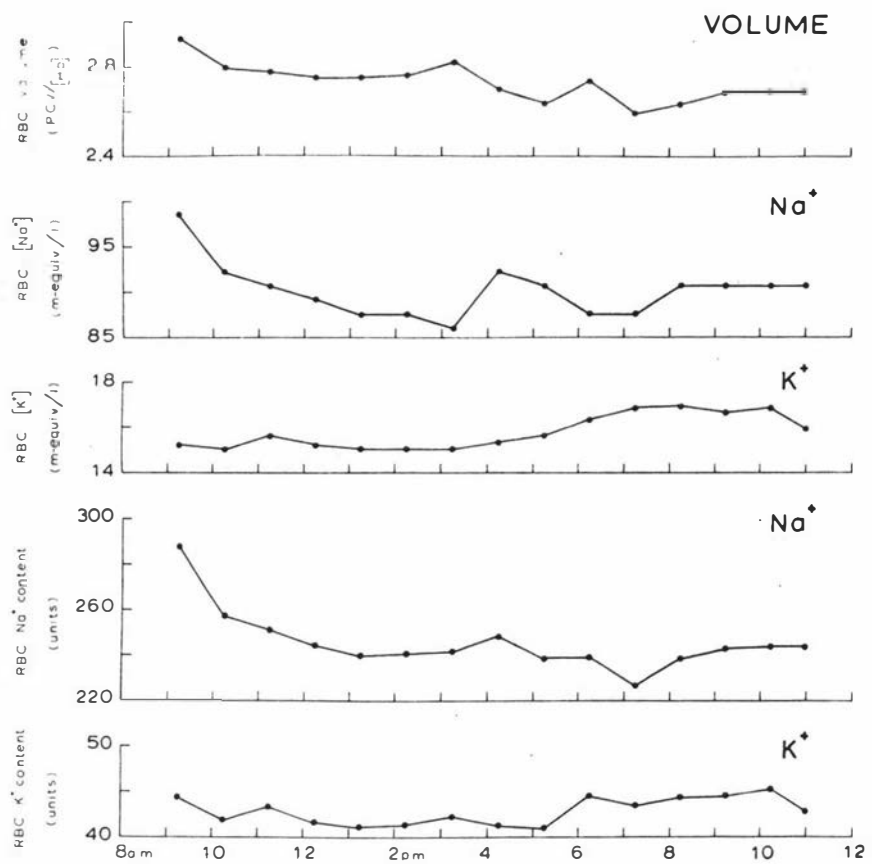

Fig 88. Erythrocyte volume, $[Na^+]$, $[K^+]$, Na^+ content and K^+ content in a sheep with ad libitum access to feed and water. (Sheep 11, 8.11.66).

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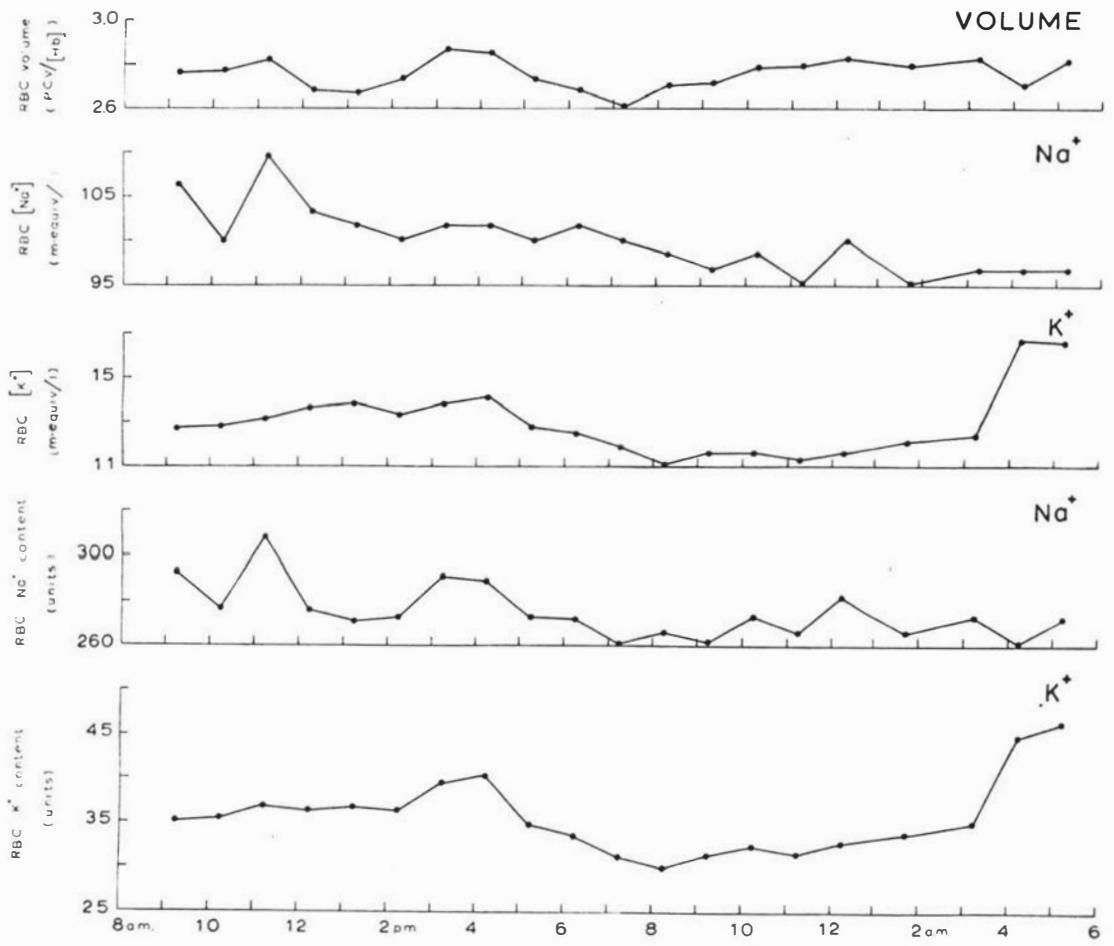

Fig 89. Erythrocyte volume, $[Na^+]$, $[K^+]$, Na^+ content and K^+ content in a sheep with ad libitum access to feed and water. (Sheep 5, 25.10.66).

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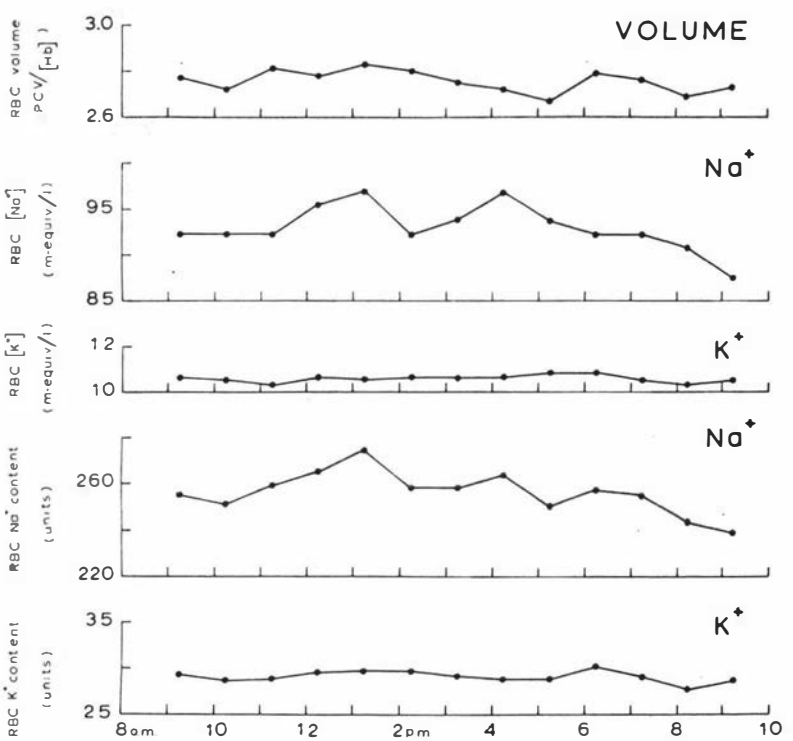

Fig 90. Erythrocyte volume, $\lceil Na^+ \rfloor$, $\lceil K^+ \rfloor$, Na^+ content and K^+ content in a sheep with ad libitum access to feed and water. (Sheep 13, 8.2.67).

peaks occurred at different times of the day. A similar general pattern was seen for erythrocyte volume and $[Na^+]$, and for $[K^+]$ on half of the days.

DISCUSSION

Experiments such as those described here can at best only give a general indication of the existence of contributions of Circadian rhythms, feeding, drinking and environmental stimuli to the excretory patterns and blood composition. Only the grossest relationships will be apparent. Correlations of smaller magnitude would require a large series of detailed experiments followed by auto-correlation and power density spectral analysis as used by Lindan et al. (1965).

The plasma values and excretory rates in sheep receiving one 2-3 hour feed a day were lower immediately before feeding than in the ad libitum fed animals, and frequently higher afterwards. The lower levels were undoubtedly a reflection of the 21-22 hour fast since the previous meal. The higher values after feeding probably reflect a greater rate of intake enforced by this short period; they may also be associated with the diurnal changes seen in the ad libitum feeding experiments.

In these ad libitum fed animals, the marked prandial and post-prandial changes in the once-daily fed animals gave way to cyclic peaks and troughs in which meal components were not conspicuous. This in itself is significant in demonstrating that the changes wrought by once-daily feeding are of a magnitude far greater than produced by the periodic feeding in sheep with constant access to feed.

The plasma and urine patterns in the ad libitum fed sheep were suggestive of a feeding component resulting in decreased excretion of water, Na^+, HCO_3^- and probably K^+, and increased plasma O.P. during periods of intensive feeding. However, the plasma electrolyte peaks were out of phase with the periods of feeding. Diurnal components were suggested in Na^+, Cl^- and K^+ excretion and in urine pH, and in erythrocyte Na^+ and K^+ content. Urinary changes such as these are seen in man.

In quadriplegics, Lindan et al. (1965) identified components in the daily excretory pattern caused by feeding, body position and diurnal effects. Excretion of creatinine was depressed by large meals, volume by even small meals. Urinary Na^+, K^+, Cl^- and nitrogen were highest 10 hours after 19 hourly meals. Less water and Na^+ and more K^+ were excreted when sitting compared with the supine position. Diurnal periodicity was present in Na^+, K^+ and Cl^- excretion, being low at night, rising from 7 a.m., and reaching a maximum at noon. The diurnal rhythm was weaker for Na^+, but stronger for K^+, than the periodicity induced by 19 hourly feeding. No diurnal cycles were seen in urine volume, creatinine or nitrogen excretion.

The experiments described in this and the previous chapter, together with the observations of Minson and Cowper (1966), have indicated the existence of both feeding and diurnal factors in urine excretion and blood composition in sheep. It would appear that in sheep the contribution of feeding to the excretory pattern is greatest with once-daily feeding, present to some extent with ad libitum feeding, but almost eliminated by 24 small meals. Minson and Cowper (1966) have eliminated environmental stimuli in their study by providing constant conditions. Ad libitum feeding has indicated the existence of diurnal changes; it also suggests that, in Chapter 2, because

of the time chosen for the once-daily feeding (10 a.m. to 1 p.m.) the changes in urine excretion with feeding, particularly the post-prandial rise in electrolyte excretion, would probably be reinforced by diurnal changes. Clearly, precise evaluation of the contributions of feeding, environmental and diurnal components to the excretory pattern under different experimental conditions is needed, and must be taken into account when designing experiments on water and electrolyte metabolism and redistributions.

CHAPTER 4

INFUSION INTO THE RUMEN OF WATER AND ELECTROLYTE SOLUTIONS

The changes in renal excretion and plasma composition observed in Chapter 2 in sheep given a single daily feed were interpreted as reflecting a phase of diversion of extracellular water and electrolytes into the rumen, followed by a phase of net absorption. Further information on the interaction of the rumen water and electrolyte content with that of the ECF might be obtained by following the absorption and excretion of solutions infused into the rumen.

In man and non-ruminants, a large number of studies of the effects of oral administration of water, Na^+ and K^+ salts have been made over many years. The responses have been diverse, and would appear to indicate that species differences, the dose rate, physiological status, posture and diurnal factors may influence the effect of the infusion.

Reports of the short-term changes in urine and plasma composition induced by intraruminal water and electrolyte infusions in domestic ruminants are few in number. Sellers and Roepke (1951) compared two-hourly urine samples before and after the administration over 45-50 minutes into the terminal oesophagus of lactating or pregnant cows either 10 gallons of water alone, or containing 0.5 gm of NaCl or KCl per lb body weight. The water alone increased the urine volume, reduced its S.G., and increased the excretion of Na^+, K^+, $PO_4^{\equiv}$ and often of Cl^-. After the administration of either of the NaCl or KCl solutions, the excretion of both Na^+ and K^+ was increased, suggesting that the cation infused displaced the second cation

from the ICF. There was a greater rise in Na^+ excretion than in K^+ in the 2 hours after the KCl infusion, suggesting it was increased earlier, or to a greater extent. The NaCl infusions were less effective in raising the excretion of the two cations. Both electrolyte infusions increased the plasma concentration of Na^+, K^+, Cl^- and inorganic phosphorus; only the increase in $[K^+]$ was greater after the KCl. Interpretation of such experimental results is made difficult by the small number of samples.

Water diureses have been produced in goats (Andersson, 1955) and in sheep (Lysov, 1960) by intraruminal infusion. In the goats, doses of water of 5½ litres and 7-8 litres resulted in a diuresis of similar time course. In both cases there was fairly constant flow for 6 hours of 400 ml/hr after the smaller dose, and 1200 ml/hr (maximum diuresis) after the larger, which returned to the preinfusion volume by 13 hours. Andersson suggested that the constant urine flow resulted from water absorption after passage to the abomasum. The small decrease in plasma $[Na^+]$ and $[Cl^-]$, and in the excretion of Cl^-, and sometimes in K^+, he attributed not to the water infusion, but to the prolonged fast during the experiment. In the sheep, the responses observed were markedly different. Thus, when 2-5 litres were infused, urine flow increased in 10-15 minutes, was maximal around the end of the second hour, then fell gradually; 90% of the dose was recovered in the urine within 5-7 hours.

Since the present experiments were commenced in July, 1966, Dewhurst and Harrison (1966), Keynes and Harrison (1967) and Dewhurst, Harrison and Keynes (1968) have reported results of a series of intraruminal and intravenous infusions in sheep. Their intraruminal infusions, 1 litre of water, 250 mM KCl, 250 mM K acetate, 125 mM NaCl or 125 mM Na acetate,

were made just after the sheep were offered their feed; hence, changes due to the infusion were superimposed upon changes due to feeding, leading to difficulties in interpretation. Urine flow was higher after KCl than after water or NaCl. In 24 hours, 80% of administered K^+ and 25% of administered Na^+ was excreted in the urine. K^+ acetate gave a smaller K^+ response than did KCl, but both solutions caused an earlier rise in K^+ excretion after the feeding depression; Na^+ excretion was also higher, the Na^+ peak preceding the K^+ peak with acetate, and following it with Cl^-. Following Na^+ infusions, there was a slower and less intense increase in the rate of excretion of Na^+, K^+ and Cl^- than after the K^+ infusions.

In the series of experiments described here, the infusions were made 15 hours after a 3 hour feed, at a time when changes in urine and plasma parameters were relatively small and consistent from day to day. Comparisons were made between infusions of 3 litres of water - equal to the largest water consumption during feeding - and the same volume of NaCl or KCl almost isotonic with blood plasma (0.15 M). A further comparison was made between these salt solutions and solutions containing the same amount of solute in one-tenth the volume of water, i.e. having an O.P. ten times that of plasma.

MATERIALS AND METHODS

Experimental design

There were six experimental treatments, involving six sheep. The animals were used for one week, then usually rested for three. Two experiments were carried out on the experimental week, with one day between. The overall number of replicates and infusions for each animal depended upon its continuing availability, and as a result, not all treatments were made

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Table 19. Intraruminal infusions: volume, concentration, sheep used and dates.

Infusion	Volume & concentration	Sheep	Date
Control		1	24. 8.66
		2	1. 8.66
		3	20. 7.66
		12	17. 4.67
		13	1. 5.67
		14	18.10.66
Water	3 litres	1	22. 8.66
		2	1. 9.66
		2	22.11.66
		3	18. 7.66
NaCl	3 litres 0.15 M	1	25. 7.66
		2	30. 8.66
		2	24.11.66
		3	16. 8.66
NaCl	0.3 litres 1.5 M	1	26. 7.66
		1	14. 9.66
		2	2. 8.66
		2	21. 9.66
KCl	3 litres 0.15 M	1	12. 9.66
		2	19. 9.66
		12	27. 2.67
		13	20. 2.67
KCl	0.3 litres 1.5 M	2	30. 3.67
		12	19. 4.67
		13	24. 4.67

in each animal. The details of the intraruminal infusions for each sheep are shown in Table 19, and the complete infusion history in Appendix 1.

All infusions were made at 10 a.m., and during the experimental period (8.30 a.m. - 4.30 p.m.) neither water nor feed was available. Feed was last offered at 4-7 p.m. on the day prior to an experiment, and water up to 8.30 a.m. on the day of the experiment.

Animals and Feed

The sheep were Romney ewes with weights of 32-40 kg. Each had a permanent fistula in the dorsal rumen, and two animals (12, 13) had also duodenal cannulae.

The animals were housed as described in Chapter 2. They were fed chaffed lucerne hay for 3 hours in the afternoon, between 2-5 p.m., except on the day preceding an experiment. On the experimental day itself, the feed was offered between 5-8 p.m. The amount offered each day was a little more than the ad libitum intake (around 1000-1200 gm), and included 5 gm of NaCl. Two batches of lucerne were used.

Infusion technique

On the evening prior to an infusion, the normal rumen stopper was replaced by a rubber stopper through which a short glass tube projected to the outside but not into the rumen. A short length of rubber tubing with a screw clamp was attached to the glass tube, and the whole tied securely to the rumen cannula.

The solution was warmed to near body temperature in a glass aspirator

bottle fitted with a tap and a length of polythene tubing. At the time of the infusion, this polythene tube was connected to the rubber tube on the cannula, and the solution run into the rumen by gravity in 2-3 minutes. Care was taken in manipulating the tap, clamp and tubing not to lose gas or rumen contents and to give a complete infusion. The animal was not upset by the procedure as long as the skin was not touched nor the rumen cannula pulled.

Sample collection

Blood samples were taken through a jugular cannula, and urine samples through a self-retaining urethral catheter as previously described. Both were inserted prior to the experimental day.

Urine was collected as 30 minute samples (occasionally 60 minutes) into collapsed vaccine bags. Blood samples were collected at 45 and 15 minutes before the infusion, 15 minutes after, and at hourly intervals after that.

Analytical methods

The method of handling samples and the chemical methods are identical with those described in Chapters 2 and 3.

For PCV both Wintrobe tubes and the microhaematocrit method were used, the former method being replaced by the latter for about half of the experiments. The results obtained from the two methods agreed closely.

Calculation of results

The new calculations were introduced:

Relative plasma electrolyte content was obtained by multiplication of the relative plasma volume by the appropriate electrolyte concentration.

% change in excretion: the overall change in excretion over the experimental period (8 hours) for each treatment, including controls, for each urinary parameter was calculated using a prediction of the excretion based on the preinfusion rate, averaged over the 90 min before infusion.

The expected excretion, assuming no change in rate, was calculated as

E = 16 x average 30 min preinfusion rate

The actual excretion (A) was obtained by adding the total 8 hour excretion

$$\% \text{ change in excretion} = \frac{A - E}{E} \times 100$$

% recovery: for administered water and electrolyte, the % of the load excreted was calculated taking into account the changes on the control days:

$$\% \text{ recovery} = \frac{A - E + Ad - C}{Ad} \times 100$$

where A and E are as above

Ad = administered water or electrolyte

C = average % change in excretion on control days. This was not always zero; for Na^+ and K^+ the average control change was used to correct the expected base line.

RESULTS

Urine excretion

(1) Volume

On 5 of the 6 control days, the urine volume showed minor fluctuations

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Table 20. Excretion of water following intraruminal infusion, expressed as % of the load for the 3 litre infusions, and as ml of the 300 ml water load retained after correction for the basal rate for the hypertonic infusions.

Infusion	% load excreted	Average
Water 3 litres	85 81 79 51	74%
KCl 3 litres 0.15M	65 54 50 38	52%
NaCl 3 litres 0.15M	40 31 23 21	29%
Infusion	**Water retention (ml)**	
KCl 0.3 litre 1.5M	133 205 625	
NaCl 0.3 litre 1.5M	31 124 465 -124	

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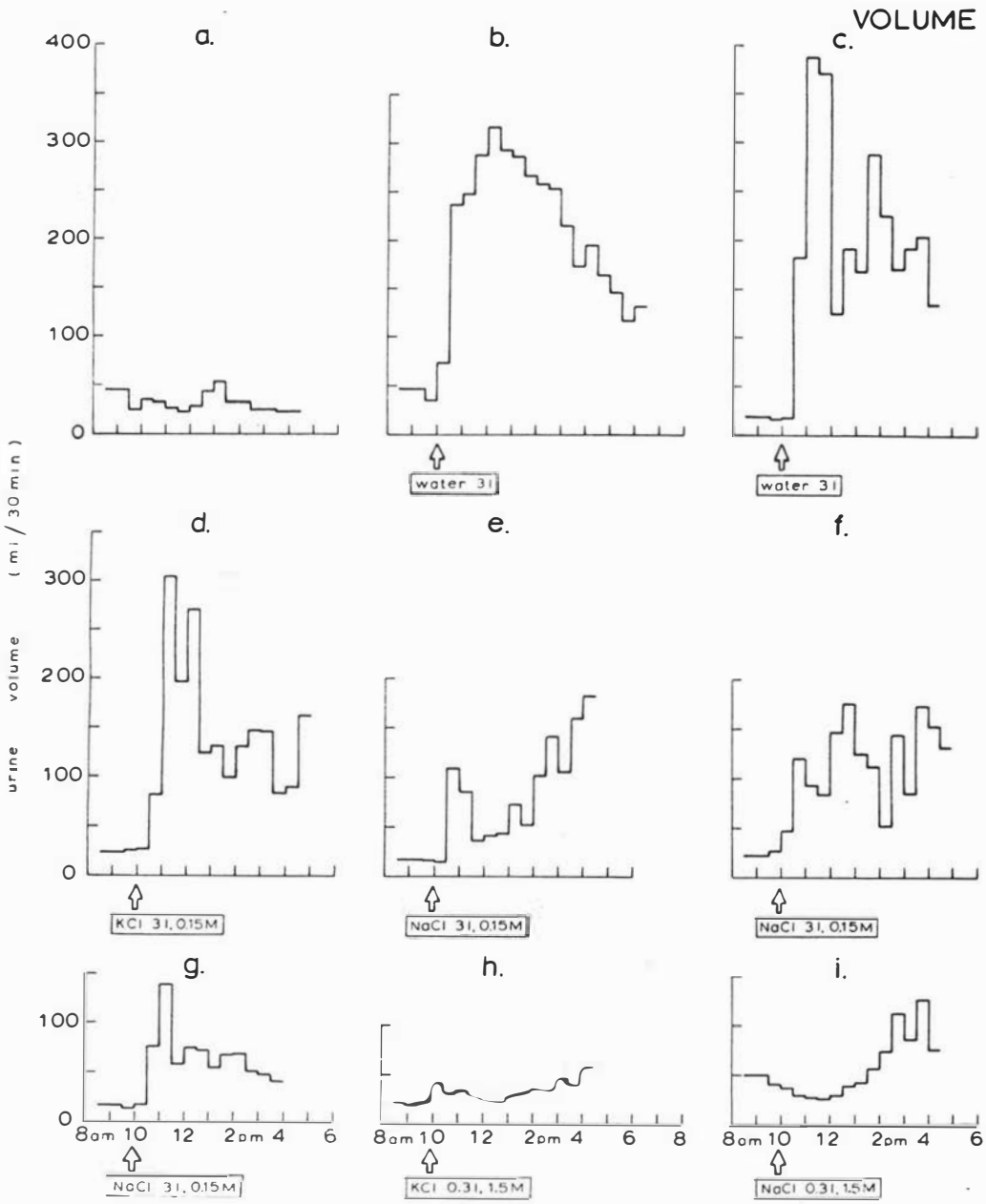

Fig 91. Urine volume following intraruminal infusions: a - control; b,c - 3 l water; d - KCl (3 l, 0.15M); e,f,g - NaCl (3 l, 0.15M); h - KCl (0.3 l, 1.5M); i - NaCl (0.3 l, 1.5M). Note particularly type (a) water diuresis in b and type (b) diuresis in c; d - after KCl (3 l, 0.15M) a pattern similar to type (b) water diuresis; variable effect of NaCl (3 l, 0.15M) in e-g. (a - sheep 1, 24.8.66; b - sheep 1, 22.8.66; c - sheep 2, 22.11.66; d - sheep 1, 12.9.66; e - sheep 2, 24.11.66; f - sheep 1, 25.7.66; g - sheep 2, 30.8.66; h - sheep 12, 19.4.67; i - sheep 1, 14.9.66).

and no consistent trend, (Fig 91a), on the sixth day the changes in flow were larger. Overall, for the 8 hours, the volume excreted was ± 10% of the predicted amount on 5 days, but 15% less on one. On 4 of the 6 days, urine total solutes changed a similar amount to that of the volume. For purposes of prediction, the base line was taken as the average flow/30 min observed over the first 90 minutes.

Two types of response to the water infusion were seen, each twice. Type (a) urine flow approximately doubled in the first 30 minutes, rapidly reached a maximum of near 350 ml/30 min between 1½ and 2½ hours, then declined slowly (Fig 91b); in type (b) there was a delay of 30 minutes before the urine flow also reached a peak at 1½-2½ hours, following which the flow usually remained between 100-200 ml/30 min (Fig 91c). The form of the response was not an individual characteristic, as one sheep showed both kinds of response. There appeared to be no difference in the efficiency of the diuresis: the peak flows were comparable, while the fraction of the load excreted was 85% and 79% for the former, and 84% and 51% for the latter (Table 20). Type (a) diuresis was associated with lower preinfusion urine osmolality than was type (b) - 600-1000 mosm/kg compared with 1350-1950 mosm/kg.

After KCl (3 l, 0.15M), the pattern of urine flow qualitatively resembled type (b) water diuresis (Fig 91d). The response was quantitatively less: the peak flow reached 250-300 ml/30 min, then continued at around 100 ml/30 min. The fraction of water excreted was also lower, averaging 52% (Table 20). The onset of diuresis occurred in less than 30 minutes on 2 days with lower preinfusion urine osmolality, and in greater than 30 minutes on the other two. There appeared to be no relationship between the fractions of the loads of water and of K^+ excreted.

After NaCl (3 l, 0.1%), the pattern of urine flow was more variable. On 2 days, urine flow reached only a low peak in the first 1½-2 hours, then increased progressively (Fig 91e). On the other 2 days, the urine volumes were much greater in the first hours after the infusion, remaining high on one day (Fig 91f), and decreasing on the other (Fig 91g). The fraction of the water load excreted was much lower than after either water or KCl, averaging 29% (Table 20). The onset of the diuresis occurred in under 30 minutes on one day, and in over 30 minutes on three. As in the case of K^+ after KCl, the % excretion of the loads of water and Na^+ were independent.

Following KCl (0.3 l, 1.5%), urine flow did not exceed 60 ml/30 min (Fig 91h), and appeared to be determined by total solute excretion. On all three days, the water excretion was less than the basal excretion + 300 ml (Table 20).

When NaCl (0.3 l, 1.5%) was infused, urine flow paralleled Na^+ excretion, over the experimental period approximating 5 ml per m-equiv Na^+. Over the 1½-2½ hours before the development of the natriuresis, the urine volume remained unchanged, or, if initially high, decreased (Fig 91i); thereafter it increased rapidly. On one day, the urine volume was greater than the basal rate + 300 ml, on the remaining three days, it was less than that (Table 20).

(2) $\underline{Na^+}$

On the 6 control days, 3 different patterns of Na^+ excretion occurred: (a) on 3 days Na^+ excretion was initially 0.1-0.2 m-equiv/30 min, and remained low (Fig 92a), varying less than 1 m-equiv from constant

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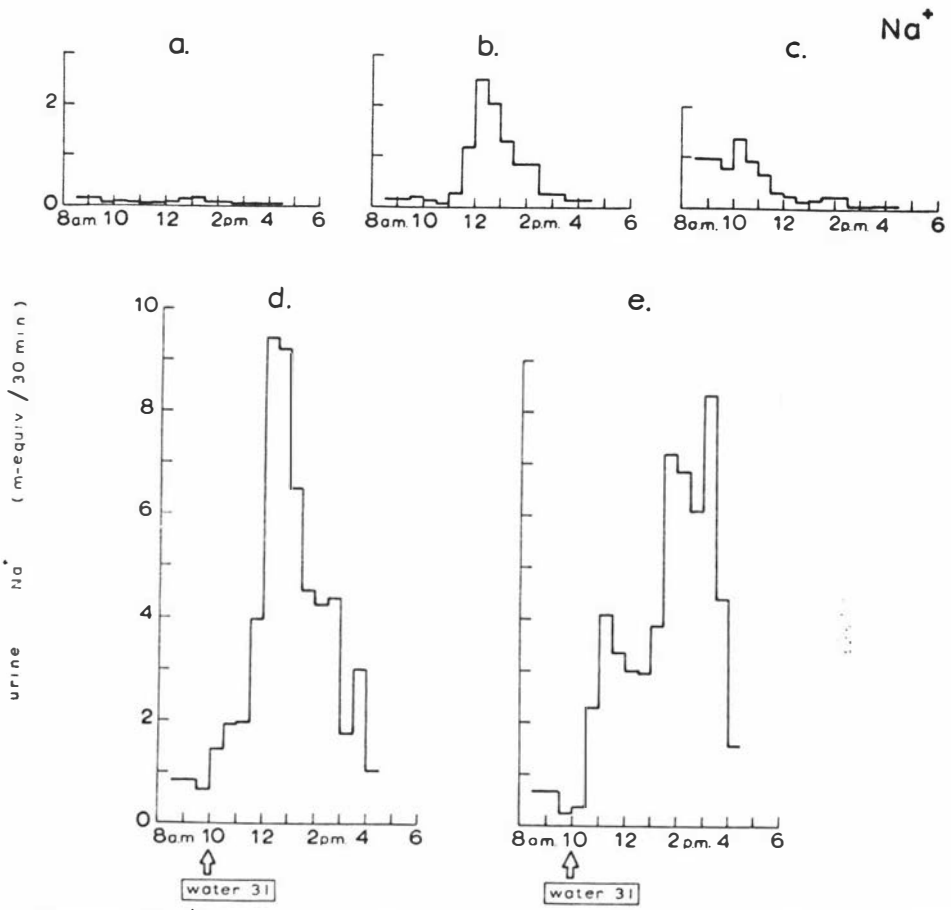

Fig 92. Urine Na^+ excretion following intraruminal infusions: a,b,c - control; d,e - 3 l water. Note 3 types of control response, a - commonly seen when the preinfusion Na^+ was low, c - when it was higher; two types of natriuresis during a water diuresis, d - single peak, e - two peaks. (a - sheep 1, 24.8.66; b - sheep 14, 18.10.66; c - sheep 2, 1.8.66; d - sheep 2, 1.9.66; e - sheep 2, 22.11.66).

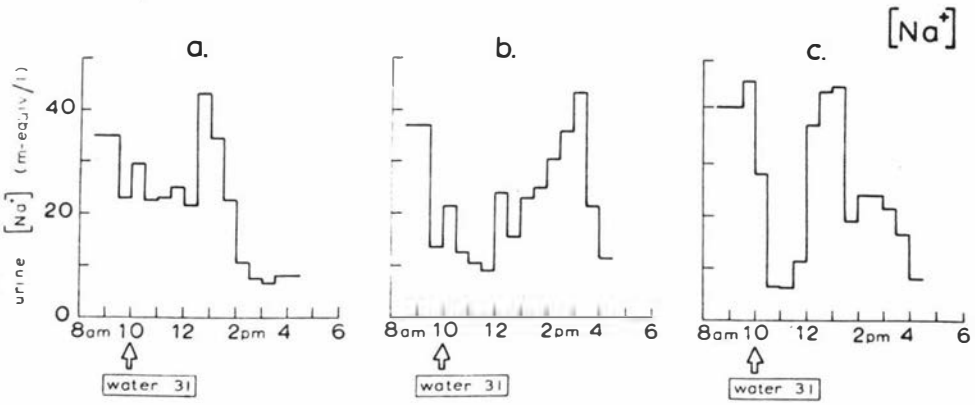

Fig 93. Urine $\lceil Na^+ \rfloor$ following intraruminal infusion of 3 l of water. Note a - unusual pattern where $\lceil Na^+ \rfloor$ remained high during peak diuresis then fell; b,c - more common fall in $\lceil Na^+ \rfloor$ during peak diuresis followed by an increase for a variable time. (a - sheep 3, 18.7.66; b - sheep 2, 22.11.66; c - sheep 2, 1.9.66).

excretion; (b) on 1 day with the same initial excretion, a peak occurred around noon (Fig 92b) resulting in an extra 8 m-equiv of Na^+ being lost; (c) on 2 days, the initial excretion was 1-2 m-equiv/30 min, began decreasing at 10.30-11 a.m., and over the 8 hours declined 50-60% (Fig 92c). In predicting the basal excretion for the infusions, a low excretion was assumed to remain low, but higher rates to decrease by 50% over the 8 hours; it was not possible to take peaks into account.

Water infusion increased Na^+ excretion - the greater the diuresis, the greater the Na^+ loss. Preinfusion Na^+ excretion was high on all 4 days, and assuming a 50% decline as a basal excretion rate, there was an extra loss of 34, 49, 52 and 53 m-equiv of Na^+. The natriuresis showed a single peak at about 2 hours on 2 days (Fig 92d), but biphasic changes with peaks around 1 and 4 hours on the other 2 days (Fig 92e). One sheep showed both kinds of natriuresis on different days. Na^+ excretion depends on factors other than the water diuresis. Na^+ excretion patterns were not associated with the two patterns of water excretion described above. Further, urine $[Na^+]$ differed markedly on one day from the pattern seen on the other 3 days, (compare Fig 93a with Fig 95b, c). On 3 out of the 4 days, urine $[Na^+]$ dropped to around 10 m-equiv/l during the peak diuresis; 2-3 hours later it had returned to the preinfusion 30-40 m-equiv/l (Fig 95b,c), resulting in a single peak on 1 day, and two peaks on 2 days. On the fourth, urine $[Na^+]$ remained high (20-40 m-equiv/l) for 4 hours, then fell to 5-10 m-equiv/l for the next $2\frac{1}{2}$ hours (Fig 93a).

For both NaCl solutions the pattern of Na^+ excretion was qualitatively similar, consisting of two phases. The natriuresis was delayed for 1-$2\frac{1}{2}$ hours after the infusion. At the beginning of the

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Table 21. Intraruminal NaCl infusion: Na^+ excretion related to other parameters.

Infusion	Fraction excreted (%)	Sheep & date	Delay in onset (hours)	Preinfusion Na^+ excretion (m-equiv/ 30 min)	Approx Na^+ rate 3rd-4th hour (m-equiv/ 30 min)	No. of previous infusions & interval since last (days)
NaCl 3 litres 0.15M	15	16. 8.66 3	2½	0.13	10	0
	19	25. 7.66 1	2	1.07	10	0
	26	24.11.66 2	2½	0.10	20	3 64 days
	36	30. 8.66 2	1½	0.42	20	1 28 days
NaCl 0.3 litres 1.5M	20	2. 8.66 2	2	0.12	20	0
	34	26. 7.66 1	1½	7.7	20	1 1 day
	40	21. 9.66 2	1½	0.17	30	2 22 days
	40	14. 9.66 1	1	0.14	30	2 50 days

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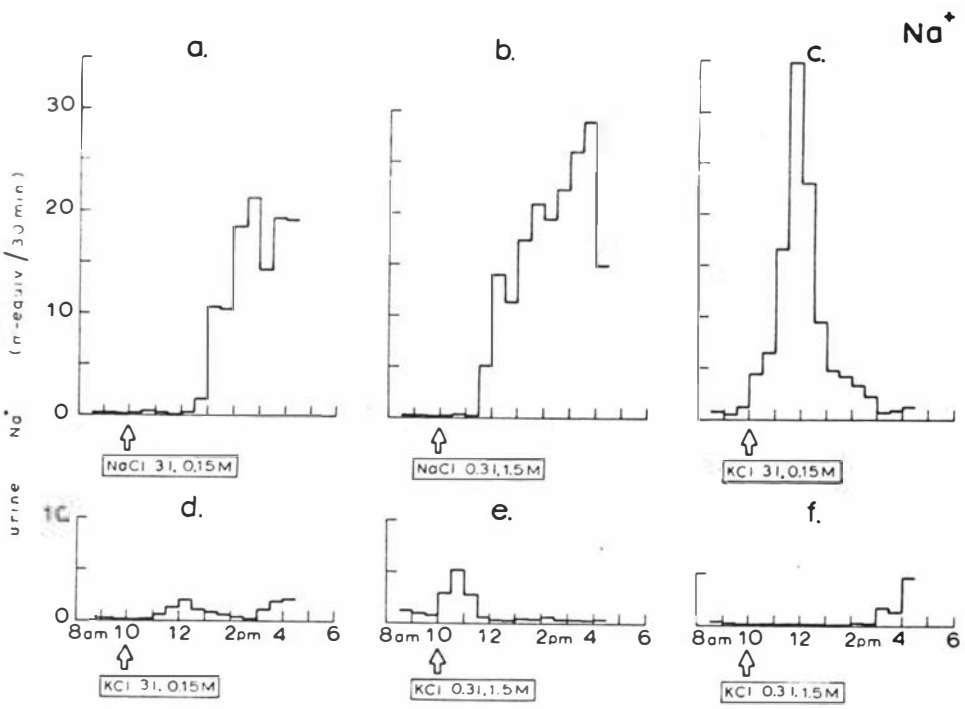

Fig 94. Urine Na^+ excretion following intraruminal infusions: a - NaCl (3 l, 0.15M); b - NaCl (0.3 l, 1.5M); c,d - KCl (3 l, 0.15M); e,f - KCl (0.3 l, 1.5M). Note delay in onset of the natriuresis after both NaCl solutions (a,b) but not in all cases after KCl (c,e); after 0.15M KCl, the largest natriuresis is shown in c, the smallest in d; after 1.5M KCl, the natriuresis was small and at a variable time after the infusion (e,f). (a - sheep 2, 24.11.66; b - sheep 2, 21.9.66; c - sheep 13, 20.2.67; d - sheep 2, 19.9.66; e - sheep 13, 24.4.67; f - sheep 12, 19.4.67).

natriuresis, Na^+ excretion increased rapidly, usually reached a peak in the next 1½-2 hours, following which it frequently continued at a high level (Fig 94a). On occasions, however, Na^+ excretion continued to increase for up to 4 hours (Fig 94b), particularly with the 1.5% NaCl. Table 21 shows for each day the fraction of the load excreted, together with the delay in onset, the average excretion during the third and fourth hours of the natriuresis, the preinfusion Na^+ excretion rate, the number of previous NaCl infusions and the time interval since the previous one. The number of replicates precludes any conclusions concerning significant relationships. As might be expected, the fraction excreted appears greater the shorter the delay in onset and the higher the level of Na^+ excretion. It might appear that Na^+ excretion was higher after the more concentrated infusion; it might also appear that prior infusions had a priming effect.

Infusion of KCl (3 l, 0.15%) increased Na^+ excretion: by 11, 25, 38 and 108 m-equiv over the following 6½ hours (Fig 94c,d). On one day, the natriuresis preceded the increase in urine flow. The temporal relationship between the natriuresis and the kaliuresis varied. On two days, the onset and peak of the Na^+ excretion preceded that for K^+, and on two days they coincided. In all cases, Na^+ excretion was decreasing again after 2 hours, at a time when K^+ excretion was increasing. During the peak of this natriuresis, urine $[Na^+]$ was greater than that occurring during peak water diuresis.

In comparison with that produced by the more dilute KCl, the natriuresis after KCl (0.3 l, 1.5%) was infused was only slight. Over the 6½ hours only 10, 6 and 0.5 m-equiv of Na^+ extra were excreted. The amounts were quantitatively similar to those on some control days, but because of

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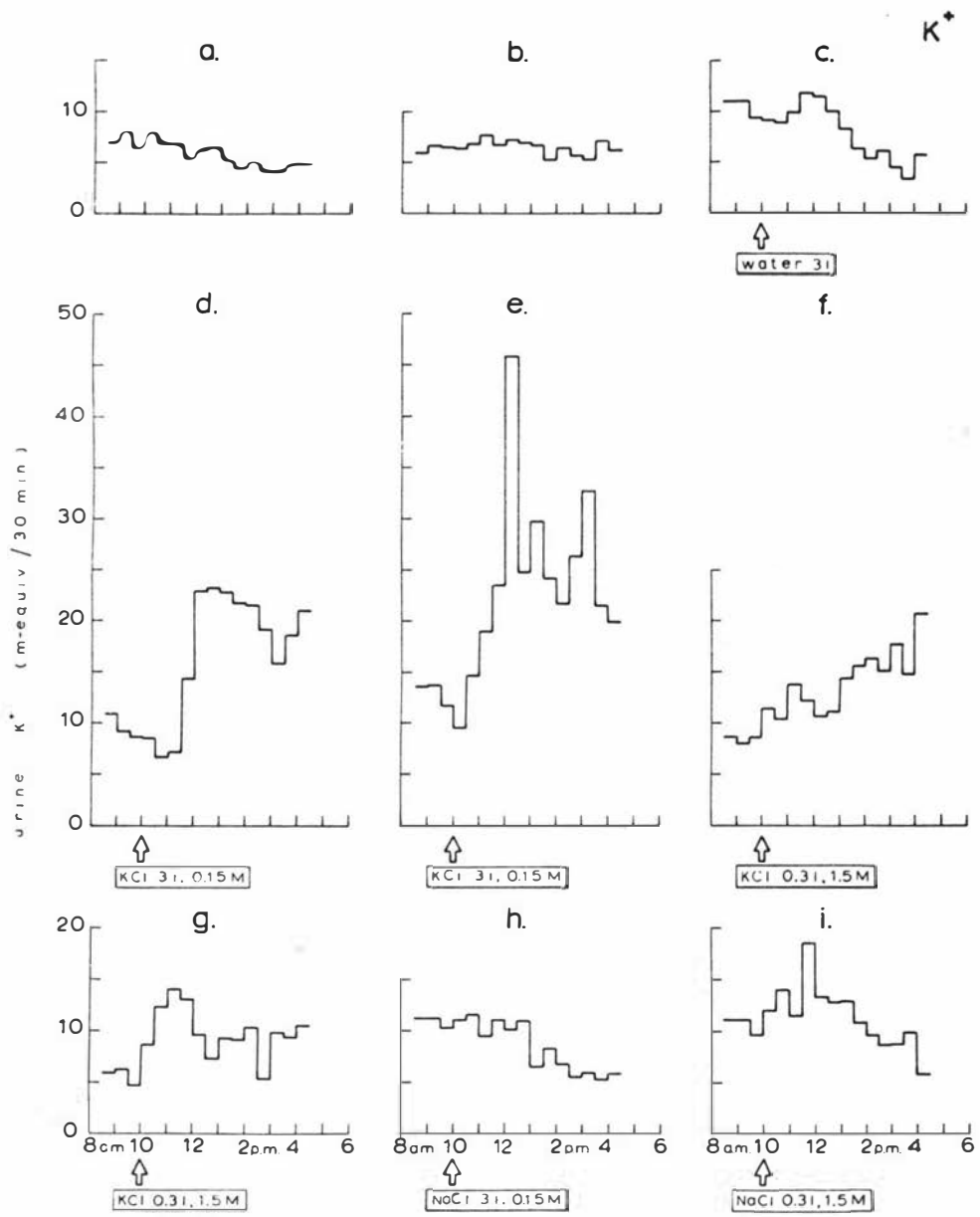

Fig 95. Urine K^+ excretion following intraruminal infusions: a,b - control; c - 3 l water; d,e - KCl (3 l, 0.15M); f,g - KCl (0.3 l, 1.5M); h - NaCl (3 l, 0.15M); i - NaCl (0.3 l, 1.5M). Note the usual control pattern in a, atypical day in b; small kaliuresis during the water diuresis in c; delay in onset, but marked kaliuresis after 0.15M KCl (d,e); earlier onset but lesser increase in K^+ excretion after 1.5M KCl (f,g); variable pattern after NaCl infusions. (a - sheep 12, 17.4.67; b - sheep 13, 1.5.67; c - sheep 1, 22.8.66; d - sheep 13, 20.2.67; e - sheep 1, 12.9.66; f - sheep 12, 19.4.67; g - sheep 13, 24.4.67; h - sheep 2, 30.8.66; i - sheep 2, 21.9.66).

their timing would appear attributable to the infusion. In each case, the Na^+ peak occurred at the time of the K^+ peak, which on one day was over the first 90 minutes (Fig 94e) and on two days was after 4 hours (Fig 94f).

(3) K^+

On 5 of the 6 control days, K^+ excretion decreased continuously over the 8 hours of observation, with only small peaks and troughs (Fig 95a); the overall decline was 13-29%. On the sixth day, K^+ excretion increased for 3 hours before declining (Fig 95b), so that overall 2% more than the predicted amount was excreted. For the infusions, the basal K^+ excretion was predicted on the assumption that the initial rate would decrease by 16%, the average for the 6 control days.

When water was administered, K^+ excretion increased a small amount during the peak water diuresis, then decreased (Fig 95c). Over the experimental period, total K^+ excretion appeared little different from that on the control days: declines from initial excretory rates were 4%, 10%, 19% and 21%, averaging 14%.

Infusion of KCl (3 l, 0.15%) was followed by a kaliuresis which began after a delay of 30 minutes on 2 days, and of 90 minutes on 2 days. The kaliuresis reached a maximum in the third hour in all experiments, coinciding with the highest plasma $[K^+]$, then declined slowly (Fig 95d,e). The fraction of the infused K^+ load excreted in the urine averaged 30% (21%, 27%, 31% and 40%), allowing for a basal rate of 16% decline.

After the concentrated KCl (0.3 l, 1.5%), the kaliuresis commenced earlier; thus some increase in K^+ excretion was evident in the first 30

Table 22. K^+ excretion following intraruminal NaCl infusion, expressed as % change from the preinfusion rate; the extra m-equiv of K^+ excreted relative to a basal excretion of 16% decline; and the same calculation based on a 20% decline. *

Infusion	8 hour excretion relative to that predicted from preinfusion rate	m-equiv of K^+ extra (or less) with basal rate declining by	
		16%	20%
NaCl 3 litres 0.15M	- 21%	- 8	- 1
	- 3%	+ 9	+ 13
	- 3%	+ 11	+ 14
	+ 13%	+ 40	+ 46
NaCl 0.3 litre 1.5M	- 10%	+ 9	+ 14
	- 11%	+ 9	+ 17
	+ 6%	+ 38	+ 44
	+ 30%	+ 34	+ 37

* 20% is the average decline in the 5 similar control experiments.

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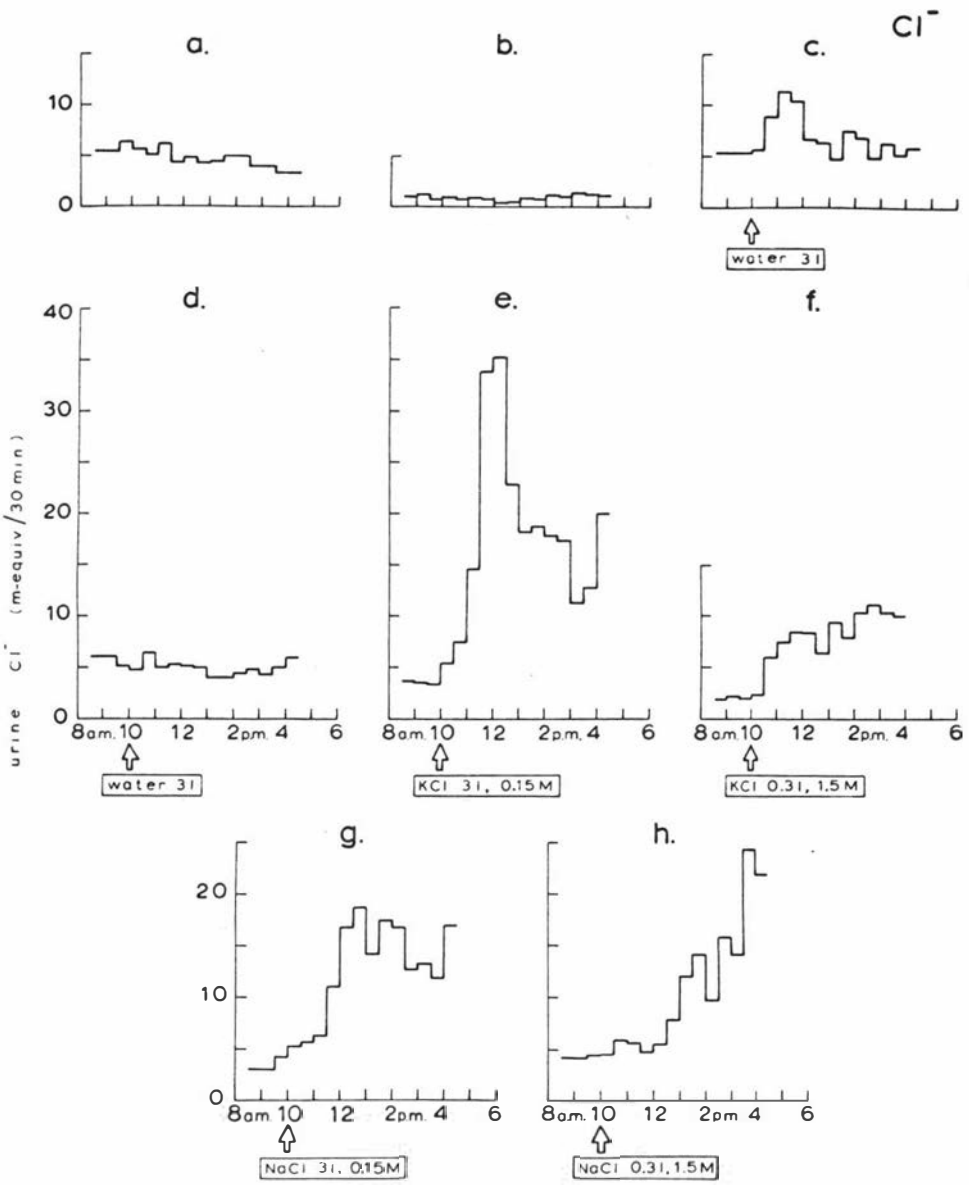

Fig 96. Urine Cl^- excretion following intraruminal infusions: a,b - control; c,d - 3 l water; e - KCl (3 l, 0.15M); f - KCl (0.3 l, 1.5M); g - NaCl (3 l, 0.15M); h - NaCl (0.3 l, 1.5M). Note the variable control changes in a and b; a small or large increase in Cl^- excretion during a water diuresis (c,d); after KCl and NaCl infusions a marked increase in Cl^- excretion, usually with little delay in onset (e-h). (a - sheep 2, 1.8.66; b - sheep 12, 17.4.67; c - sheep 2, 22.11.66; d - sheep 1, 22.8.66; e - sheep 13, 20.2.67; f - sheep 2, 30.3.67; g - sheep 2, 30.8.66; h - sheep 2, 2.8.66).

minutes in all. Over the early post-infusion period, K^+ excretion increased to 12-14 m-equiv/30 minutes, thereafter, in two cases excretion rate continued to increase to the end of the experimental period (Fig 95f) while in the third, excretion fell and was maintained at a lower, almost constant rate. The preinfusion level was lower on this day (Fig 95g). The fraction of the K^+ load excreted was 16%, 18% and 21%, averaging 18%.

After each of the NaCl infusions, on 2 of the 4 days K^+ excretion decreased over the experimental period (Fig 95h), while on the other 2 days, a K^+ peak lasting 3-4 hours preceded the Na^+ peak (Fig 95i). The overall changes in K^+ excretion for the 8 days are shown in Table 22. Quantitatively, the amount of Na^+ and of K^+ excreted did not appear to be related.

(4) Cl^-

On the control days, Cl^- excretion was variable, both quantitatively and qualitatively (Fig 96a,b). On two occasions there was a progressive fall, amounting to 17% and 31%. On 4 occasions there were successive peaks and troughs, the overall changes being decreases of 11% and 19% on 2 days, no change on one, and an increase of 31% on one.

When water was administered, a small or large increase in Cl^- excretion occurred during the maximum water diuresis on each of the 4 days. Over the whole experimental period, the excreted amount was not obviously different from on control days: 3 decreased 11-22% and one increased 25% (Fig 96c,d).

After the NaCl and KCl infusions, in general, Cl^- excretion paralleled that of the cation (compare Fig 96e-h with Fig 94, 95). Increased

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Table 23. Intraruminal infusions: % change in HCO_3^- excretion from the preinfusion rate.

Infusion	No. of observations	% change in HCO_3^- excretion
Control	4	- 1% - 15% - 25% - 34%
Water 3 litres	3	+ 9% + 50% + 74%
NaCl 3 litres 0.15M	3	+ 59% + 70% + 94%
NaCl 0.3 litre 1.5M	2	+ 13% + 92%
KCl 3 litres 0.15M	4	- 38% + 47% + 55% + 68%
KCl 0.3 litre 1.5M	3	- 33% + 39% + 59%

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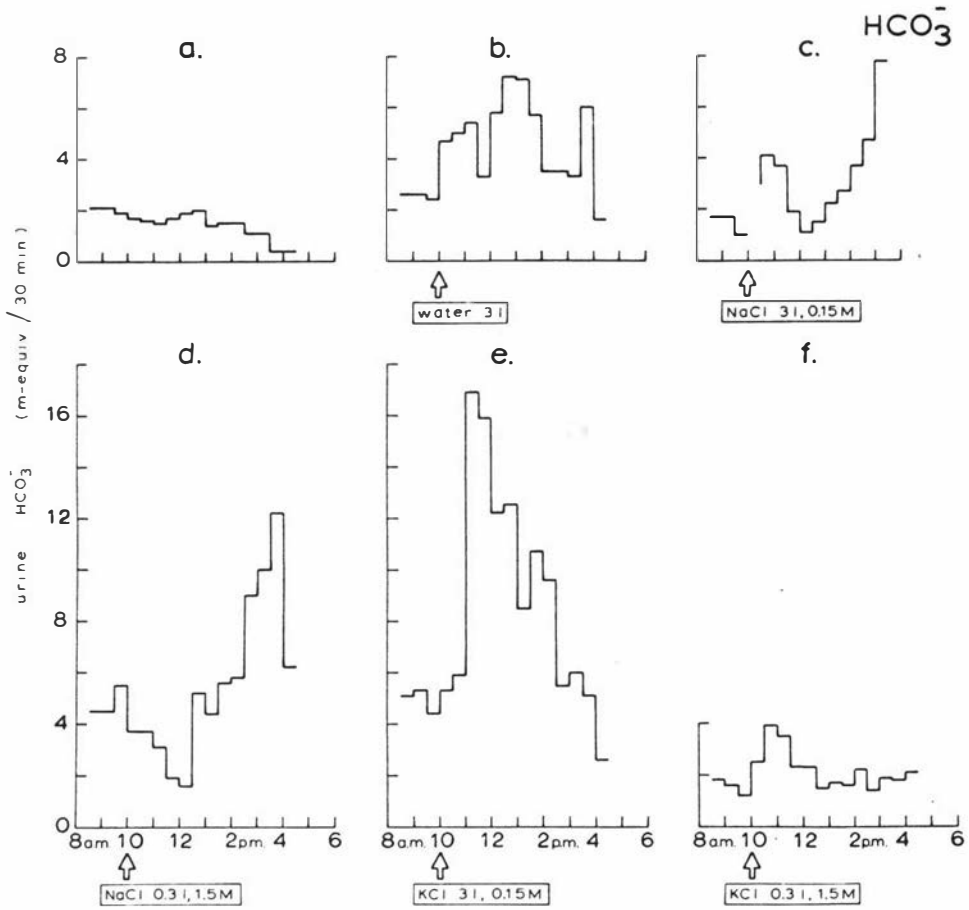

Fig 97. Urine HCO_3^- excretion following intraruminal infusions: a - control; b - 3 l water; c - NaCl (3 l, 0.15M); d - NaCl (0.3 l, 1.5M); e - KCl (3 l, 0.15M); f - KCl (0.3 l, 1.5M). Note the usual control pattern of an overall decline, particularly during the latter 3-4 hours; increased HCO_3 excretion after all types of infusion. (a - sheep 14, 18.10.66; b - sheep 2, 1.9.66; c - sheep 3, 16.8.66; d - sheep 1, 14.9.66; e - sheep 13, 20.2.67; f - sheep 13, 24.4.67).

Cl^- excretion began soon after the infusion although excretion of the administered cation was delayed: where Na^+ preceded K^+ after KCl (3 l, 0.15M), some Cl^- was excreted with the Na^+; furthermore, a small amount of Cl^- was excreted before the Na^+ after both NaCl infusions. The average fraction of the Cl^- load excreted was similar to that for the administered cation when the dilute solutions were infused. For the two more concentrated solutions, Cl^- loss was a little less than the cation fraction.

(5) HCO_3^-

The excretion of HCO_3^- was not estimated on every day. On 3 control days, HCO_3^- excretion decreased over the experimental period by 15-34%; the changes were not uniform, the main decrease occurring during the last 3-4 hours (Fig 97a). On one day of low HCO_3^- excretion, overall there was no trend.

All types of infusion increased HCO_3^- excretion (Fig 97b-f), but not on every occasion (Table 23). HCO_3^- excretion accompanied that of Na^+ as frequently as it did that of K^+.

When water was administered, the maximum HCO_3^- output occurred after the peak of water diuresis and appeared to be related to Na^+ rather than to water excretion. More HCO_3^- was excreted over the 8 hours than on control days (Table 23).

Following NaCl (3 l, 0.15M), there was a small HCO_3^- peak preceding the Na^+ excretion, and much larger excretion accompanying the natriuresis (Fig 97c). When concentrated NaCl (0.3 l, 1.5M) was administered, HCO_3^- excretion was raised during the natriuresis. For both solutions, HCO_3^- excretion was considerably greater than for the controls (Table 23).

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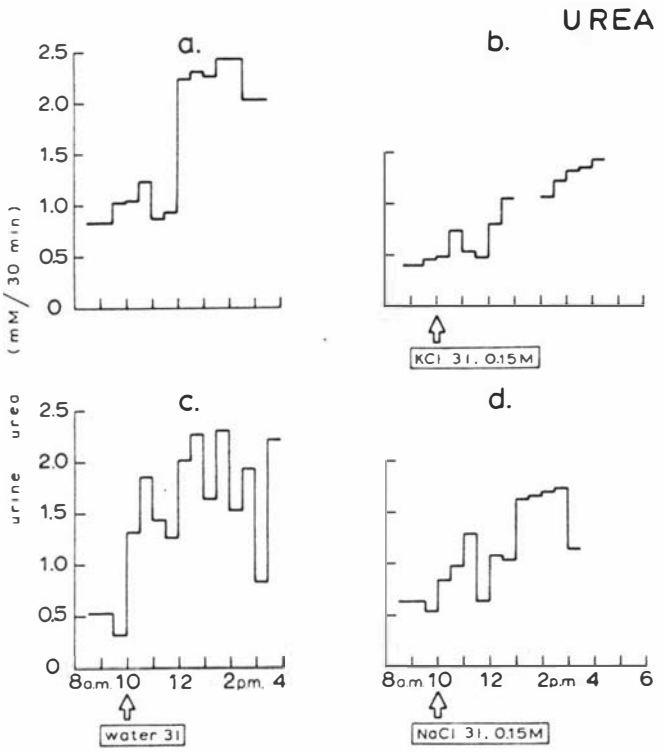

Fig 98. Urine urea excretion following intraruminal infusions: a - control; b - KCl (3 l, 0.15M); c - 3 l water; d - NaCl (3 l, 0.15M). Note abrupt increase in urea excretion in controls and after infusions. (a - sheep 3, 20.7.66; b - sheep 2, 19.9.66; c - sheep 2, 1.9.66; d - sheep 3, 16.8.66).

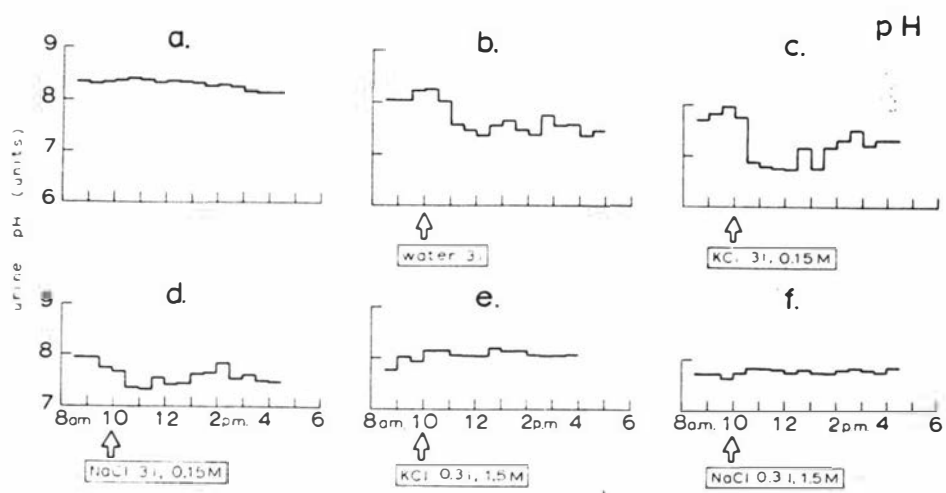

Fig 99. Urine pH following intraruminal infusions: a - control; b - 3 l water; c - KCl (3 l, 0.15M); d - NaCl (3 l, 0.15M); e - KCl (0.3 l, 1.5M); f - NaCl (0.3 l, 1.5M). Note small decline in pH on control days; more marked fall during the diuresis following water or 0.15M NaCl or KCl (b-d); slight increase in pH after the 1.5M infusions (e,f). (a - sheep 12, 17.4.67; b - sheep 3, 18.7.66; c - sheep 12, 27.2.67; d - sheep 1, 25.7.66; e - sheep 2, 30.3.67; f - sheep 1, 26.7.66).

On one day after each of the KCl infusions, HCO_3^- excretion decreased progressively over the postinfusion period, but on the other occasions HCO_3^- excretion was raised (Table 23). When the more dilute KCl solution was infused, HCO_3^- reached an early peak, then returned to the preinfusion level (Fig 97e). Quantitatively, the extra HCO_3^- was related to the sum of the extra Na^+ and K^+ lost, rather than to K^+ alone. After the KCl (0.3 l, 1.5M), the increase in HCO_3^- and in K^+ excretion occurred at nearly the same time (Fig 97f).

(6) Urea

Urea was not estimated on all days. On each of the 4 control days on which it was measured, urea was found to increase, frequently discontinuously, over the experimental period (Fig 98a). In one case the increase over the initial rate was 20%, in the others it was 75-100%. Similar increases occurred after the infusions (Fig 98b). The greatest losses were observed after the water load and the dilute NaCl (up to 200%) (Fig 98c,d).

(7) pH

pH was not estimated on all days. In general, changes in urine pH paralleled those of $[HCO_3^-]$. On the 4 control days on which pH was measured, it did not change until the last 3-4 hours of the experiment when a steady decrease occurred. On none of these days did the pH fall below 7.0 (Fig 99a). When the urine flow started to increase after infusion of water (1 day), dilute KCl (2 days) and dilute NaCl (1 day), the urine pH decreased 0.5-1.0 units, and remained at that lower pH except after the KCl, when there was a later small rise (Fig 99b-d). After one of the KCl

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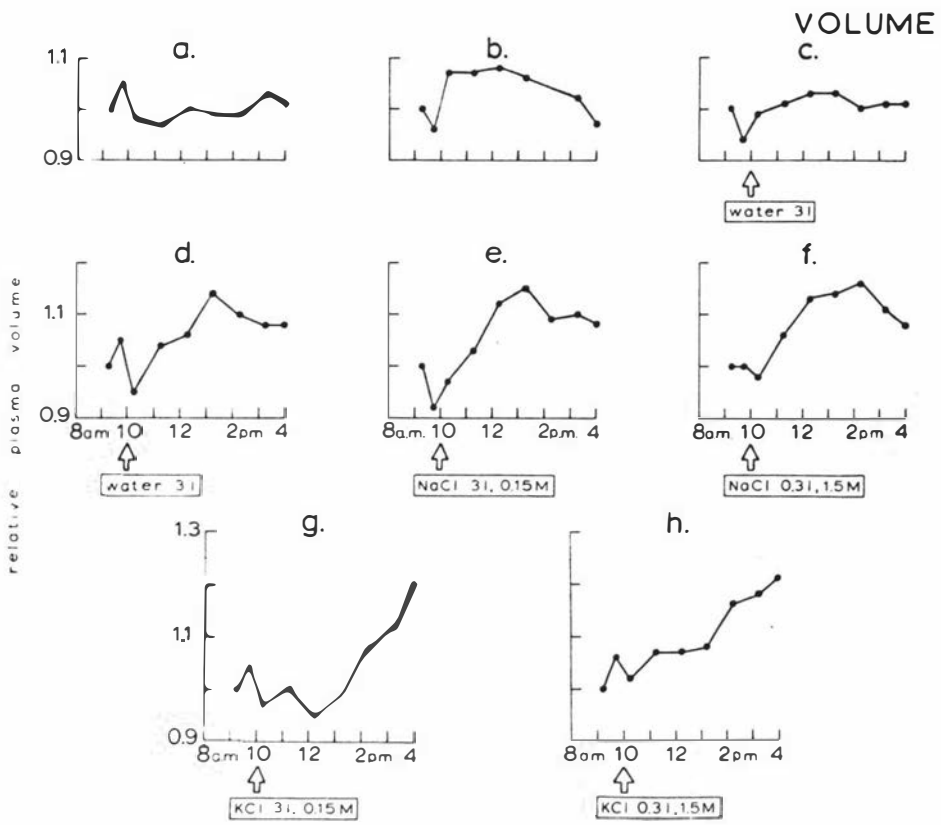

Fig 100. Relative plasma volume following intra-ruminal infusions: a,b - control; c,d - 3 l water; e - NaCl (3 l, 0.15M); f - NaCl (0.3 l, 1.5M); g - KCl (3 l, 0.15M); h - KCl (0.3 l, 1.5M). Note the variable changes in controls and after water infusion, slightly greater in d than on control days; considerable increase in plasma volume after both NaCl solutions, reaching a peak around 3 hours (e,f); decreased volume for 2-3 hours after 0.15M KCl (g) but no change after 1.5M KCl (h), followed by an increase in volume with both. (a - sheep 12, 17.4.67; b - sheep 14, 18.10.66; c - sheep 2, 22.11.66; d - sheep 2, 1.9.66; e - sheep 2, 24.11.66; f - sheep 1, 26.7.66; g - sheep 1, 12.9.66; h - sheep 12, 19.4.67).

infusions, when the $[HCO_3^-]$ fell to 2-10 m-equiv/l, the urine became acid.

The urine pH increased slightly after the concentrated NaCl and KCl (0.3 l, 1.5M) (Fig 99e,f).

(8) Total Solutes

On all 6 control days, total solute excretion fluctuated little over the experimental period, decreasing by 2-10% on 5, and by 15% on one. The water diuresis increased solute excretion, particularly during the early period; the increase over the whole experimental period was 10-30%. All infusions of electrolytes increased the total solute excretion, usually in parallel with the excretion of the infused electrolyte. The average % recovered compared with the 900 mosm administered was 37% for the dilute KCl, 15% for the concentrated KCl, 27% for the dilute NaCl, and 30% for the concentrated NaCl. The basal excretion was not corrected for the small control decrease.

Plasma Composition

(1) Volume, O.P. and Total solute content

The relative plasma volume on control days was variable, with no consistent trends as a group, (Fig 100a,b). Changes which occurred often seemed abrupt, but overall trends rarely exceeded ± 8% of the 9.15 a.m. volume. The [plasma protein] remained more nearly constant; the small changes that did occur were usually parallel to those occurring in the calculated relative plasma volume. Plasma O.P. decreased a little over each day, to a greater extent when the initial O.P. was higher. On 5 of the 6 days, at the end of the period the O.P. lay between 283-286 mosm/kg

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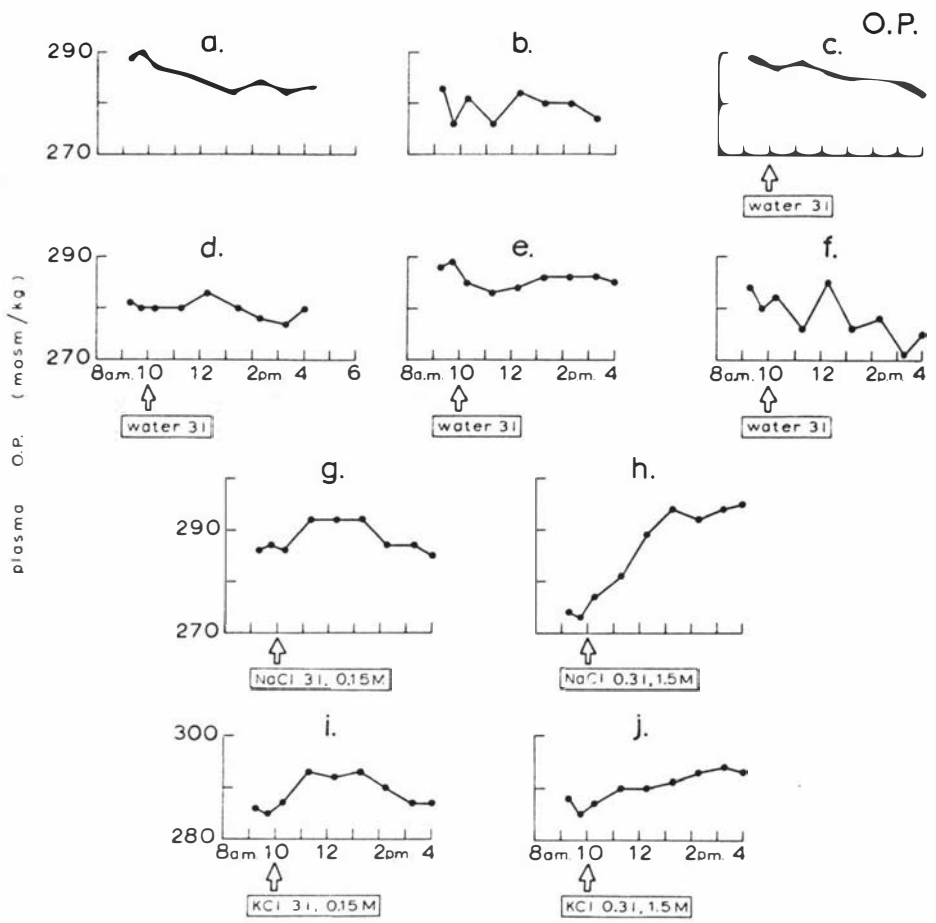

Fig 101. Plasma osmolality following intraruminal infusions: a,b - control; c-f - 3 l water; g - NaCl (3 l, 0.15M); h - NaCl (0.3 l, 1.5M); i - KCl (3 l, 0.15M); j - KCl (0.3 l, 1.5M). Note the overall declining O.P. on control days and after water infusion; no fall in O.P. over the first 1½ hours after water infusion in c,d but a prominent drop in e; a greater rise in O.P. after 1.5M NaCl than after 0.15M NaCl (g,h); one of the variable effects of 0.15M KCl (i); a steady increase in O.P. after the 1.5M KCl (j). (a - sheep 2, 1.8.66; b - sheep 3, 20.7.66; c - sheep 2, 22.11.66; d - sheep 2, 1.9.66; e - sheep 1, 22.8.66; f - sheep 3, 18.7.66; g - sheep 2, 24.11.66; h - sheep 1, 26.7.66; i - sheep 12, 27.2.67; j - sheep 13, 24.4.67).

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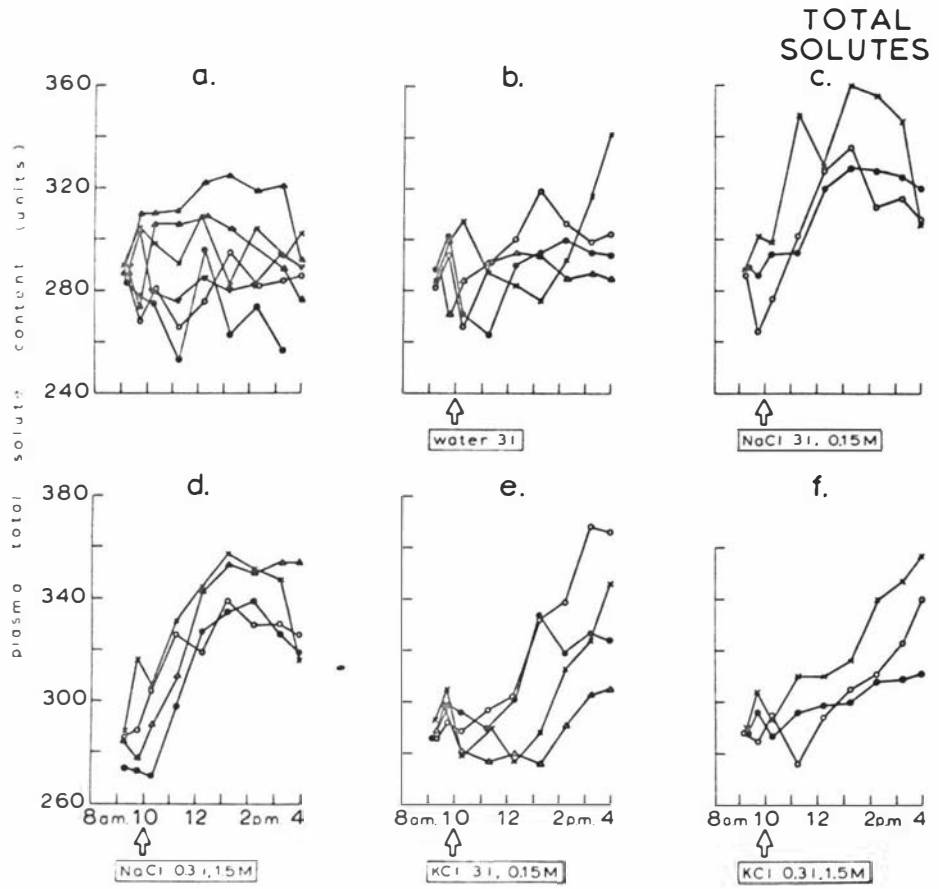

Fig 102. Plasma total solutes following intraruminal infusions: a - control; b - 3 l water; c - NaCl (3 l, 0.15M); d - NaCl (0.3 l, 1.5M); e - KCl (3 l, 0.15M); f - KCl (0.3 l, 1.5M).
(a - ○ - sheep 1, 24. 8.66
● - sheep 3, 20. 7.66
× - sheep 2, 1. 8.66
△ - sheep 14, 18.10.66
▲ - sheep 13, 1. 5.67
▽ - sheep 12, 17. 4.67

b - ○ - sheep 2, 1. 9.66
● - sheep 1, 22. 8.66
× - sheep 3, 18. 7.66
△ - sheep 2, 22.11.66

c - ○ - sheep 2, 24.11.66
● - sheep 2, 30. 8.66
× - sheep 1, 25. 7.66

d - ○ - sheep 1, 14. 9.66
● - sheep 1, 26. 7.66
× - sheep 2, 21. 9.66
△ - sheep 2, 2. 8.66

e - ○ - sheep 2, 19. 9.66
● - sheep 12, 27. 2.67
× - sheep 1, 12. 9.66
△ - sheep 13, 20. 2.67

f - ○ - sheep 2, 30.3.67
● - sheep 13, 24.4.67
✖ - sheep 12, 19.4.67
).

(Fig 101a,b). No consistent trend in plasma total solutes occurred from day to day; changes appeared to be related to changes in plasma volume, and were of a similar magnitude (Fig 102a).

During a water diuresis, plasma volume, O.P. and total solute changes did not appear different from those on control days. The changes in O.P. on all 4 days are shown in Fig 101c-f. On 2 days, the O.P. had not dropped below the preinfusion value after 1½ hours (Fig 101c,d) at a time when the urine flow had increased to over 300 ml/30 min. On the other 2 days drops did occur, however, in only one case was it prominent. On none of the 4 days did the plasma volume increase immediately after the infusion; on 2 it decreased. The change in volume 6 hours after the infusion had been given was on 2 occasions similar to that occurring on control days, i.e. within 8% of the 9.15 a.m. volume, (Fig 100c). On the other 2 days there was a slightly greater increase, 8% and 14% (Fig 100d). Changes in total solute content (Fig 102b) did not appear to differ from those seen in the controls, with the possible exception of one animal.

Both NaCl infusions resulted in considerable expansion of the plasma volume and increase in its total solute content. However, the changes in O.P. were considerably greater after the concentrated NaCl than after the 0.15M NaCl. Plasma volume changes were similar for both solutions, reaching a maximum after 3 hours, when Na^+ excretion was increasing rapidly, then declining (Fig 100e,f; Table 24). After the dilute NaCl, plasma O.P. increased little for the first 2-3 hours, then fell (Fig 101g). The increase was far greater after the concentrated NaCl: in 3 of the 4 it reached a plateau 3 hours after the infusion (Fig 101h). Total solutes reflected this difference in O.P. Over the first 3-4 hours post-infusion, total

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Table 24. Plasma volume and total solute content relative to the 9.15 a.m. value after intraruminal infusion of NaCl solutions.

Infusion	Plasma volume relative to the 9.15 a.m. volume		Total solute content relative to 9.15 a.m.	
	Maximum (after 3½ hours)	After 6 hours	Maximum (after 3-4 hours)	After 6 hours
NaCl 3 litres 0.15M	114-130% (average 120%)	106-114% (average 109%)	113-126% (average 119%)	107-110% (average 108%)
NaCl 0.3 litre 1.5M	114-118% (average 116%)	108-118% (average 111%)	118-124% (average 122%)	110-123% (average 116%)

solutes reached a maximum, then declined (Fig 102c,d), however, the changes were quantitatively greater after NaCl (0.3 l, 1.5M) than after the dilute infusion, particularly after 6 hours (Table 24). For both solutions there was an apparent inverse relationship between the increase in plasma volume and total solute content on the one hand, and the rate of Na^+ excretion on the other, i.e. the greater the Na^+ retention, the larger the increase in plasma volume and total solutes.

Following KCl (3 l, 0.15M) there was an initial drop in plasma volume to as low as 93% of the 9.15 a.m. value during the first 2-3 hours; there followed a rapid increase to 110-120% on 3 days, and to 105% on the fourth (Fig 100g). O.P. changes were variable: on 2 days there was an increase for 3 hours after the infusion followed by a return to the preinfusion level (Fig 101i); on one day the O.P. remained elevated; on the fourth day there was an initial decrease before the increase. Total solutes did not change over the first 2-3 hours on 2 days, but decreased a little on the other 2 days when Na^+ excretion was high. After this initial period, all 4 increased to 105-120% (Fig 102e).

After infusion of KCl (0.3 l, 1.5M), plasma volume remained unchanged for the first 2-3 hours, and then increased to 105%, 115% and 120% after 6 hours (Fig 100h). Over the whole period, O.P. increased steadily by 7-10 mosm/kg (Fig 101j). Total solutes were almost unchanged for 2-3 hours, then rose to 107%, 116% and 121% 6 hours after the infusion (Fig 102f).

For all infusions, [plasma protein] changes paralleled those of the calculated relative plasma volume.

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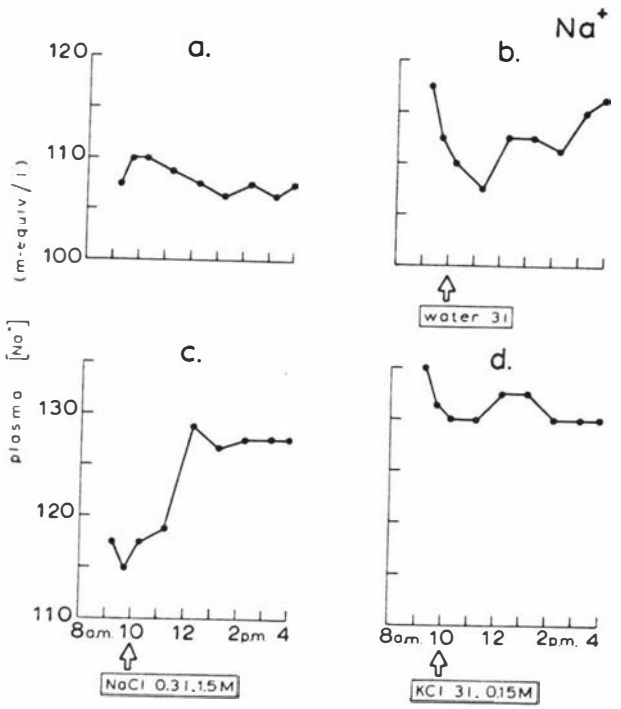

Fig 103. Plasma [Na^+] following intraruminal infusions: a - control; b - 3 l water; c - NaCl (0.3 l, 1.5M); d - KCl (3 l, 0.15M). (a - sheep 2, 1.8.66; b - sheep 3, 18.7.66; c - sheep 2, 2.8.66; d - sheep 2, 19.9.66).

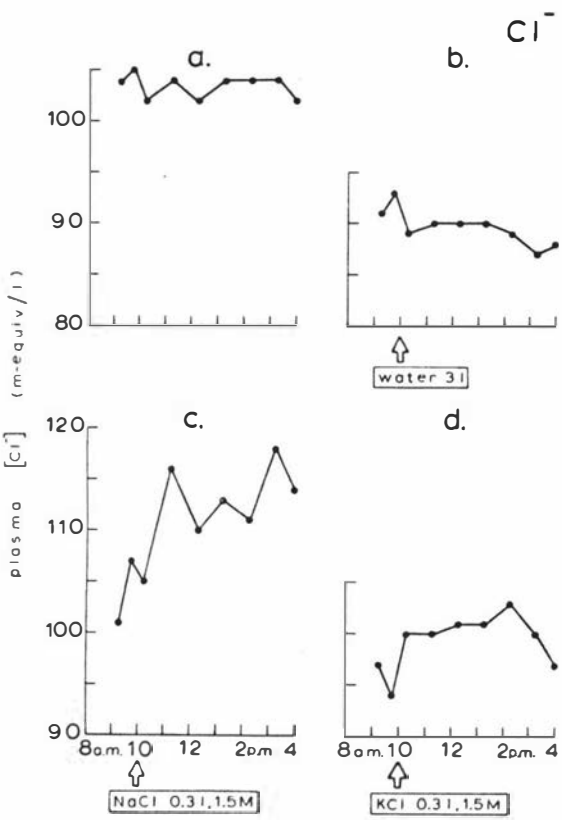

Fig 104. Plasma [Cl^-] following intraruminal infusions: a - control; b - 3 l water; c - NaCl (0.3 l, 1.5M); d - KCl (0.3 l, 1.5M). (a - sheep 2, 1.8.66; b - sheep 2, 1.9.66; c - sheep 1, 26.7.66; d - sheep 13, 24.4.67).

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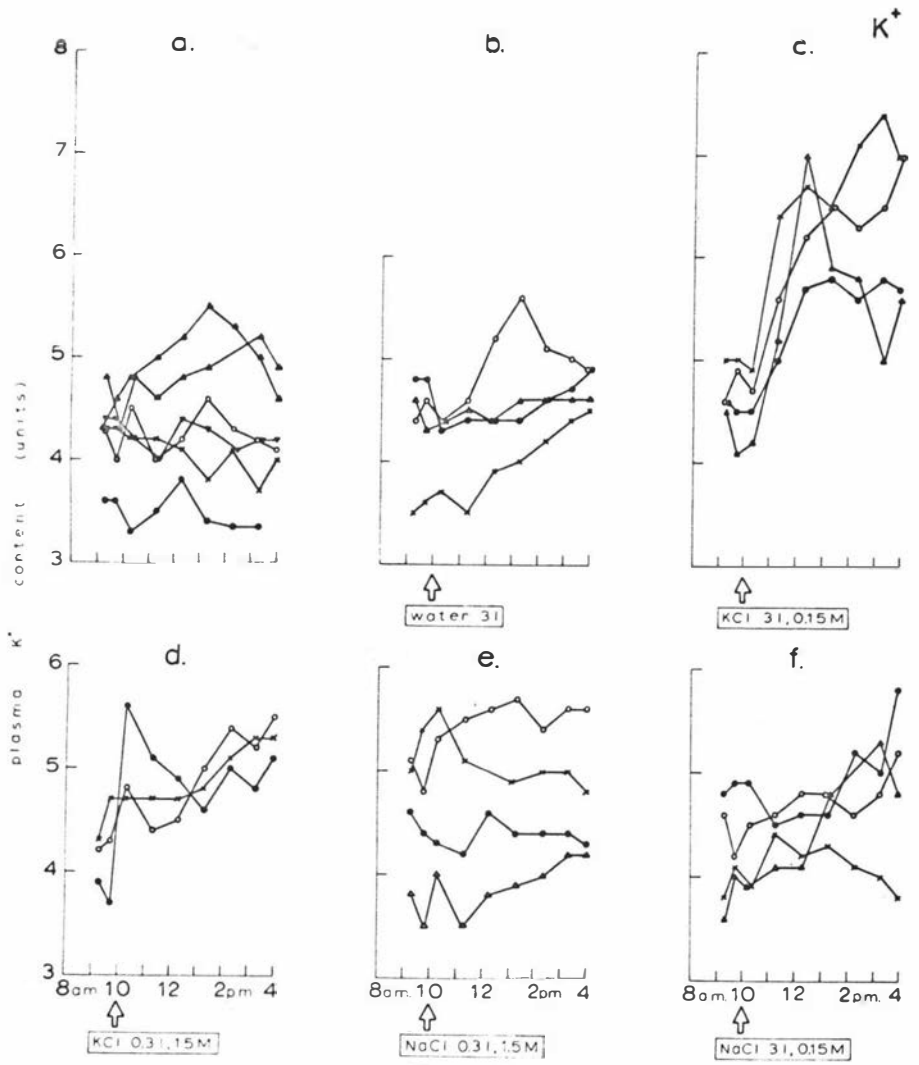

Fig 105. Plasma K^+ content following intraruminal infusions: a - control; b - 3 l water; c - KCl (3 l, 0.15M); d - KCl (0.3 l, 1.5M); e - NaCl (0.3 l, 1.5M); f - NaCl (3 l, 0.15M).
(a - ○ - sheep 1, 24. 8.66
⊙ - sheep 3, 20. 7.66
× - sheep 2, 1. 8.66
△ - sheep 14, 18.10.66
▲ - sheep 13, 1. 5.67
▽ - sheep 12, 17. 4.67

b - ○ - sheep 2, 1. 9.66
⊙ - sheep 1, 22. 8.66
× - sheep 3, 18. 7.66
△ - sheep 2, 22.11.66

c - ○ - sheep 2, 19. 9.66
⊙ - sheep 12, 27. 2.67
× - sheep 1, 12. 9.66
△ - sheep 13, 20. 2.67

d - ○ - sheep 2, 30. 3.67
⊙ - sheep 13, 24. 4.67
× - sheep 12, 19. 4.67

e - ○ - sheep 1, 14. 9.66
⊙ - sheep 1, 26. 7.66
× - sheep 2, 21. 9.66
△ - sheep 2, 2. 8.66

f - ○ - sheep 2, 24.11.66
⊙ - sheep 2, 30. 8.66
× - sheep 1, 25. 7.66
△ - sheep 3, 16. 8.66).

(2) $[Na^+]$ and $[Cl^-]$

Plasma $[Na^+]$ changes (Fig 103) did not always parallel those of O.P. and at times were considerably greater, particularly after water infusion and in the controls, in both of which cases the changes in O.P. were small. However, after the electrolyte infusions, $[Na^+]$ and O.P. in general showed a similar trend, although there were greater and more abrupt changes in the $[Na^+]$ (Fig 103c). With KCl (3 l, 0.15M) there was slower increase of $[Na^+]$ compared with O.P. over the first 2 hours, when $[K^+]$ increased markedly (Fig 103d); however, the difference was greater than the increase in $[K^+]$.

Plasma $[Cl^-]$ (Fig 104) remained almost unchanged on control days, but after a water infusion, decreased slightly. Following infusion of the electrolytes, in general $[Cl^-]$ and O.P. were similar, but not precisely so. An exception was after KCl (0.3 l, 1.5M), (Fig 104d), where $[Cl^-]$ increased to a peak, then decreased while O.P. kept increasing.

(3) $[K^+]$ and K^+ content

On control days and after water infusion, the trends in plasma $[K^+]$ and K^+ content were variable. For the controls, on 2 days there was an increase in $[K^+]$ (Fig 106a), on one there was a decrease and on 3 there was no change (Fig 106b), while for the water infusion, 2 increased and 2 decreased (Fig 106c). There was a similar lack of pattern for K^+ content (Fig 105a,b).

The two KCl infusions produced changes in $[K^+]$ and K^+ content of quite different time course and magnitude. After KCl (3 l, 0.15M), the

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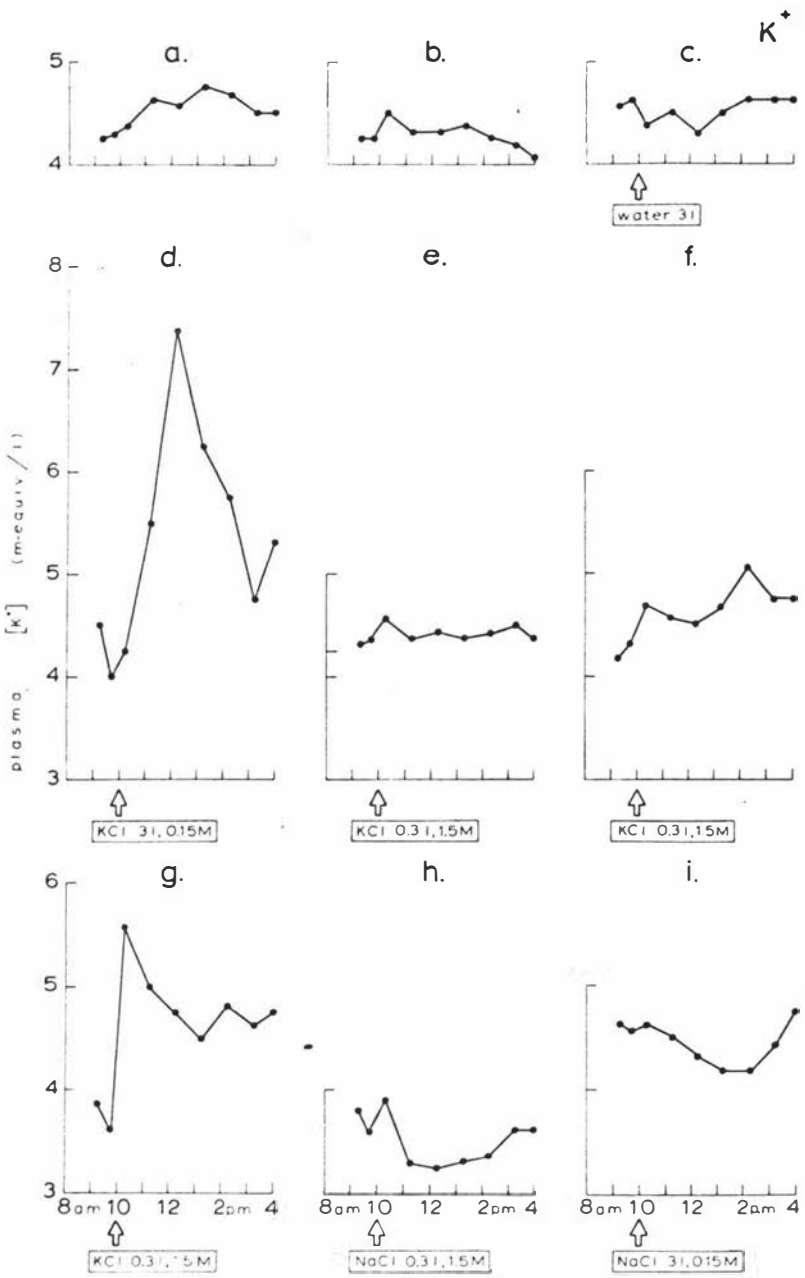

Fig 106. Plasma $[K^+]$ following intraruminal infusions: a,b - control; c - 3 l water; d - KCl (3 l, 0.15M); e-g - KCl (0.3 l, 1.5M); h - NaCl (0.3 l, 1.5M); i - NaCl (3 l, 0.15M). Note variable changes in controls and after water infusion (a-c); a large increase in $[K^+]$ after 0.15M KCl (d), less after 1.5M KCl (e-g) and with a variable time course in the latter; a fall in $[K^+]$ after both NaCl infusions. (a - sheep 13, 1.5.67; b - sheep 1, 24.8.66; c - sheep 2, 22.11.66; d - sheep 13, 20.2.67; e - sheep 12, 19.4.67; f - sheep 2, 30.3.67; g - sheep 13, 24.4.67; h - sheep 2, 2.8.66; i - sheep 2, 24.11.66).

plasma $[K^+]$ was unchanged for 15 minutes, but increased steeply over the next 2 hours to a maximum of 5.5-7.4 m-equiv/l, corresponding to an increase of 1.1, 1.5, 2.2 and 3.1 m-equiv/l. Subsequently, the concentration decreased at a variable rate, but not to the original level (Fig 106d). Changes in K^+ content were similar, remaining constant for 15 minutes, but increasing to 130-140% of the initial level over the next 2-3 hours (Fig 105c). Thereafter on 2 days when K^+ excretion was lower and the plasma total solute content increased, K^+ content fell to 125%; but on the other 2 there was a further small increase to 140-150%.

After KCl (0.3 l, 1.5M), the changes in plasma $[K^+]$ and K^+ content started earlier as shown by rises, although small on 2 days, during the first 15 minutes. The pattern of $[K^+]$ changes differed on the 3 days. On one day, $[K^+]$ showed no substantial change all day (Fig 106e), on a second, a second increase occurred after 3 hours (Fig 106f); while on the third day, there was a maximum increase of 1.8 m-equiv/l at 15 minutes, followed by a decline (Fig 106g). K^+ content followed $[K^+]$ over the first 2 hours after the infusion, but for the latter 4 hours there was a more uniform slow increase (Fig 105d). At 6 hours, the K^+ content was 118%, 127% and 134% of the initial value, the smaller increase occurring on a day of lower increase in plasma total solutes.

Both NaCl infusions caused marked drops in plasma $[K^+]$. NaCl (0.3 l, 1.5M) lowered $[K^+]$ on 3 of the 4 days for up to 4 hours by 0.5-1.0 m-equiv/l (Fig 106h); on the fourth it was unchanged all day. Since plasma volume increased considerably on all days, the change in K^+ content was more variable, (Fig 105e). On the day of constant $[K^+]$, K^+ content increased immediately and remained elevated all day; on 2 days there was a decrease to

90-95% for 1-2 hours; on one day K^+ content increased only at the end of the period.

On all 4 days, NaCl (3 l, 0.15M) reduced the $[K^+]$ over the first half of the post-infusion period by up to 0.8 m-equiv/l (Fig 106i). With the concurrent increase in plasma volume, K^+ content increased on 3 days by about 10%, but on the fourth, a day on which urinary K^+ excretion declined as in the controls, there was an increase in K^+ content of 10% (Fig 105f). Over the last 3 hours of the experiment, $[K^+]$ increased again to the original preinfusion level. Over this time, K^+ content increased on 3 days to 118%, 121% and 126%, but returned to the initial level on one day.

DISCUSSION

Interpretation of experiments of this type is hindered by the limitations of the techniques. An example of this is the lack of parallelism in changes in plasma O.P. and $[Na^+]$ where these are relatively small. O.P. determinations were very accurate, to 1 mosm/kg, whereas the smallest detectable increment in $[Na^+]$ was 2.5 m-equiv/l, and the estimations made to 5.0 m-equiv/l with confidence.

The second problem was the lack of constancy in the control experiments, and the variation between days. If the results are to be analysed, some compromise is necessary, and a base line for the infusions has to be chosen from the control experiments. In the case of Na^+ excretion, there appeared to be two types of response depending on the initial rate. In the case of K^+, the other important cation, on 5 of 6 control days K^+ declined steadily by 13-29%, but on the sixth increased by 2%. The average would be a decline of 16% for all 6, but of 20% for the 5 similar days. Of

the two, 16% was chosen, being the overall average. For the NaCl infusions, the effect on the urinary K^+ excretion in excess of the controls of using a base line of 16% decline and not of 20% is shown in Table 22.

These limitations must be borne in mind in the following discussion in which the treatments will be considered as entities.

(1) Control

All the experiments reported in this chapter were performed during the last 8 hours of a 24 hour day which started with a 3 hour feed between 4-7 p.m. Thus, they were commenced after a fast of approximately 13 hours, and the control experiment was, in fact, observation of the changes associated with a fast of $13\frac{1}{2}$-$21\frac{1}{2}$ hours. The once-daily feeding experiments had shown that this period was one of the most constant for urine excretion and plasma composition for the day, and that infusions performed around feeding time, such as those of Keynes and Harrison (1967), would be difficult to interpret.

Plasma changes were small and followed no trend except for O.P. which decreased slowly. Although urinary excretion was not constant, the variation was systematic. Urine volume varied less than $\pm$ 10% of that predicted. Na^+ excretion followed one of three patterns, one for high excretion and two for low excretion. Total solutes showed similar changes to those in urine flow on 4 of the days; K^+ and HCO_3^- declined progressively 13-29% and 15-34% respectively. Urea increased 75-100%. pH decreased a little towards the end of the observation period. Although excretion was not constant, the changes were small compared with the infusions, and interpretation of the infusion results was not usually difficult.

(2) Water infusion

Excretion of water and of electrolytes after water loading is influenced by species differences, the state of hydration, diurnal factors, temperature and by the magnitude of the load. Blood dilution occurs with the slower excretion of moderate loads given at night (Blombert, Gerbrandy, Molhuysen, Devries and Borst, 1951), after large loads (Chanutin, Smith and Mendel, 1924; Cross, 1948), or after inhibition of the diuresis with pitressin (Smirk, 1933o). Species differences are apparent in the height and spread of the water excretion curve in the experiments of Smirk (Heller and Smirk, 1932a,b; Smirk, 1932; Smirk, 1933a,b,c), and the diuresis was increased by cold, and reduced by warmth and previous water depletion. A water diuresis can be inhibited by isotonic reduction of the ECF volume (Cort, 1954). The effect on urine excretion of an intraruminal infusion of 3 litres of water 18 hours after feeding contrasts sharply with that of consumption of a similar volume during feeding, when no large diuresis results. Keynes and Harrison (1967) infused 1 litre at feeding time in sheep without producing a diuresis, however, the animals had access to drinking water, and the normal prandial intake was depressed.

In the present experiments, an average of 74% of the load was excreted in 6½ hours. The fraction excreted from this relatively large load (79-86 ml/kg) compares with that reported by others in ruminants and in certain studies in man and the dog. Moderate loads (2 litres and 50 ml/kg in man and dog respectively) were completely excreted in 4 hours during the day (Chanutin et al.,1924; Klisiecki, Pickford, Rothschild and Verney, 1933; Verney, 1946, 1947; Blombert et al., 1951), while large loads (100 ml/kg in the dog) were more slowly excreted and caused expansion of the ECF volume.

In goats, Andersson (1955) produced a maximum water diuresis of 1200 ml/hour with 7-8 litres of water, and a flow of 400 ml/hour with 5½ litres. In the present experiments, 3 litres produced a diuresis of up to 600-700 ml/hour. Lysov (1960) infused 2-5 litres of water in sheep, and recovered 90% in 5-7 hours.

The time course of the diuresis followed one of two patterns, both of which have been described by other workers: type (a) in which there was a steady decline after the peak, and type (b) in which there was a fairly constant flow after the peak. The sheep in Lysov's experiments showed type (a), while Andersson's goats showed a pattern similar to type (b). The cause of the differing form of the diuresis was not established, but was not an individual characteristic. It may be related to the state of hydration since type (a) diuresis was associated with lower preinfusion urine O.P.

In non-ruminants, in the first hours of a water diuresis some extra Na^+, K^+ and Cl^- were excreted (Chanutin et al., 1924; Klisiecki et al., 1933; Eggleton, 1943; Verney, 1946, 1947; Gross, 1948; Blomhert et al., 1951; Reid and Hills, 1965). In the cow, Sellers and Roepke (1951) found increased Na^+ excretion and a small rise in K^+, Cl^- and $PO_4^{\equiv}$ for 2 hours after water loading. Andersson (1955) described a fall in K^+ and Cl^- excretion in goats undergoing a water diuresis, but believed fasting could account for some of these observations. In the present experiments, total solute excretion increased 10-30%, compared with a small decrease on control days. Urea excretion was about doubled over the controls. Urea recycling from the collecting duct to the loop of Henle ceases during water diuresis, hence urea loss in the urine is usually greater (Ullrich, 1960; Gottschalk,

1961). Small increases in the excretion of K^+ and Cl^- occurred during the maximum diuresis, but overall differed little from the controls. It is not clear why Andersson failed to observe the transient increase in K^+ and Cl^- excretion since there was a similar overall decrease in both as in these sheep. He also failed to note any increase in Na^+ excretion, which contrasts with the prominent changes in Na^+ as well as in HCO_3^- excretion seen in these experiments: an extra 34-53 m-equiv, averaging 47 m-equiv, and 30 m-equiv respectively.

During the water diuresis there was an accompanying natriuresis which might have been solely of renal origin, but could also have resulted from increased Na^+ absorption from the gut along with the water. The measurements made in these experiments were inadequate to differentiate between the two. The greater the urine volume, the greater the Na^+ loss; this does not differentiate between absorption together from the gut, or renal excretion together. This could be determined by examination of the flow and composition of fluid leaving the rumen and that entering the duodenum. In the initial phase, during the peak diuresis, the natriuresis would appear to be of renal origin since on 3 of the 4 days urine $[Na^+]$ fell as the flow increased, but overall the rate of Na^+ excretion increased. On the fourth day there was a failure to lower $[Na^+]$ at this time, however, it did fall later. Less expected was the high Na^+ excretion after the peak diuresis when on 3 days $[Na^+]$ rose to the preinfusion level although the flow rate was still elevated. This could reflect a failure of the renal tubules to reabsorb Na^+ from very low concentrations after 2-3 hours, possibly through a weakened stimulus. On the other hand, it may be excretion of Na^+ absorbed from the gut. This is not negated by the observation of separation of the

water and Na^+ peaks since this also occurs after infusion of 0.15M NaCl. The reason for the different $[Na^+]$ pattern on the fourth day is not known.

Normally in man the anion accompanying the increased cation excretion during a water diuresis is predominantly Cl^-. In the sheep, however, the rise in HCO_3^- was much greater and appeared to follow the Na^+ excretion rather than the water excretion. However, in man initially excreting a HCO_3^--rich urine, Reid and Hills (1965) noted increased excretion of HCO_3^- along with Na^+ and K^+ during maximal water diuresis. They suggest the lower luminal $[HCO_3^-]$ might reduce H^+ secretion, or passive HCO_3^- reabsorption, hence lower Na^+ and K^+ reabsorption in the distal nephron, and increase the excretion of all three ions. On the other hand, HCO_3^- may passively follow the increase in cation excretion, so that the overall rate of H^+ secretion may not be altered, part of the H^+ previously reacting with HCO_3^- being excreted as increased titratable acidity and ammonia. Urine pH fell during the water diuresis (also after NaCl and KCl (3 1, 0.15M)), an observation also made in man by others when there was initially a high pH (Eggleton, 1946; Barclay, Cooke, Kenney and Nutt, 1947; Knudsen, 1960; Reid and Hills, 1965).

The stimulus to the diuresis is usually believed to be the decreased plasma O.P. (Verney, 1946; 1947). Up to 2½ litres of water were absorbed and excreted without any change in plasma volume or composition obviously different from those on control days. Particularly noteworthy was the failure to observe reduced plasma osmolality as the stimulus for the diuresis, except perhaps on one day. On another day, the drop in O.P. was no greater than on a control day, and on two days there was no decrease at all. Nor did an increase in plasma volume appear to be the stimulus

for the diuresis. Others have reported only a small change in plasma volume and O.P. after a moderate water load (Chanutin et al., 1924; Bloomhert et al., 1951). Moreover, Cordova and Lococo (1964) report a diuresis in man after 1 litre of water, beginning after 15-20 minutes, and reaching peak flows of 21.4 and 11.9 ml/min at 40-60 minutes, with no detectable change in PCV, [plasma protein], plasma O.P., Na^+, K^+ and Cl^-; the only decrease was in the albumin : globulin ratio. Thus the present experiments are not alone in failing to demonstrate clearly that reduced plasma osmolality is the stimulus for ADH inhibition. Nevertheless, reports that osmoreceptors in the portal system may play a part in triggering the diuresis after water loading (Haberich, Azis and Nowacki, 1964, 1965, 1966; Aziz, Nowacki and Haberich, 1966; Haberich, Azis and Ohm, 1967; Haberich, 1968) may explain the diuresis in spite of lack of detectable systemic effects.

(3) KCl infusion

Little comparison is possible with other reports of the effect of K^+ infusion since these are largely restricted to intravenous infusion in monogastrics, and to intraruminal infusion when the sheep were being fed. In the latter experiments (Keynes and Harrison, 1967; Dewhurst, Harrison and Keynes, 1968), the effects on Na^+ and K^+ excretion were superimposed on the already large changes associated with feeding.

The excretion of the extra water after both the concentrated and dilute KCl was less than might have been expected. After KCl (0.3 l, 1.5M) the K^+ was eliminated with little increase in urine volume, and even in the case of KCl (3 l, 0.15M) the water excreted averaged 52% which is lower than the fraction excreted after water (74%). Nevertheless, a greater % of the

water load than that of K^+ was excreted during the experimental period, possibly because some K^+ was temporarily absorbed into the intracellular pool rather than being excreted. The difference in water recovery from dilute KCl compared with water infusion is in the range 0.5–1.0 litre. However, after the KCl there was a 10–20% expansion of the plasma volume by the end of the experimental period. If the ECF is expanded to the same extent, this accounts for most of the above difference in water excretion. The time course of the diuresis was similar to type (b) water diuresis - that with a peak followed by a fairly constant flow - but in both phases the flow rate was lower.

If water were absorbed directly from the rumen, one might expect absorption following a water load would be greater and earlier than following dilute KCl infusion since the water would be expected to lower the rumen O.P. by about 120–140 mosm/kg, while the 0.15M KCl would raise it a very small amount. In fact, the observed patterns of water absorption and excretion were similar following both types of infusion if allowance were made for the expansion of the ECF volume by KCl infusion. Consequently, if water is significantly absorbed in the rumen, the rate must be independent of the osmotic gradient. It would appear more likely that the rumen is relatively impermeable to water, and that absorption occurs lower in the digestive tract where osmotic differences have a smaller effect on the rate of water absorption. This could occur either by modification of the composition of the digesta leaving the rumen before reaching the absorbing region, or by absorption in the small intestine following equilibration to isotonicity in the duodenum (Hindle and Code, 1962; Grim, 1962; Fordtran et al., 1965). The similarity in water absorption from the two solutions would depend on their stimulation

of rumen outflow to a comparable extent, as would be likely when the volume infused is the same (Ash, 1962). Further evidence of the low rumen permeability is the lack of shrinkage of the plasma volume during the initial 2-3 hours after the concentrated KCl, even though K^+ absorption was low, suggesting water cannot readily pass into the rumen.

Of particular interest is the different absorption and excretion of K^+ after the two KCl infusions. In the period immediately following infusion, absorption and the onset of excretion were more rapid with the more concentrated KCl, as may be seen from the early rise in plasma K^+ content and in K^+ excretion. On the other hand, over the next 2-3 hours absorption and excretion from the dilute KCl were much greater. Towards the end of the observation period both of these parameters were increasing steadily in the case of the 1.5M KCl, but had reached their peak and were declining in the case of the dilute KCl. At the end of the experimental period, the rise in plasma K^+ content was about one-third less in the group receiving the concentrated KCl and the average K^+ excretion was similarly lower (18% compared with 30% of the infused load).

Some characteristics of the permeability of the rumen to K^+ can be inferred from the above observations. It would seem that direct K^+ absorption through the rumen wall is less than that from other areas of the gut, being small unless there is a considerable increase in rumen $[K^+]$ as would occur immediately after the infusion of the 1.5M KCl. The whole question of the permeability of the rumen wall to water and electrolytes will be discussed further in Chapter 7, the general discussion.

This work verified previous observations that the excretion of K^+

is independent of plasma $[K^+]$, since increases in the former frequently occur with no detectable change in $[K^+]$, or even a decrease. The role of intracellular determinants of K^+ excretion cannot be assessed from these experiments but may be of major significance.

Increased Na^+ excretion occurred at the time of peak K^+ excretion after both KCl infusions, but especially after the dilute KCl. Indeed, the natriuresis after KCl (0.3 l, 1.5M) was similar in magnitude to peaks on control days, but the coincidence with the K^+ peak suggested it was an effect of the infusion. Six hours after either infusion, the plasma total solute content increased to 105-121%, hence some Na^+, the major ECF cation, must have accumulated in the ECF at the expense of Na^+ in the gut or ICF.

There are 5 possible explanations for the increased Na^+ excretion following KCl infusion:

(a) The increased Na^+ excretion could arise from release of ICF Na^+ by K^+ (Sellers and Roepke, 1951; Liddle, Bennett and Forsham, 1953; Laragh and Capeci, 1955).

(b) The extra Na^+ could be derived from enhanced Na^+ absorption from the rumen in the presence of raised $[K^+]$ (Stacy and Warner, 1966; Scott, 1967).

(c) The excreted Na^+ may result from increased outflow of ruminal fluid to more distal areas where Na^+ might be absorbed, particularly for the dilute infusion, when the volume was increased.

(d) For the same infusion, the Na^+ might be of renal origin, secondary to the diuresis.

(e) The Na^+ might be the result of K^+-induced inhibition of the renal Na^+-H^+ exchange (Berliner et al., 1951; Liddle et al., 1953; Anderson and Pickering, 1962).

In the present experiments, it would seem unlikely that the natriuresis was purely of renal origin (d,s) because of the simultaneous increase in the ECF Na^+ content. There may, however, be some loss during the maximum urine flow, since on 2 days of large early natriuresis the plasma total solute content decreased for 2-3 hours. On the other hand, there is a dissociation between Na^+ loss and the urine flow: the $[Na^+]$ in the urine did not fall to the low levels seen during the peak water diuresis; on one day, the Na^+ excretion rose before the urine flow. Direct evidence that K^+-H^+ competition can reduce Na^+ reabsorption is not available, and Liddle et al. (1953) showed titratable acidity and ammonia increased along with the natriuresis and kaliuresis following K phosphate administration.

Nor is it likely that all the Na^+ originated in the gut (b,c). Even when the gut is bypassed by intravenous infusion (see Chapter 6) a natriuresis is produced, confirming reports in a number of species, including sheep and cattle (Anderson and Pickering, 1962; Keynes and Harrison, 1967). The possibility of enhanced absorption cannot be eliminated in the dilute infusions, but was delayed considerably, if it occurred, after KCl (0.3 l, 1.5M), since the plasma total solute content remained almost unchanged for 2-3 hours.

A large contribution of ICF Na^+ appears to be occurring. In general, the larger the increase in plasma K^+ content, the larger the increase in total solute content, which would be principally Na^+ and Cl^- increase, and perhaps some HCO_3^-. This would be compatible with part of the absorbed K^+ entering the ICF in exchange for Na^+. The displacement of either Na^+ or K^+ from the ICF by infusion of the other cation has been observed in other species (Elkington and Winkler, 1944; Gamble, 1947; Elkington et al.,

1948; Laragh and Capeci, 1955; Irvine et al., 1960; Reinhardt and Behrenbeck, 1967).

Cl^- excretion differed little from K^+ excretion except where there was a large natriuresis: the overall average Cl^- excretion for the 0.15M KCl was 31% (the same as the K^+), but on individual days it exceeded the K^+ when Na^+ was high. After KCl (0.3 l, 1.5M), Cl^- excretion was a little lower than K^+ on 2 days, and considerably lower on one day of low preinfusion Cl^- excretion, perhaps related to an altered acid-base status.

Urine pH appeared to depend on the $[HCO_3^-]$. During the diuresis following the 3 litres of dilute KCl, both $[HCO_3^-]$ and pH of the urine decreased, the latter becoming acid on one day when the $[HCO_3^-]$ fell to 2-10 m-equiv/l. After the concentrated KCl, both urine pH and $[HCO_3^-]$ increased a little.

Irrespective of the change in $[HCO_3^-]$, HCO_3^- excretion increased 40-70% on 3 of the 4 days after KCl (3 l, 0.15M), and on 2 of 3 days after KCl (0.3 l, 1.5M), but on the other two days it was indistinguishable from the controls. The increase could result from increased filtered load of HCO_3^-, reduced H^+ secretion, or excretion of HCO_3^- in company with the extra cations without overall change in the secretion of H^+. Competition between K^+ and H^+ is frequently invoked as the cause of the increased HCO_3^- excretion after K^+ loading (Berliner et al., 1951; Roberts et al., 1953; Anderson and Pickering, 1962). However, in the present experiments three observations do not support this hypothesis: increased HCO_3^- did not invariably occur; the rise in HCO_3^- excretion did not parallel that of K^+, but rather the extra $Na^+ + K^+$; on one day after the dilute KCl, the increased HCO_3^- preceded the

rise in K^+. It would appear, therefore, that the HCO_3^- excretion is dependent on the excretion of either cation rather than the secretion rate of H^+. Measurement of changes in H^+ secretion rate after infusion would help to distinguish between the relative importance of the two factors.

(4) NaCl infusion

The only studies of intraruminal saline infusion in ruminants involving sequential sampling were carried out during feeding (Keynes and Harrison, 1967; Dewhurst et al., 1968) so that the effects of the saline infusion alone cannot be visualized. Oral saline loads in monogastric animals were excreted more slowly than similar volumes of water (Adolph, 1923; Gross, 1948; Blombert et al., 1951; Kellogg, Bureak and Isselbacher, 1954; McCance and Widdowson, 1963), so that plasma volume expansion was greater and the constituents were diluted more (Haldane and Priestley, 1916; Chanutin et al., 1924; Lyons, Jacobson and Avery, 1944; Blombert et al., 1951; Markley, Bocanegra, Morales and Chiapperi, 1957). The dog and rat excreted most of the Na^+ and water together after a delay of about one hour (Gross, 1948; Kellogg et al., 1954), but man had a variable pattern of excretion according to diurnal and postural factors. During the day, a hypotonic urine was formed for the first 1½ hours, followed by a mild, protracted saline diuresis, but at night there was merely a brief water diuresis (Blombert et al., 1951). Markley et al. (1957) also noted a diurnal variation in response, and that a saline diuresis occurred in recumbent subjects. Compared with isotonic saline, solid NaCl or hypertonic saline was excreted more slowly in the dog (Gross, 1948), and extra water was lost.

In the present experiments, water excretion following the two NaCl infusions was markedly different. After the NaCl (0.3 l, 1.5M) there was an osmotic diuresis of about 5 ml of urine for each m-equiv of Na^+. The urine flow did not increase until Na^+ excretion began. On the other hand, solute and water were independently excreted from the NaCl (3 l, 0.15M); either could show the larger fractional excretion. The excretion of a dilute urine for 2-3 hours, followed by a mild saline diuresis, as seen in these intra-ruminal infusions of isotonic NaCl, is similar to the response following an oral saline load in man during the day (Blomhert et al., 1951). The time course of the diuresis was very variable - 3 patterns on 4 days - but in each case the onset was delayed around 30 minutes, as for the other two 3 litre infusions.

Water excreted from the dilute NaCl was considerably less than from a similar volume of water: an average of 29% compared with 74%, confirming the observations in several species (Adolph, 1923; Gross, 1948; Blomhert et al., 1951; Kellogg et al., 1954; McCance and Widdowson, 1963). Furthermore, in agreement with Adolph (1923), it was also less than from the dilute KCl infusion. Over the first 3½ hours after the NaCl infusion, the time of peak diuresis after water and KCl (3 l, 0.15M), the extra water excretion was 200-400 ml on 3 days, and 800 ml on the fourth. However, this need not indicate water absorption any more slowly from the NaCl than from the other infusions since over this period the plasma volume was expanded to 113-125% of the preinfusion level; if equally distributed through the ECF, this would account for 0.8-1.3 litres of water. Over this period of time, the water excreted in excess of the basal amount, plus the water accumulated in the ECF, ranged from 1200-1900 ml, approximating the water excreted during

the same time from a water diuresis (1000-1750 ml). Over the whole $6\frac{1}{2}$ hour post-infusion period, the water excreted together with the increase in ECF volume also were in the same range (1500-2500 ml) as the water excreted from a 3 litre water load. It seems, therefore, that the difference in the extent of the diuresis after dilute NaCl compared with water or dilute KCl is due to a difference in renal response rather than in absorption from the gut. The above evidence further supports the conclusion made earlier from the KCl infusion that water absorption from the gut after the 3 litre infusions seems independent of the osmotic gradient.

Although the pattern of Na^+ excretion was very similar from day to day, and with both NaCl infusions, the number of variables possibly involved made difficult accurate quantitative comparison of the effects of the two concentrations of NaCl (Table 21). Two variables in particular - the preinfusion Na^+ excretion rate and adaptation after repeated infusions of Na^+ - may be of importance in influencing the excretion of infused Na^+. The importance of each of these needs to be examined so that it can be taken into account in comparing the response to the two NaCl infusions. In the present experiments, the picture is complicated by there being in the group receiving NaCl (0.3 l, 1.5M) three days when the sheep had previously been exposed to a Na^+ load compared with two such days in the other group, however, one of the three was only one day after the previous infusion, probably too soon for any adaptive changes to have occurred. Overall, it appeared that the NaCl (0.3 l, 1.5M) resulted in slightly greater elimination of Na^+.

Over the first 3 hours, before Na^+ excretion had reached its maximum, the plasma total solute content increased to a peak; it then declined after excretion had reached its height. The NaCl (0.3 l, 1.5M)

caused a marked increase in plasma O.P., but only the same expansion of the volume as the other NaCl infusion; the plasma total solute content was greater following the concentrated NaCl both during the pre-natriuresis period and also at the end of the experimental period. With this more concentrated infusion, both absorption and excretion appeared to be greater than with the NaCl (3 l, 0.15M). This is in contrast with the situation over the 10 hours after NaCl administration in dogs (Gross, 1948).

Na^+ excretion can be increased by an increase in GFR, or a decrease in tubular reabsorption, or both. There is a species difference in the lability of the GFR, and Potter (1966, 1968) has indicated that there may even be a breed difference in sheep during intravenous NaCl infusion. The GFR may have increased with such a large increase in the plasma volume; however, the size of the natriuresis is such that a decrease in fractional tubular reabsorption probably also is involved.

The stimulus for the decreased tubular reabsorption could be related either to the expansion of the plasma volume or to reduction in the rate of aldosterone secretion. Isosmotic expansion of the ECF has been shown to induce natriuresis via a humoral substance which decreases the proximal tubular Na^+ reabsorption (Mills *et al.*, 1961; de Wardener *et al.*, 1961; Levinsky and Lalone, 1963; Cortney *et al.*, 1965; Cirksena *et al.*, 1965; Dirks *et al.*, 1965; Watson, 1966; Landwehr *et al.*, 1967). Although aldosterone secretion appears to be more concerned with long term adjustments to altered Na^+ status (see Chapter 1), a decrease in its secretion in these experiments cannot be ruled out, since the strongest stimuli to its secretion are of haemodynamic origin (e.g. haemorrhage).

The nature of the stimulus for the release of any of these humoral

factors has not been clearly established, but they appear related to the circulation. The sensitivity of the receptor mechanism seems to vary with the physiological status. The pre-existing level of Na^+ excretion may alter the sensitivity of the receptors to changing ECF volume, since Espiner, Tunci, Jagger, Pauk and Lauler (1967) observed a smaller increase in aldosterone secretion in persons on a low Na^+ diet compared with those on a high Na^+ diet after contraction of the plasma volume with ethacrynic acid. The current work suggests that, if expansion of the plasma volume is the stimulus, the receptor mechanism is of low sensitivity since this volume increased up to 25% before excretion of absorbed Na^+ was under way. An alternative for the receptor could be a parameter of flow, possibly in the central venous pool (see Chapter 7).

Changes in the distribution of K^+ were more variable. On 7 of the 8 days, K^+ excretion over the experimental period exceeded that on control days, by 9-40 m-equiv. As well, a rise in plasma K^+ content was observed on all but one day. Extra K^+ of either cell or gut origin must be excreted or accumulated in the ECF, or both. Oral NaCl-induced kaliuresis or displacement of ICF K^+ into the ECF has been reported in man (Gamble, 1947), the cow (Sellers and Roepke, 1951), the rat (Kellogg et al., 1954) and the dog (Reinhardt and Behrenbeck, 1967). Thus, at least part of the extra K^+ is likely to be of cell origin. The contribution of gut K^+ cannot be gauged in these experiments but is more probably of significance after the 3 litre infusions.

The stimulus to the kidney for the excretion of this displaced K^+ is obscure. Although intraruminal infusion of NaCl into cows increased the plasma $[K^+]$ (Sellers and Roepke, 1951), thus affording a possible

explanation for the kaliuresis, in 7 of 8 of the present experiments, the plasma $[K^+]$ actually decreased by up to 1.0 m-equiv/l over the first 4 hours, coincident with the increase in plasma volume. Furthermore, the amount of K^+ excreted did not appear to be related to the Na^+ excretion. In addition, if intracellular displacement of K^+ is occurring, the stimulus cannot be raised ICF $[K^+]$ unless large osmotic water shifts also take place, which is unlikely since plasma O.P. changed little after 0.15M NaCl.

Excretion of the administered anion, Cl^-, frequently paralleled that of Na^+, however, HCO_3^- excretion was always increased by the NaCl infusions and affected the recovery of Cl^- in the urine. As discussed for the KCl infusions above, increased HCO_3^- excretion accompanies that of Na^+ as readily as it does K^+, and does not appear to be specifically related to K^+ excretion but rather to increased cation loss.

One of the more remarkable features of the NaCl infusions was the long delay before the Na^+ was excreted in the urine. Since this is not the usual observation in monogastric animals, it was decided to conduct further NaCl loading experiments bypassing the rumen entirely. It was hoped these infusions would cast further light on the delayed renal response to the saline load.

CHAPTER 5

INFUSION OF NaCl INTO THE DUODENUM OF SHEEP

Intraduodenal infusion of NaCl was undertaken to determine whether intestinal absorption of Na^+ resulted in more rapid and complete renal elimination than that following intraruminal administration. For this reason, the experimental conditions were maintained as for the intraruminal infusions, but the study was restricted to the examination of the effects of Na^+ loading at two different levels. These were infused in small and comparable volumes since it was feared the administration of large volumes could cause gut distention or rapid flow artefacts.

Short-term changes in urine and blood composition after intraduodenal saline loading do not appear to be reported in the literature. However, more direct studies of absorption from the small and large intestine in monogastric animals have been made frequently and have demonstrated active Na^+ absorption (Curran and Solomon, 1957; Cooperstein and Brockman, 1959; Curran, 1960; Clarkson et al., 1961; Schultz and Zalusky, 1964a,b). Bruce et al. (1966) collected digesta at various levels of the gut of sheep to study the sites of net addition and absorption of water and electrolytes on a daily basis. They concluded that net absorption of K^+, Cl^- and water occurred principally in the small intestine, and of Na^+ mainly in the large intestine, together with much of the remaining K^+, Cl^- and water.

MATERIALS AND METHODS

Na^+ loading with 1.5M NaCl was selected for study against a control of 0.15M NaCl of the same volume. Because the Na^+ load which could be

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Table 25. Intraduodenal NaCl infusion: rates of infusion and total dose.

Sheep	Date	Conc. (M)	Rate of infusion (ml/min)	Total load	
				Water (ml)	Na^+ (m-equiv)
15	31. 1.67	1.5	30	300	450
	2. 2.67	0.15	30	300	45
13	22. 3.67	1.5	20	200	300
	20. 3.67	0.15	20	200	30
12	5. 4.67	1.5	20	200	300
	3. 4.67	0.15	20	200	30
14	3.10.66	1.5	1	150	225
	20.10.66	1.5	50 ml then 1 ml/min	50 + 150	300
	3.11.66	1.5	20	200	300
	16.11.66	0.15	20	200	30

tolerated by the sheep without causing diarrhoea had to be determined, there was a variation in the rate and volume of the infusion, as shown in Table 25. In fact, only the infusion of 300 ml of 1.5M NaCl (sheep 15) appeared to cause the animal any distress or produce significant diarrhoea.

The experimental conditions were as similar as possible to those for the intraruminal infusions (see Chapter 4): feed was last offered at 4-7 p.m., the infusions were commenced at 10 a.m. and no drinking water was available during the experimental period.

Animals

The 4 Romney ewes (weight 29-32 kg) were housed and fed as described in Chapter 4. Each sheep had a permanent perspex duodenal cannula inserted about 10 cm below the pylorus and brought to the exterior through a stab incision in the skin. Although the skin around the cannula was kept free of wool and cleaned regularly, after some months the cannula usually began leaking, at which time the sheep was discarded. Two animals (12, 13) also had rumen fistulae.

Infusion

Just prior to the infusion, the usual screw cap was removed from the cannula and, after the insertion of a glass rod to remove any plugs of digesta, was quickly replaced by a cap constructed to take connecting internal and external tubing which were filled with the warmed infusion solution. The external polythene tube fitted over the end of a Luerlock syringe and was clamped with a bulldog clip; the internal short, soft rubber tube was closed at the end but had a lateral slit which acted as a Bunsen valve. The

changeover of the two caps did not disturb the animal unless the skin was touched.

For infusions of 20-30 ml/min, the large syringe was operated manually with reference to a stop watch. For infusions of 1 ml/min, a Sage infusion pump was used to move the plunger of the syringe at a constant speed. At the end of the infusion, the tubing was reclamped and not recapped until the end of the experiment.

Sample collection

As described in Chapter 4.

Analytical methods

As described in Chapters 2 and 3.

Calculation of results

As described in Chapters 2 and 4.

RESULTS

(1) Control

See Chapter 4.

(2) 0.15M NaCl

Two distinct responses in Na^+ metabolism followed the 30 or 45 m-equiv dose of Na^+, related to the preinfusion Na^+ excretion. On the 2 days when it was low - 0.13 and 0.04 m-equiv/30 min - all the administered Na^+ was retained, the Na^+ excretion remained low, and the plasma volume and

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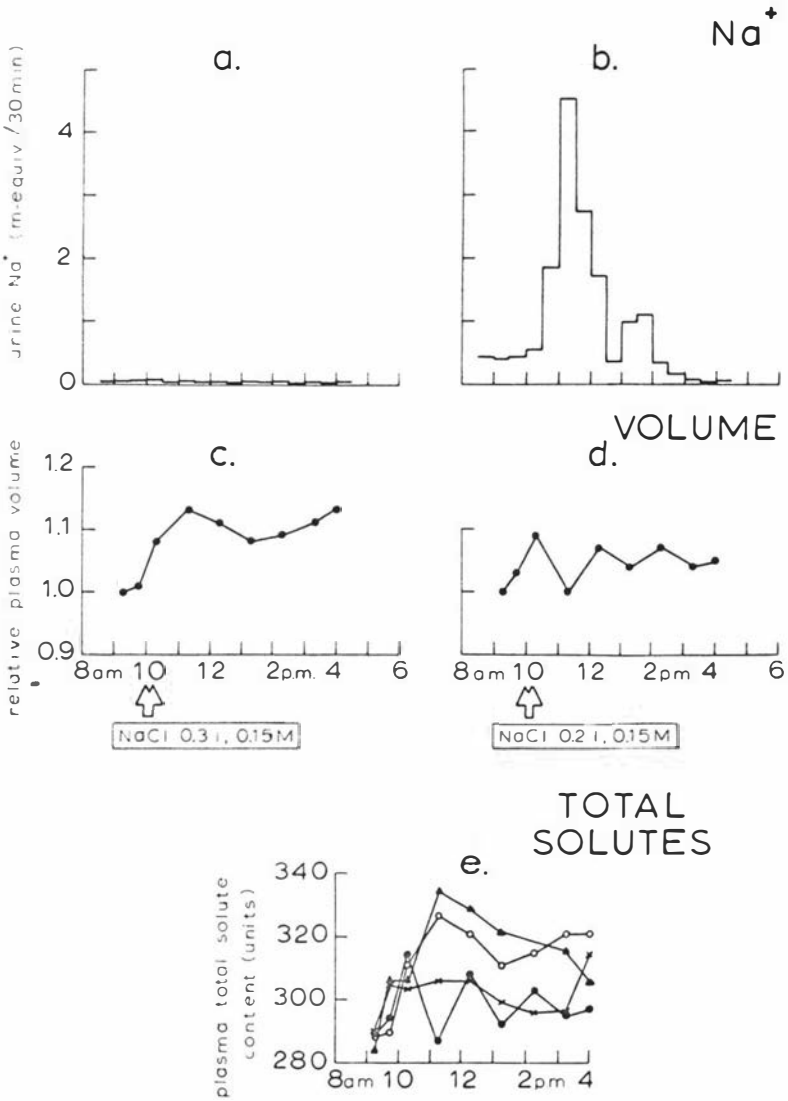

Fig 107. Urine Na^+ excretion, relative plasma volume and total solute content following intraduodenal 0.15M NaCl infusion. Note low preinfusion Na^+ excretion, complete Na^+ retention and raised plasma volume in a,c; higher preinfusion Na^+ excretion, a biphasic natriuresis and little change in plasma volume in b,d; raised total solutes after low Na^+ (▲ , ○) but not higher (● , ×). (a,c - sheep 15, 2.2.67; b,d - sheep 12, 3.4.67; e - ▲- sheep 14, 16.11.66; ○- sheep 15, 2.2.67; ●- sheep 12, 3.4.67; × - sheep 13, 20.3.67).

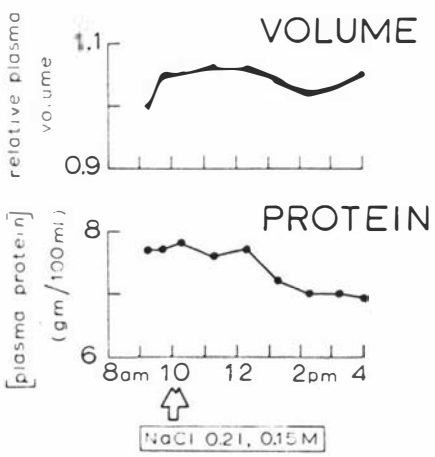

Fig 108. Relative plasma volume and [plasma protein] following intraduodenal 0.15M NaCl infusion. Note the fall in [plasma protein] over the final 3-4 hours not accompanied by a fall in relative plasma volume. (Sheep 13, 20.3.67).

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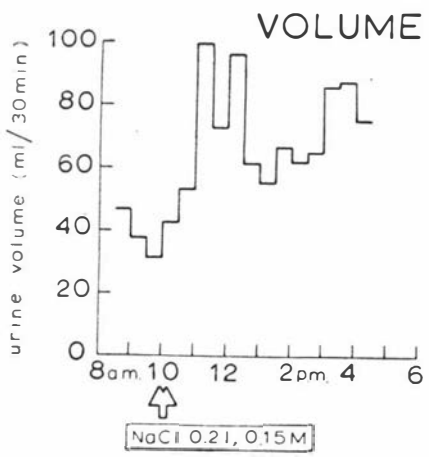
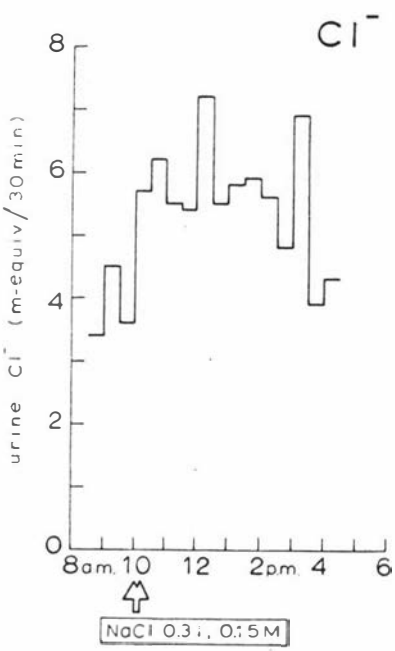


Fig 109. Urine volume following intraduodenal 0.15M NaCl infusion. Note the biphasic diuresis. (Sheep 13, 20.3.67).

Fig 110. Urine Cl^- excretion following intraduodenal 0.15M NaCl infusion. (Sheep 15, 2.2.67).



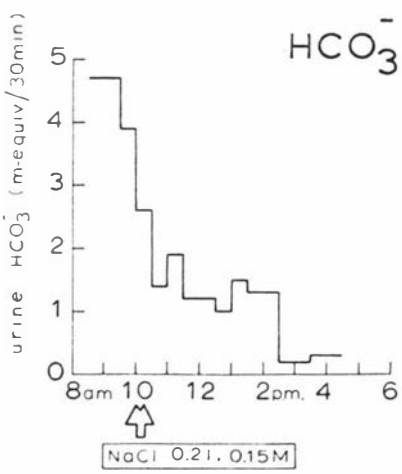


Fig 111. Urine HCO_3^- excretion following intraduodenal 0.15M NaCl infusion. Note the marked drop for 1½ hours after infusion and the overall falling excretion. (Sheep 14, 16.11.66).

total solute content increased early and remained elevated for several hours (Fig 107a,c,e). In contrast, when Na^+ excretion was higher - 0.4 and 0.7 m-equiv/30 min - 41% and 94% of the Na^+ was excreted in the urine, and the plasma volume and total solutes were almost unchanged (Fig 107b,d,e). Na^+ excretion began in the first 30 minutes and followed a biphasic course with the first peak higher than the second.

As in the controls, the plasma O.P. decreased, but was not paralleled by $[Na^+]$ or $[Cl^-]$. Unlike previous observations, on 3 days [plasma protein] changes failed to support the calculated relative plasma volume: over the final 3-4 hours, the [plasma protein] decreased while the calculated plasma volume did not increase at the same time (Fig 108).

Urine flow was qualitatively similar regardless of the Na^+ excretion pattern: the urine volume increased after 30-60 minutes, and formed two peaks (Fig 109). However, more than the infused volume was excreted when there was a natriuresis, and less than the infusion volume in the other two cases. Water and total solute excretion were unrelated. Total solutes increased during a natriuresis, but otherwise followed K^+ excretion.

The fractional Cl^- excretion was independent of that for Na^+, ranging from 7% to 105% of the infused Cl^-. In all, Cl^- excretion was raised, at least during the first 2 hours after infusion and usually for much longer (Fig 110). HCO_3^- excretion was estimated on one day when there was no natriuresis, and showed a marked drop for 1-1½ hours after the infusion, and overall a 61% decline (Fig 111). Urine pH and urea excretion were no different from the controls.

On all 4 days, the plasma $[K^+]$ and K^+ content increased and

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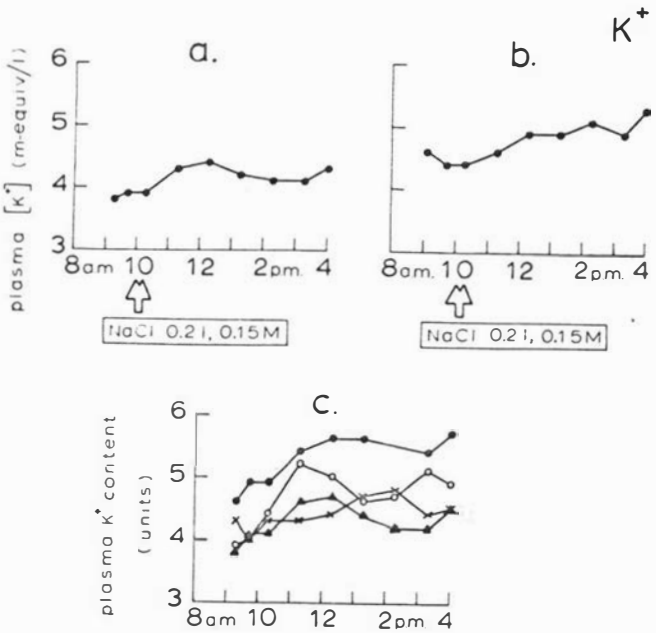

Fig 112. Plasma $[K^+]$ and K^+ content following intraduodenal 0.15M NaCl infusion. Note the increase in $[K^+]$ in a and b but the differing time course; maximum K^+ content after 1-2 hours, but higher values after 6 hours when Na^+ was retained (●, ○). (a - sheep 13, 20.3.67; b - sheep 14, 16.11.66; c - ● - sheep 14, 16.11.66; ○ - sheep 15, 2.2.67; × - sheep 12, 3.4.67; ▲ - sheep 13, 20.3.67).

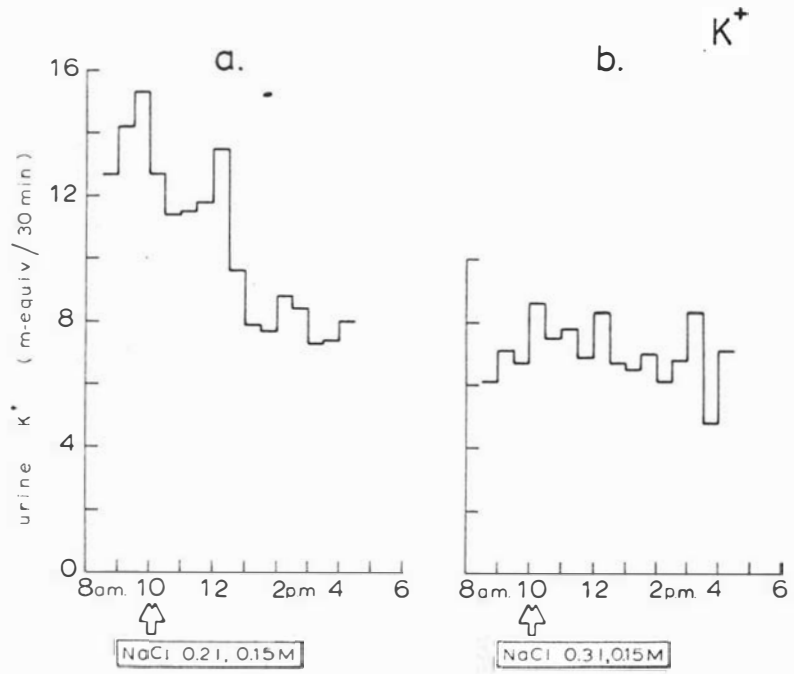

Fig 113. Urine K^+ excretion following intraduodenal 0.15M NaCl infusion. Note the two patterns of excretion in a, b. (a - sheep 12, 3.4.67; b - sheep 15, 2.2.67).

remained elevated (Fig 112), although the time course was variable. The K^+ content showed a maximum rise to 117%, 118% and 133% at 1-2 hours, and to 114% at 4 hours in the other. When Na^+ was completely retained, the increase in K^+ content at 6 hours was greater, to 118% and 123% compared with 107% and 113% when there was a natriuresis. K^+ excretion followed one of two basic patterns which were also seen with other NaCl infusions (Fig 113a,b), either decreasing almost continually, more slowly towards the end of the experiment, or increasing for a variable time after the infusion. On the 2 days when some infused Na^+ was excreted, K^+ excretion increased a little between the two Na^+ peaks (Fig 113a). The total K^+ excretion appeared independent of the total Na^+ and whether a natriuresis occurred; in addition, it differed little from that on control days: on 2 days, K^+ excretion declined by 29% and 25%, within the control range, and on 2 days there was a decrease of 7% and an increase of 6%, representing extra K^+ loss.

(3) 1.5M NaCl

It is obviously impossible to present a Na^+ load to the duodenum in vivo under conditions comparable to those in which it was presented to the rumen since the surface area involved and the dilution by local contents are different. Since both these factors are unpredictable, the simplest approach would be to use the same total Na^+ load. However, even this was unsuitable since watery diarrhoea commenced approximately 30 minutes after the 300 ml infusion and continued for the entire 6½ hour post-infusion period. Consequently, the load infused had to be smaller, and even on 2 days a brief period of extra faecal water loss occurred. In sheep 13, on 22.3.67, diarrhoea occurred for only 1½ hours, from 12.30 to 2 p.m. In sheep 12 (5.4.67) the faeces were slightly moister from 3 to 3.30 p.m.

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Table 26. Intraduodenal 1.5M NaCl infusion: Na^+ excretion related to other parameters.

Date	Na^+ load (m-equiv)	% excretion	Excretion above basal (m-equiv)	Preinfusion Na^+ (m-equiv/30 min)	Delay in onset (hours)	Previous infusion (days)
3.10.66	225	61	144.2	2.0	1½	-
20.10.66	300	51	152.2	0.30	2	17
3.11.66	300	49	145.8	0.18	2	14
22. 3.67	300	42	124.9	0.34	1	2
5.4.67	300	42	126.1	0.01	1½	2
31. 1.67	450	27	121.8	3.20	0	-

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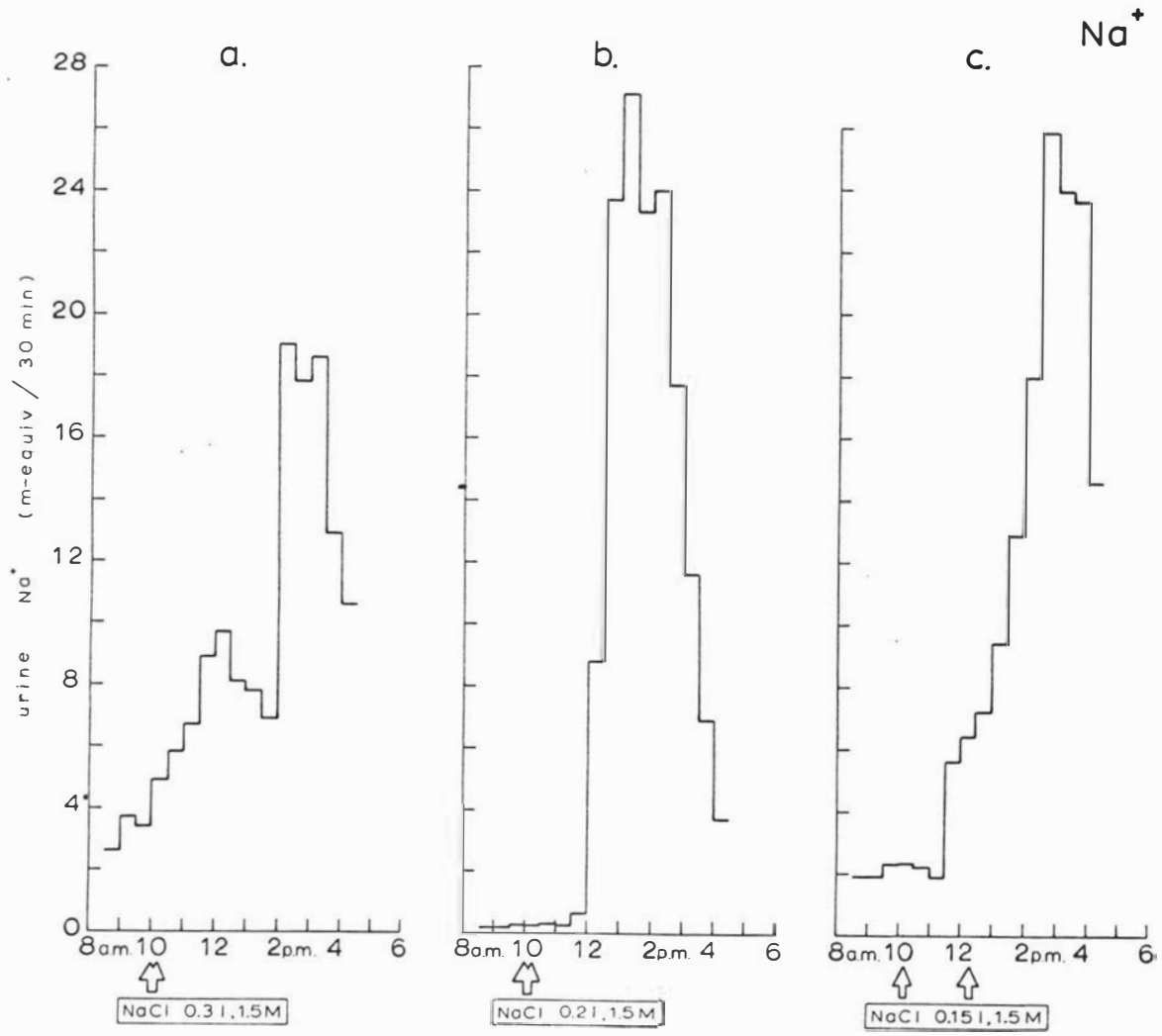

Fig 114. Urine Na^+ excretion following intraduodenal 1.5M NaCl infusion: a - 450 m-equiv Na^+, b - 300 m-equiv Na^+, c - 225 m-equiv Na^+. Note 1-2 hours delay in onset of the natriuresis in b,c but not in a; a single Na^+ peak with lesser loads (b,c) but biphasic excretion with the largest load (a); earlier peak excretion with the shorter infusions (b compared with c). (a - sheep 15, 31.1.67; b - sheep 14, 3.11.66; c - sheep 14, 3.10.66).

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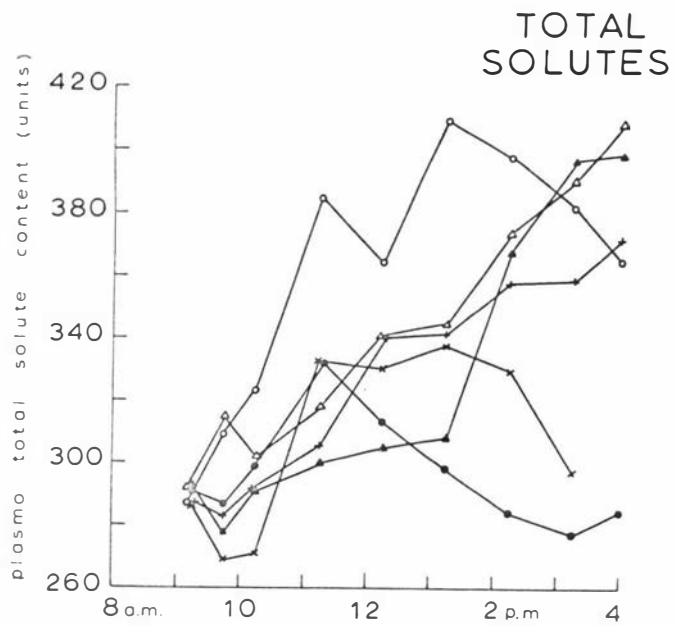

Fig 115. Plasma total solute content following intraduodenal 1.5M NaCl infusion: 225 m-equiv Na^+ (△), 300 m-equiv Na^+ (+, o, O, x), 450 m-equiv Na^+ (▲). (△ - sheep 14, 3.10.66; + - sheep 14, 20.10.66; o - sheep 14, 3.11.66; O - sheep 13, 22.3.67; x - sheep 12, 5.4.67; ▲ - sheep 15, 31.1.67).

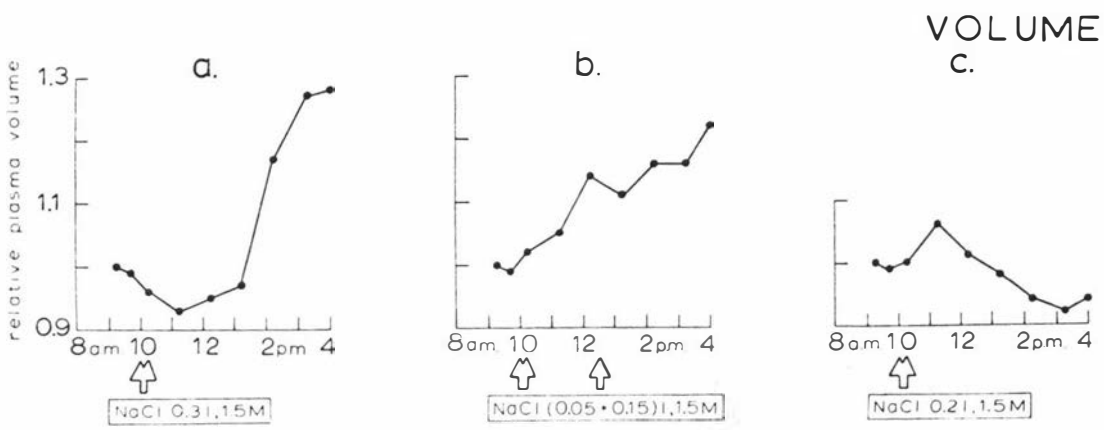

Fig 116. Relative plasma volume following intraduodenal 1.5M NaCl infusion: a - 450 m-equiv Na^+, b - 300 m-equiv Na^+, c - 300 m-equiv Na^+. Note the 3 hour decrease in volume before the expansion after the largest load (a); increasing volume over the whole observation period with the longer infusions (b); a peak volume with the shorter infusions (c). (a - sheep 15, 31.1.67; b - sheep 14, 20.10.66; c - sheep 13, 22.3.67).

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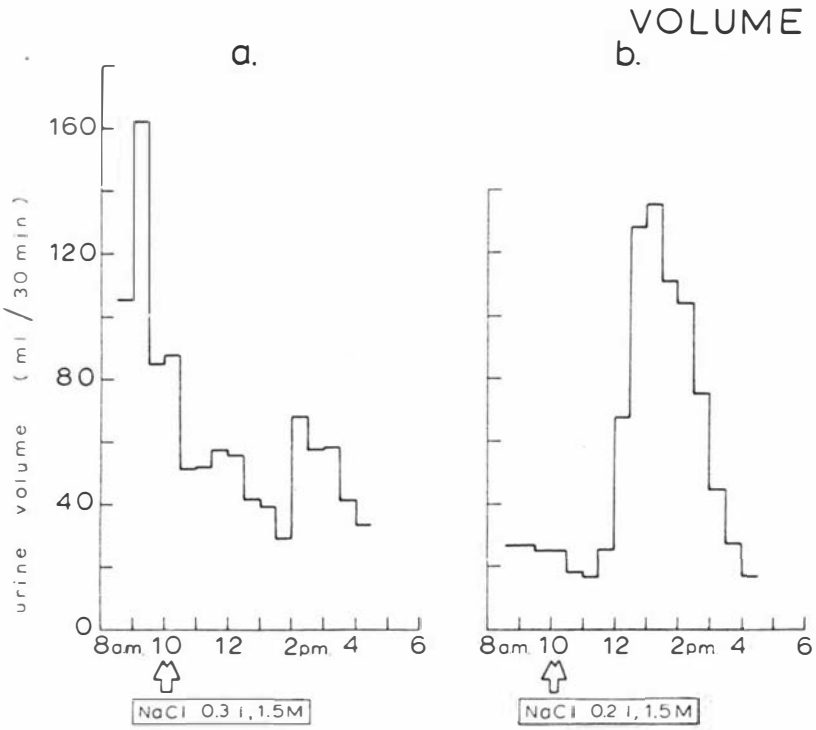
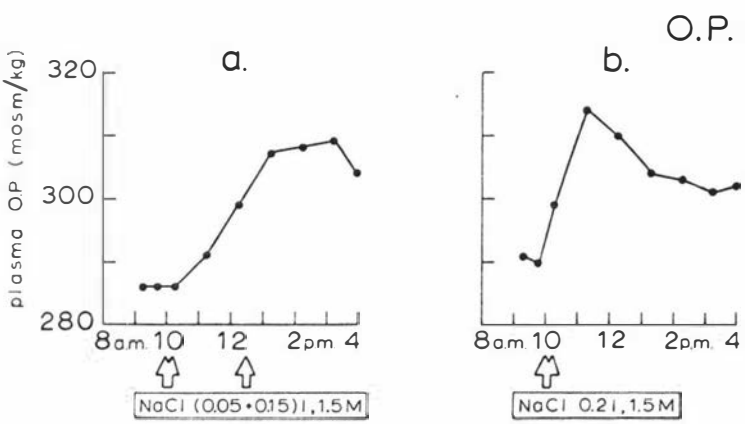


Fig 117. Urine volume following intraduodenal 1.5M NaCl infusion. Note the high preinfusion volume and fall for 4 hours with the largest load (a) and the usual single peak of water excretion (b) at the time of natriuresis. (a - sheep 15, 31.1.67; b - sheep 14, 3.11.66).

Fig 118. Plasma O.P. following intraduodenal 1.5M NaCl infusion. Note the later peak of O.P. with slower rate of infusion (a). (a - sheep 14, 20.10.66; b - sheep 13, 22.3.67).



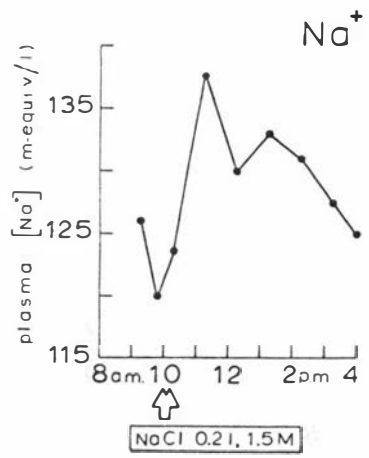


Fig 119. Plasma $[Na^+]$ following intraduodenal 1.5M NaCl infusion. Note the greater fall in $[Na^+]$ than in O.P. (Fig 118b) over the later period. (Sheep 13, 22.3.67).

On the day when diarrhoea was persistent, Na^+ and water metabolism over the first 4 hours after infusion differed considerably from that on the other 5 days. In brief, this sheep presented a picture of early moderate natriuresis with little Na^+ retention in the ECF, followed by a phase in which the excreted Na^+ was larger but exceeded by the amount absorbed, resulting in expansion of the ECF. Na^+ excretion increased without delay, and reached a moderate peak at 2 hours (Fig 114a). The plasma total solute content increased slowly over 3 hours to 107% (Fig 115). The plasma volume decreased to 95% over 75 minutes, but expanded back to the original volume over a further 3 hours (Fig 116a). The urine flow was high before infusion, and decreased for 4 hours (Fig 117a). After this initial period there were marked increases in Na^+ and water excretion, in plasma volume (to 128%) and total solute content (to 138%).

After the other 5 infusions, Na^+ excretion increased after 1-2 hours, usually reaching an earlier peak with the shorter infusions (Fig 114b) than with the prolonged ones (Fig 114c). The details of the natriuresis, shown in Table 26, reveal decreasing fractional excretion as the load increased, although the absolute excretion above the basal amount fell into 2 groups: 144-152 m-equiv for one sheep, and 122-126 m-equiv for the other 3 sheep. This amount appeared independent of the preinfusion excretion, the dose or the number of previous infusions. An osmotic diuresis was associated with the excretion of Na^+, so that urine flow paralleled Na^+ excretion over the latter half of the experiment (compare Fig 117b with Fig 114b). Total solute excretion, in general, followed Na^+.

The longer infusions were characterized by a late peak of plasma O.P., after 3-5 hours (Fig 118a), associated with increasing plasma volume

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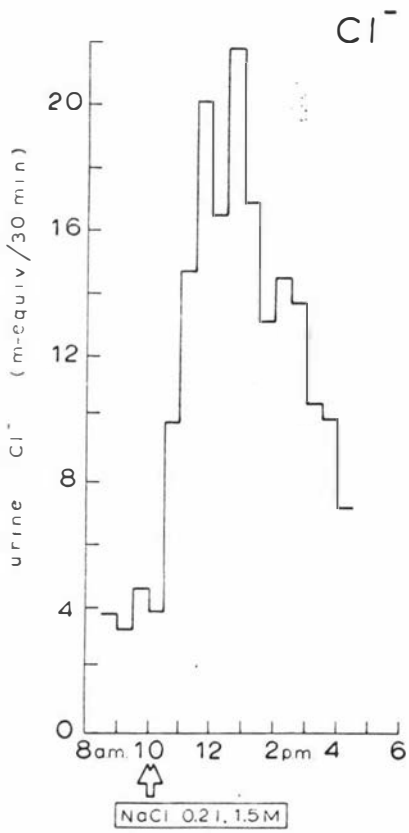

Fig 120. Urine Cl^- excretion following intraduodenal 1.5M NaCl infusion. Note the early peak of Cl^- excretion. (Sheep 13, 20.3.67).

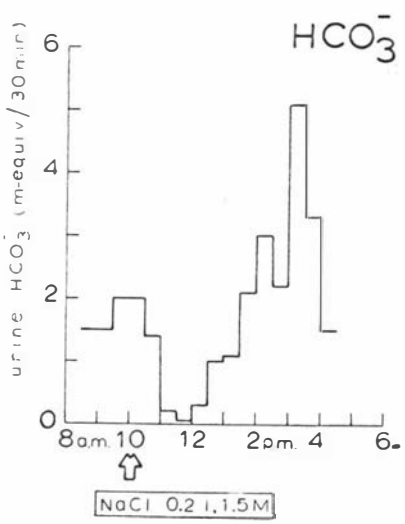

Fig 121. Urine HCO_3^- excretion following intraduodenal 1.5M NaCl infusion. Note the fall in HCO_3^- immediately after the infusion and peak excretion at the time of the natriuresis. (Sheep 14, 3.11.66).

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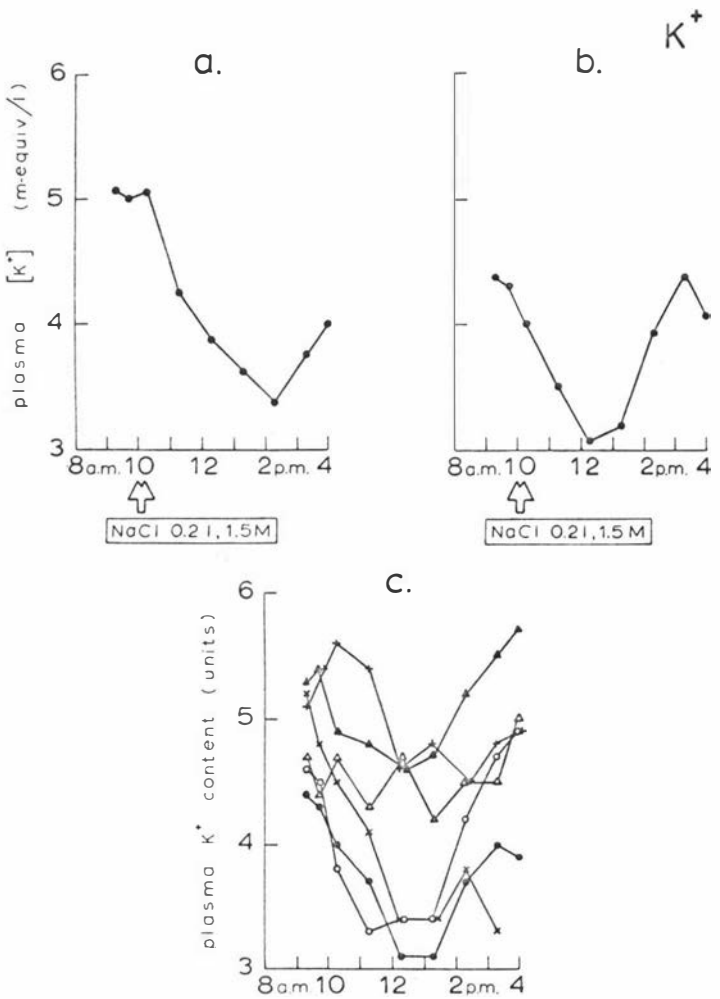

Fig 122. Plasma $[K^+]$ and K^+ content following intraduodenal 1.5M NaCl infusion. Note the fall in plasma $[K^+]$ in both cases, but the uncommon return to the initial value in b; fall in K^+ content on all days. (a - sheep 14, 3.11.66; b - sheep 13, 22.3.67; c - ▲ - sheep 14, 3.10.66; △ - sheep 14, 20.10.66; + - sheep 14, 3.11.66; ○ - sheep 13, 22.3.67; × - sheep 12, 5.4.67; ○ - sheep 15, 31.1.67).

over the whole period (to 122% and 130%) (Fig 116b) and thus also a steadily increasing total solute content (to 130% and 133%) (Fig 115). After the 10 minute infusions, the maximum plasma O.P. occurred earlier, at 1½ hours in 2 cases and 2½ hours in the third (Fig 118b). These infusions were further distinguished by a peak in plasma volume which occurred at 3½ hours on 2 days, and at 1½ hours in the third case, preceding a short period of diarrhoea. On this latter day, the plasma volume was below the preinfusion level from the start of the period of diarrhoea (Fig 116c) as was the total solute content for the last 2 hours of the observation period. In general, total solute content changes were in the same direction as volume, but of greater magnitude (Fig 115), owing to the maximum in O.P. Plasma $[Na^+]$ and $[Cl^-]$ followed O.P. with the exception that $[Na^+]$ returned to the preinfusion level while the O.P. was still raised (Fig 119).

Over the entire observation period, the fractions of Na^+ and Cl^- excreted were similar. For 150 minute infusions, the two were almost in step, but for shorter infusions, Cl^- excretion exceeded that of Na^+ in the early period, but was less during the peak natriuresis (Fig 120).

On the 2 days when HCO_3^- was measured, its excretion decreased for 3 hours then increased during the natriuresis (Fig 121); overall an extra 4% and 52% were excreted. On another 2 days urine pH fell by 0.4 and 1.2 units during the initial period before increasing again. On 3 days, urea excretion did not differ from that on control days.

Plasma $[K^+]$ decreased in all cases after the infusion, and remained low (Fig 122a) in all but one (Fig 122b). Plasma K^+ content fell rapidly to a minimum at 2-3 hours (30% lower), returning to the preinfusion value in 3 of the 6 cases (Fig 122c), these being the 3 where the total solute

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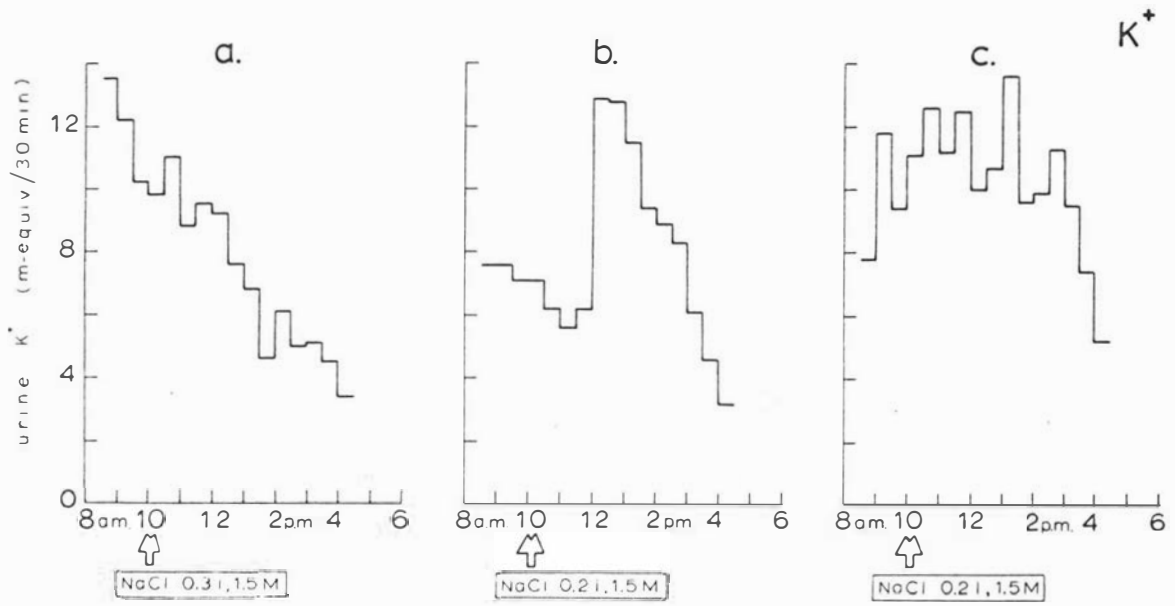

Fig 123. Urine K^+ excretion following intraduodenal 1.5M NaCl infusion. Note the three patterns of K^+ excretion. (a - sheep 15, 31.1.67; b - sheep 14, 3.11.66; c - sheep 12, 5.4.67).

content was still increasing at the end of the experiment.

K^+ excretion was variable. On 2 days there was a falling rate of excretion for most of the day, overall declining by 34% and 23% (Fig 123a). On 2 days there was an initial decrease followed by a peak, overall increasing by 1% and 6% (Fig 123b). On 2 days, K^+ increased immediately after the infusion, overall increasing by 5% and 59% (Fig 123c). Only the second of these patterns is distinct from any seen after intraruminal NaCl infusion.

DISCUSSION

Although the aim of this part of the study was to compare the effect of intraruminal and intraduodenal NaCl infusion, it was found that the dose used in the intraruminal infusions (450 m-equiv of Na^+) caused persistent diarrhoea when given into the duodenum, and was too great to obtain a physiological response. Water was drawn into the gut as evidenced by the initially decreasing plasma volume. Over the same period, a moderate natriuresis began without delay, and this Na^+ was apparently of gut origin since ECF total solute content (Na^+) increased a little. In the latter part of the experiment, a massive increase in Na^+ and water absorption and excretion was revealed as rapid expansion of the plasma volume and increase in total solute content coupled with a marked natriuresis and osmotic diuresis.

The time over which an intraduodenal NaCl load was administered influenced the time course of the absorption and excretion of Na^+. When the infusion was prolonged over 150 minutes, the Na^+ excretion, plasma volume and total solute content were still increasing at the end of the 6½ hour post-infusion period. When the infusion was given over 10 minutes, at 20 ml/min, all 3 parameters were declining at the end of the period having reached a

maximum earlier.

Although the amount of Na^+ infused differed considerably, the amount of Na^+ excreted in excess of the basal rate was remarkably similar on all 6 days. This could be a coincidence, and have no physiological significance, or, on the other hand, it may be caused by constant absorption and/or renal excretion. A saturated renal excretory mechanism appears unlikely in view of the varying time course of excretion, the range of peak excretion rates and the absence of maintained peak rates. Constant absorption in the presence of constant excretion should result in the same accumulation of Na^+ in the ECF. However, this was not observed: on 2 days, the plasma total solute content had returned to the preinfusion value, while on the other 4, total solutes were elevated to about the same extent. Consequently, if the absorption rate is limiting, it is necessary to postulate a greater penetration of Na^+ into the ICF on the 2 days.

As for the intraruminal 1.5M NaCl infusions, the natriuresis was delayed 1-2 hours, except when prolonged diarrhoea occurred. Although none of the intraduodenal experiments resulted in an exactly comparable time course of the natriuresis, the greater difference was shown by changes in plasma composition. In particular, the shorter intraduodenal infusions resulted in elimination of the 3 hour plateau in total solute content preceding the natriuresis after intraruminal infusion. The occurrence of the similar time lag in onset of the natriuresis, coupled with increasing plasma total solute content with both routes of administration suggests that the delay is in stimulating an excretory receptor mechanism rather than in absorption from the gut.

The handling of the smaller NaCl load after infusion of 0.15M

NaCl depended on the preinfusion excretion rate of Na^+. Where the Na^+ excretion was initially low, the Na^+ load was completely retained and the plasma volume and total solute content increased. On the other hand, where the preinfusion Na^+ was higher, the onset of the natriuresis was rapid and the plasma volume and total solutes were virtually unchanged. It thus appears that a natriuresis can occur without any increase in plasma volume, O.P. or total solute content after 0.15M NaCl, or even concurrently with a drop in plasma volume after 1.5M NaCl. The present experiments would therefore suggest that the primary stimulus to the natriuresis of Na^+ loading is <u>not</u> plasma volume expansion. Moreover, it is unlikely that the stimulus for natriuresis arises from any receptors in the arterial circulation since the rate of absorption of such a small load would produce insignificant changes at this site. It would seem more likely that the receptors triggering the natriuresis are located between the site of absorption and the heart. More will be said about this in Chapter 7 in relation to other experiments. The diuresis following 0.15M NaCl may share the same receptor mechanism since there was a similar biphasic response in both urine flow and Na^+ excretion. Although the natriuresis was completely suppressed when the preinfusion excretion of Na^+ was low, the diuresis was merely reduced.

Cl^- excretion after either NaCl infusion was not remarkable. The fraction excreted from the 0.15M infusions was independent of that for Na^+, which is not unexpected since the Na^+ excretion was still small compared with K^+ excretion. For the hypertonic NaCl the overall Cl^- and Na^+ excretion were similar although Cl^- excretion was the greater during the early period, but less than Na^+ in the later period. This Cl^- pattern fits in with HCO_3^- excretion, which falls during the first period then increases to

coincide with the peak of Na^+ excretion. The initial drop in HCO_3^- excretion after intraduodenal infusion is in contrast with the observations after intraruminal infusion. This may be accounted for by the diffusion of HCO_3^- into the intestine. Since the equilibrium $[HCO_3^-]$ is higher in distal parts of the intestine (Swallow and Code, 1967), it may be that the intraduodenal infusions progress further down the digestive tract and hence draw more HCO_3^- into the gut than do the intraruminal infusions. On the other hand, increased reabsorption of HCO_3^- could be of renal origin, although it is unlikely that this should occur with the intraduodenal but not the intraruminal infusions. Urine pH also decreased during the initial period, probably related to the lower $[HCO_3^-]$ of the urine. The association of HCO_3^- excretion with the natriuresis was also seen after intraruminal infusions.

After the two types of infusion, K^+ excretion showed one of several different patterns, all of which were seen with the intraruminal infusions. In contrast to this variability of the urinary changes, all 4 days showed increased plasma K^+ content with the 0.15M NaCl, and on all 6 days of 1.5M NaCl infusion, the plasma K^+ content dropped sharply over the first 2-3 hours. In the former case, the extra K^+ would appear to be of ICF origin, as the increase was greater when Na^+ was completely retained. The net loss of plasma K^+ with the hypertonic infusions could not in every case be accounted for by urine changes; it is unlikely to have entered cells since it is more likely that Na^+ would displace K^+, so probably has diffused into the gut contents.

CHAPTER 6

INTRAVENOUS INFUSION OF KCl AND NaCl

When the infusions were made into the rumen or duodenum, the rate of absorption into the ECF would not of necessity be constant, so that the excess water and electrolytes would not be presented to the ICF for exchange, nor to the kidneys for excretion, at a steady rate. By infusing similar solutions intravenously at a constant rate, the role of ICF-ECF exchange and renal excretion can be seen more clearly.

Intravenous infusions of Na^+ and K^+ salts have been reported frequently in man, dog and rodents, although few similar experiments have been performed in ruminants. However, it might be expected that the response to intravenous infusion would vary less between the different species, so that results on monogastric animals become more relevant to the present observations.

In monogastrics, hypertonic saline loading increases excretion of Na^+, Cl^-, water and K^+, decreases the fractional tubular reabsorption of Na^+, and may increase plasma volume, $[Na^+]$ and $[Cl^-]$, and decrease plasma CO_2 content and $[K^+]$ (Wolf, 1947; Green and Farah, 1949; Baldwin, Kahana and Clarke, 1950; Goodyer, Relman, Lawrason and Epstein, 1950; Selkurt and Post, 1950; Crawford and Ludemann, 1951; Stein, Bercovitch and Levitt, 1964; Dirks et al., 1965). However, Luomanmäki and Salminen (1964) observed in man an increase in plasma $[K^+]$ along with a small decrease in serum and urine pH. GFR was raised in some studies (Selkurt and Post, 1950; Baldwin et al., 1950) but not in others (Green and Farah,

1949; Crawford and Ludemann, 1951).

The response elicited by isotonic NaCl infusion was more variable, being affected by factors including state of hydration, posture and time of the day. There may be a hypotonic diuresis, an isotonic saline diuresis, or almost complete retention of the load. A separated excretion of Na^+ and water in the form of a hypotonic diuresis, sometimes followed by a saline diuresis, was reported by Thompson (1900), Murphy (1950), Blomhert et al. (1951), 8-13 hours after prehydration by Ladd (1951a,b), and in recumbent but not sitting subjects by Strauss, Davis, Rosenbaum and Rossmeisl (1951). Saline diuresis has been described in man and dog by Chanutin et al. (1924), Gross (1948), Baldwin et al. (1950), Crawford and Ludemann (1951), and by Blomhert et al. (1951) only with higher rates of infusion, and only during the day. Recent micropuncture studies during saline loading uniformly describe a saline diuresis with reduced proximal tubular fractional Na^+ reabsorption (Cortney et al., 1965; Dirks et al., 1965; Cirksena et al., 1965; Watson, 1966; Landwehr et al., 1967); the uniformity in these experiments may be partly caused by the similarity of conditions demanded by this technique. Expansion of the plasma volume may occur (Crawford and Ludemann, 1951; Strauss et al., 1951), as well as reduced plasma $[K^+]$ and increased K^+ excretion (Baldwin et al., 1950; Strauss et al., 1951), although these K^+ changes were variable in the experiments of Crawford and Ludemann (1951).

Intravenous K^+ salts produce increased excretion of K^+, water, Na^+, Cl^- and HCO_3^-, and an alkaline urine. Plasma $[K^+]$ usually rises, $[Na^+]$ is unchanged, and plasma pH and $[HCO_3^-]$ fall (Miller, 1923, 1926a,b; Winkler and Smith, 1942; Schwartz, Smith and Winkler, 1942; Baldwin et al.,

1950; Berliner et al.,1951; Roberts et al., 1953). After KCl infusion in dogs, there were increases in plasma O.P. and $[Cl^-]$, PCV and $[Hb]$, although the plasma volume was unchanged, as estimated by Evan's Blue dilution (Maxwell, 1965).

Anderson and Pickering (1962) infused 1.0% KCl into cows at 7-9 ml/min. Plasma $[K^+]$ and K^+ excretion were both raised, the latter equalling the rate of administration by the end of 2 hours. GFR and plasma $[Na^+]$ were unchanged, but Na^+ excretion increased 2-50 fold, with the peak at 1-2 hours, paralleled by water excretion. In preliminary experiments, Keynes and Harrison (1967) produced similar results with KCl or K acetate in sheep, but a depressed natriuresis when adrenal vein effluent was collected, and reduced kaliuresis when the adrenal circulation was occluded. Further results of their experiments in defining the possible role of the adrenals in K^+ metabolism may be of great value.

Potter (1966) studied intravenous NaCl loading in Merino sheep, and recently (Potter, 1968) compared the response to NaCl loading in Dorset Horn sheep which were drinking either water or 1.3% saline water. The experimental procedure was complex: feed and water were removed, 1 litre of water was administered orally, the sheep were catheterized and cannulated, isotonic saline was infused for 1 hour, 10% saline for 1 hour at twice the rate, isotonic saline for 2 hours, then the feed and water were replaced. This rapid changing of the infusion could result in the effects of one infusion persisting during the next infusion time. In the Merinos, during hypertonic saline there was increased excretion of Na^+, Cl^- and water, raised plasma O.P., $[Na^+]$ and $[Cl^-]$, and decreased $[K^+]$. The GFR was variable, but tubular fractional Na^+ reabsorption decreased.

During the initial isotonic NaCl, plasma $[K^+]$ began falling, and K^+ excretion reached a peak then also began falling, both declines continuing during the 1% NaCl. The quantitative relationship between changes in K^+ metabolism and hypertonic NaCl infusion cannot be assessed because of the carry-over of the effects of the isotonic saline infusion. The same experiments on Dorset Horns produced similar results, with the exception that GFR was clearly raised, K^+ excretion increased, and the drop in plasma $[K^+]$ caused clinical signs and often death ensued in 2-3 days unless K^+ was infused. When NaCl was infused into Dorset Horns drinking 1.3% saline water, GFR increased to a greater extent, Na^+ and Cl^- were more rapidly eliminated, the elevated plasma levels returned more quickly, less K^+ was lost in the urine and no distress was caused. The blood volume expanded in both groups of Dorset Horns, more in those drinking water.

The dose rate used by Potter produced plasma $[Na^+]$ of near 200 m-equiv/l, which was considered excessive. For the present experiments, a dose rate of 1 ml/min for 1.5M NaCl was selected as being low enough to prevent excessive rises in plasma $[Na^+]$ nor to cause significant expansion of the ECF per se. 0.15M NaCl at the same rate of infusion was used as a volume control. The KCl selected was 1.0M, not 1.5M, as a precaution against severe hyperkalaemia, and to be similar in absolute amount/kg to that administered to cows by Anderson and Pickering (1962).

MATERIALS AND METHODS

Experimental design

The experimental conditions were as similar as possible to those for the intraruminal and intraduodenal infusions (see Chapter 4): feed

Table 27. Sheep used for intravenous infusions given at 1 ml/min for 120 minutes

Infusion	Sheep	Date	m-equiv of cation infused
NaCl 0.15M	2	12.12.66	19 ***
	1	12. 1.67	18
	15	6. 3.67	18
	13 *	11. 4.67	10.8
NaCl 1.5M	1	10. 1.67	180
	15	13. 2.67	180
	2	14. 3.67	180
	13 **	13. 4.67	108
KCl 1.0M	14	5.12.66	120
	15	16. 2.67	120
	2	16. 3.67	120

* 0.09M

** 0.9M

*** 127 ml

was last offered at 4-7 p.m., the infusions were commenced at 10 a.m. and no drinking water was available during the experimental period.

Three solutions were infused, each at 1 ml/min for 120 minutes, as summarized in Table 27:

(i) 0.15M NaCl

(ii) 1.5M NaCl

(iii) 1.0M KCl

Animals

The 5 Romney ewes (weight 27-34 kg) were housed and fed as described in Chapter 4. Sheep 1, 2 and 13 had a rumen fistula, and sheep 13, 14 and 15 had a duodenal cannula.

Infusion

Both jugular veins were cannulated, one cannula being used for sampling and the other for infusion of the warmed solution at a constant rate from a Luerlock syringe driven by a Sage pump. The cannula was extended by an additional length of cannula-tubing to a sawn-off needle on the syringe using small pieces of rubber tubing as connectors. The heparinized saline left in the cannula was aspirated before connecting up the tubing, and after the infusion the cannula was reheparinized as after a routine blood sample.

Sample collection

As described in Chapter 4.

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Table 28. Excretion of Na^+, Cl^- and water after intravenous infusion of 0.15M NaCl.

Sheep & Date	Amount infused		Preinfusion Na excretion (m-equiv/30 min)	Excretion above basal		
	Na^+, Cl^- (m-equiv)	water (ml)		Na^+ (m-equiv)	Cl^- (m-equiv)	water (ml)
1 12.1.67	18	120	0.06	0.1	2.9	-13
15 6.3.67	18	120	0.07	2.9	3.5	122
13 11.4.67	10.8	120	0.97	3.0	19.3	350
2 12.12.66	19	127	1.60	33.0	20.1	691

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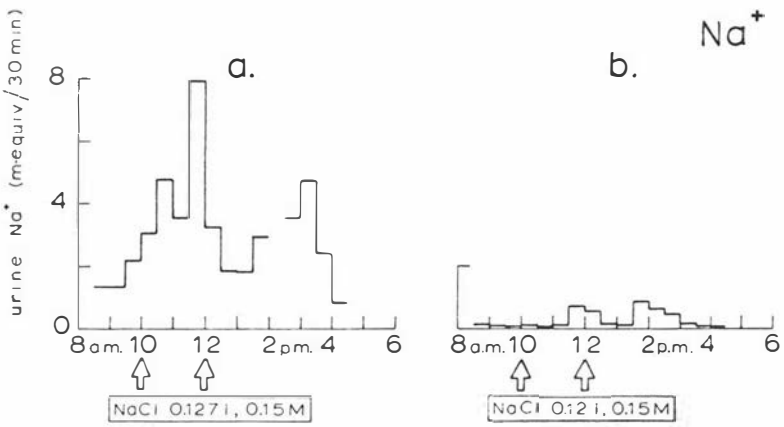

Fig 124. Urine Na^+ excretion following intravenous 0.15M NaCl infusion. Note the biphasic natriuresis in both a and b, although small in magnitude in the latter; delay in onset in b, not in a. (a - sheep 2, 12.12.66; b - sheep 15, 6.3.67).

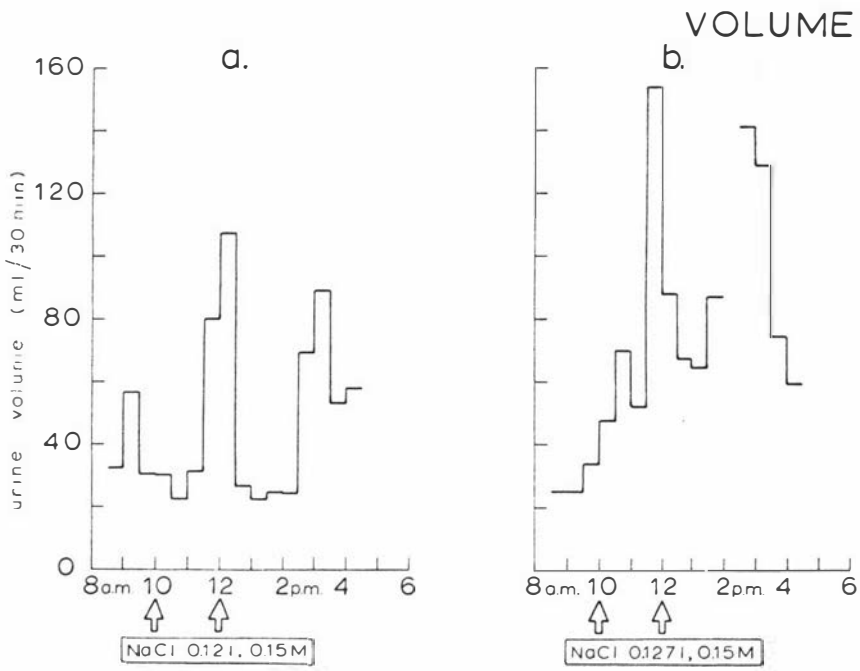

Fig 125. Urine volume following intravenous 0.15M NaCl infusion. Note the biphasic diuresis at the time of natriuresis. (a - sheep 15, 6.3.67; b - sheep 2, 12.12.66).

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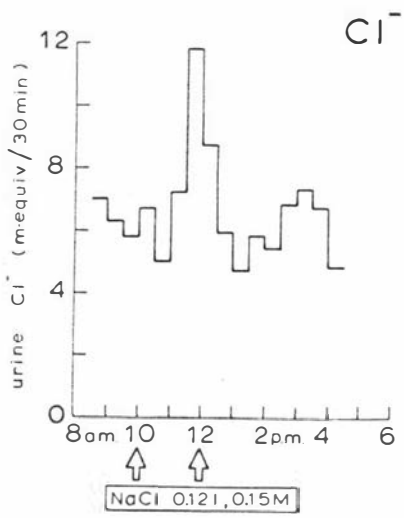

Fig 126. Urine Cl^- excretion following intravenous 0.15M NaCl infusion. Note the greatest increase in excretion during the infusion. (Sheep 15, 6.3.67).

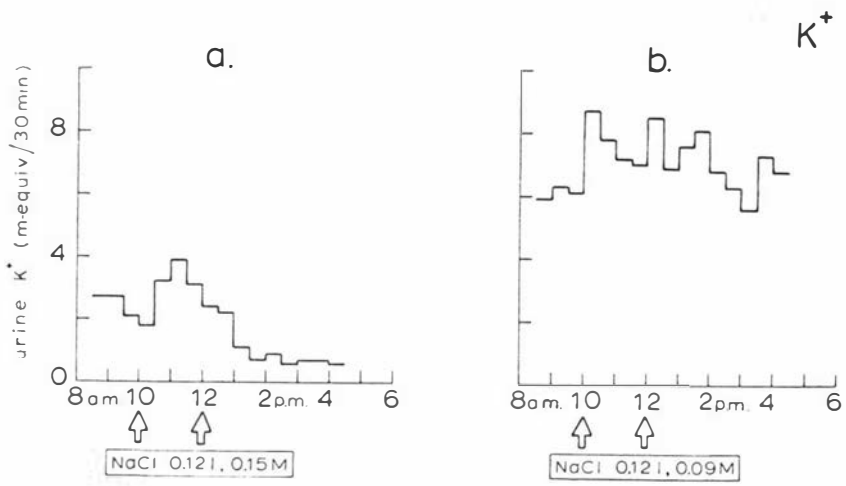

Fig 127. Urine K^+ excretion following intravenous 0.15M or 0.09M NaCl infusion. Note some kaliuresis during the infusion in both cases. (a - sheep 1, 12.1.67; b - sheep 13, 11.4.67).

Analytical methods

As described in Chapters 2 and 3.

Calculation of results

As described in Chapters 2 and 4.

RESULTS

(1) Control

See Chapter 4.

(2) 0.15M NaCl

Excretion of the administered Na^+, Cl^- and water in the urine appeared to be less when the preinfusion Na^+ excretion was low (Table 28). Excretion of water accompanied that of Na^+: on the 3 days of significantly raised Na^+ excretion, 2 peaks of both Na^+ (Fig 124) and water excretion (Fig 125) were apparent; furthermore, on the day when Na^+ excretion was only very slightly raised, the urine flow barely increased (by 5-10 ml/30 min). However, urine flow on each day was independent of total solute excretion (which followed K^+ closely). Only on one day was there an immediate onset of natriuresis (Fig 124a), on the other 3, it was delayed 60 - 90 minutes (Fig 124b). Cl^- excretion was increased on all days, mainly during the infusion on 3 days (Fig 126), later on the fourth.

The overall excretion of K^+ and HCO_3^- on 3 days was no different from that on control days (declines of 13-37% and 22-58% respectively). On the fourth day, both were a little greater, more particularly K^+

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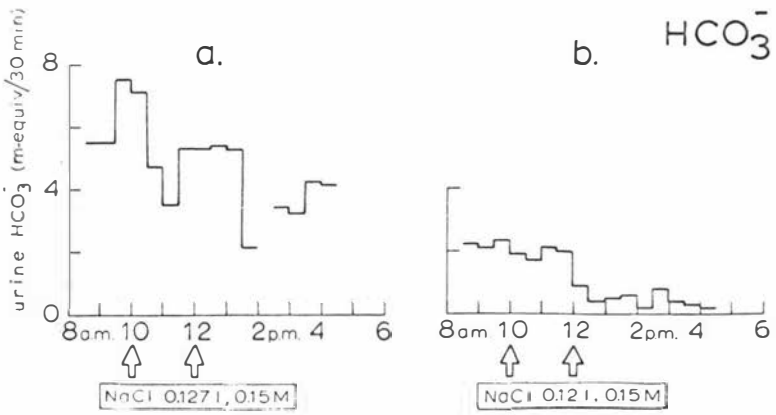
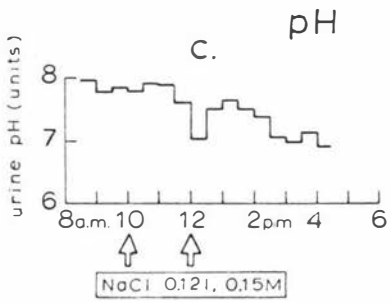


Fig 128. Urine HCO_3^- excretion and urine pH following intravenous 0.15M NaCl infusion. Note the overall fall in both parameters; the acid urine and very low HCO_3^- on the day shown in b,c. (a - sheep 2, 12.12.66; b,c - sheep 15, 6.3.67).



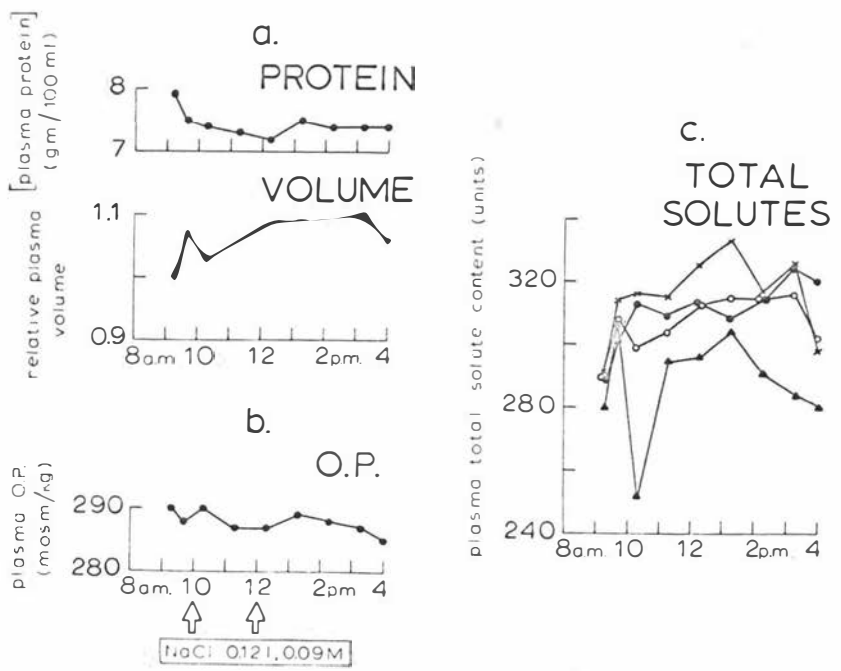


Fig 129. [Plasma protein], relative plasma volume, plasma O.P. and total solute content following intravenous 0.15M or 0.09M NaCl infusion. Note the small increase in volume, O.P. and total solutes, but overall fall in O.P.; protein changes consistent with those in volume. (a,b - sheep 13, 11.4.67; c - ● - sheep 2, 12.12.66; ○ - sheep 13, 11.4.67; × - sheep 15, 6.3.67; ▲ - sheep 1, 12.1.67).

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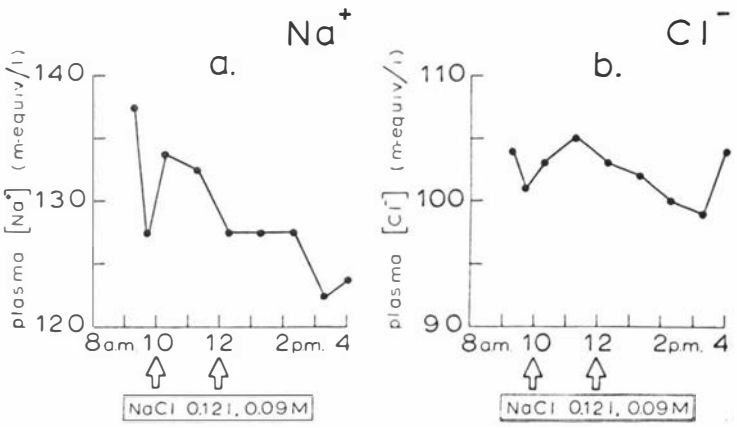

Fig 130. Plasma $[Na^+]$ and $[Cl^-]$ following intravenous 0.09M NaCl infusion. Note the overall fall in $[Na^+]$; the small increase in $[Cl^-]$ during the infusion. (Sheep 13, 11.4.67).

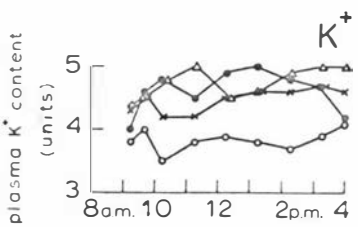

Fig 131. Plasma K^+ content following intravenous 0.15M or 0.09M NaCl infusion. Note the lack of consistent change. (Δ - sheep 2, 12.12.66; × - sheep 13, 11.4.67; O - sheep 15, 6.3.67; O - sheep 1, 12.1.67).

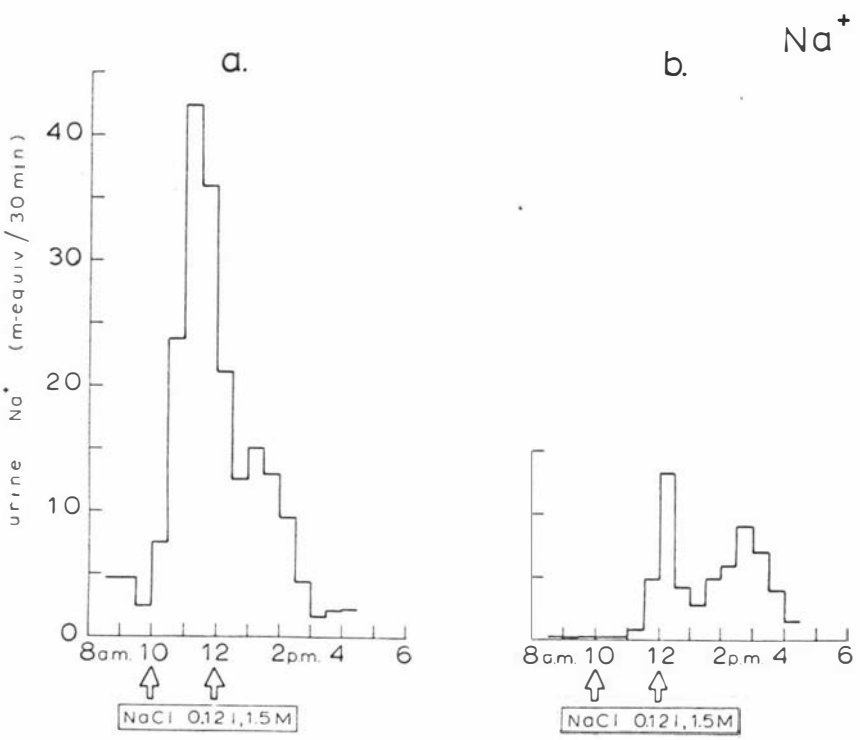

Fig 132. Urine Na^+ excretion following intravenous 1.5M NaCl infusion. Note the earlier onset and the higher and earlier peak excretion when the preinfusion Na^+ was higher (a) compared with low levels (b). (a - sheep 15, 13.2.67; b - sheep 2, 14.3.67).

(Fig 127a) which increased by 16%, accompanied by a 2% increase in HCO_3^-. In all, however, there was some increase in K^+ excretion during the infusion (Fig 127a,b). HCO_3^- excretion tended to decline (Fig 128a), even when the initial HCO_3^- was already low (Fig 128b), which included both days of low Na^+ excretion. On these occasions urine $[HCO_3^-]$ became low and urine pH fell more than usual, to below pH 7.0 (Fig 128c).

On all days the plasma volume increased, but remained within the range of the controls except for the largest increase of 10% which occurred on a day when more than the infused Na^+ was excreted (Fig 129a). Similarly, plasma O.P. (Fig 129b) and total solute content (Fig 129c) showed a slight increase at some stage in all 4 cases. This increase was of the same order of magnitude as in the controls, but contrasts with the variability of the direction of change in the latter. [Plasma protein] changes were small and consistent with the calculated relative plasma volumes (Fig 129a). Plasma $[Na^+]$ decreased over the experimental period (Fig 130a), but the drop was large compared with that in O.P.; on 2 days, the $[Na^+]$ dropped markedly during the preinfusion period. In general, plasma $[Cl^-]$ followed O.P. (Fig 130b); on 3 days $[Cl^-]$ showed a small increase during the infusion. Both plasma $[K^+]$ and K^+ content changes (Fig 131) were inconsistent, and similar to controls.

(3) <u>1.5M NaCl</u>

Since the infusion of 0.9M NaCl gave similar results to 1.5M NaCl, it is considered together with the latter in this section.

On 2 days when the preinfusion Na^+ excretion was high (9.5 and 3.9 m-equiv/30 min), a natriuresis began within 30 minutes, reached a

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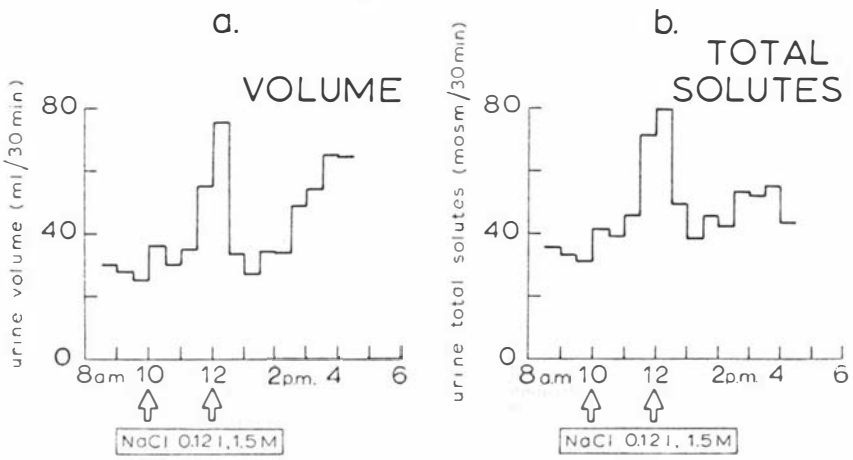

Fig 133. Urine volume and total solute excretion following intravenous 1.5M NaCl infusion. Note the similar effect on both parameters of the infusion. (Sheep 2, 14.3.67).

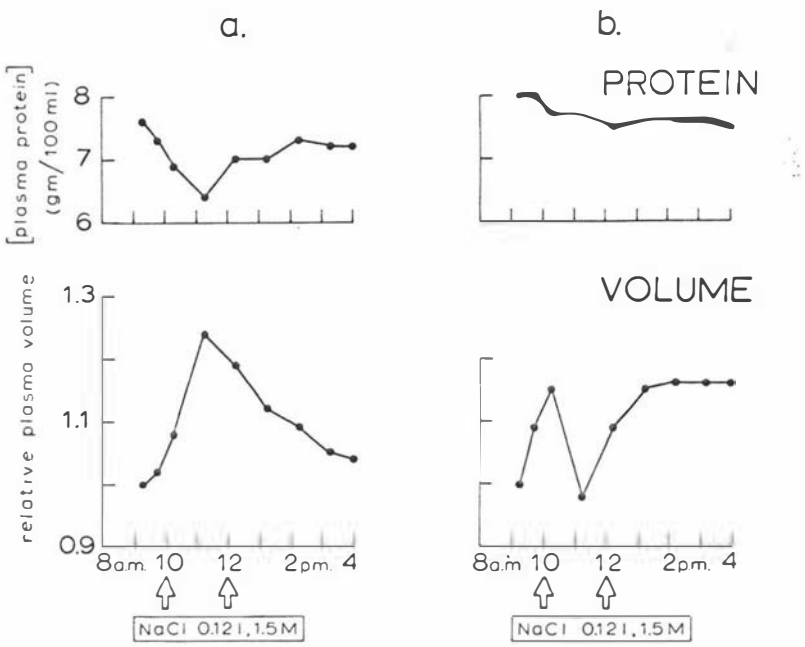

Fig 134. [Plasma protein] and relative plasma volume following intravenous 1.5M NaCl infusion. Note the typical response in a; the unusual day in b when a large fall in plasma volume occurred (at 11.15 a.m.) although changes in protein were not unusual. (a - sheep 2, 14.3.67; b - sheep 1, 10.1.67).

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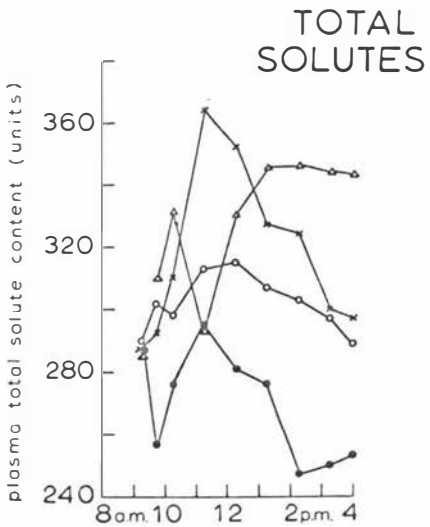

Fig 135. Plasma total solute content following intravenous 1.5M or 0.9M NaCl infusion. Note the increase during the infusion.
(△ - sheep 1, 10.1.67;
× - sheep 2, 14.3.67;
○ - sheep 13, 13.4.67;
● - sheep 15, 13.2.67).

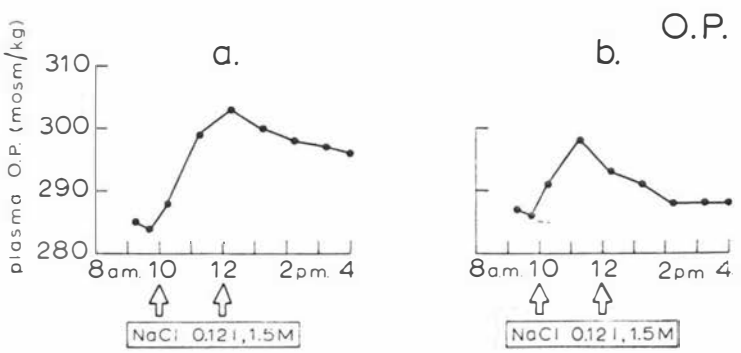

Fig 136. Plasma O.P. following intravenous 1.5M NaCl infusion. Note the peak O.P. in both; failure to return to the pre-infusion value only in a. (a - sheep 1, 10.1.67; b - sheep 15, 13.2.67).

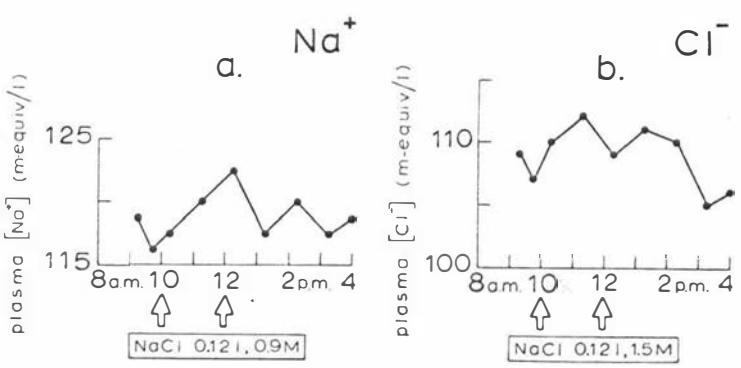

Fig 137. Plasma $[Na^+]$ and $[Cl^-]$ following intravenous 0.9M or 1.5M NaCl infusion. Note the increase in both parameters during the infusion. (a - sheep 13, 13.4.67; b - sheep 15, 13.2.67).

maximum in the second hour of 30 and 42 m-equiv/30 min, then after the infusion dropped rapidly to near the initial rate (Fig 132a) or below. Overall 110 and 9 m-equiv were retained from the 180 m-equiv load. When the preinfusion Na^+ was lower (0.12 and 0.18 m-equiv/30 min), the onset and bulk of the natriuresis were later and the peak excretion was lower. After a delay of 60 minutes Na^+ excretion rose to a maximum of 7 and 13 m-equiv/30 min in the post-infusion period in which the greater part of the natriuresis occurred (Fig 132b). The overall retention on these days was 57 of 108 and 124 of 180 m-equiv.

Urine flow and total solute excretion changed together, and reflected peaks of both Na^+ and K^+ (Fig 133a,b). Overall, the urine volume was 112 ml more and 99 ml less than the infused volume for low Na^+, and 8 ml more and 295 ml less for high Na^+.

The plasma volume and total solute content increased during the infusion (Fig 134, 135), and on only one day failed to return to the preinfusion value (Fig 134b, 135). The largest increase in volume (23%) and total solutes (30%) occurred on the day when Na^+ excretion was delayed for 60 minutes after the larger Na^+ load (Fig 134a). On the one day when the two were still elevated at the end of the observation period, the extra Na^+ calculated to be in the ECF was around 100 m-equiv compared with unexcreted Na^+ of 110 m-equiv. On this day plasma O.P. also remained high (Fig 136a) although on the other 3 it returned to the initial O.P. after reaching a peak in 1½ or 2½ hours, representing an increase of 7-18 mosm/kg (Fig 136b). [Plasma protein] changes supported the calculated relative plasma volume (Fig 134a) with one exception where a large rise in [Hb] and PCV was not accompanied by a rise in [plasma protein] (see Fig 134b),

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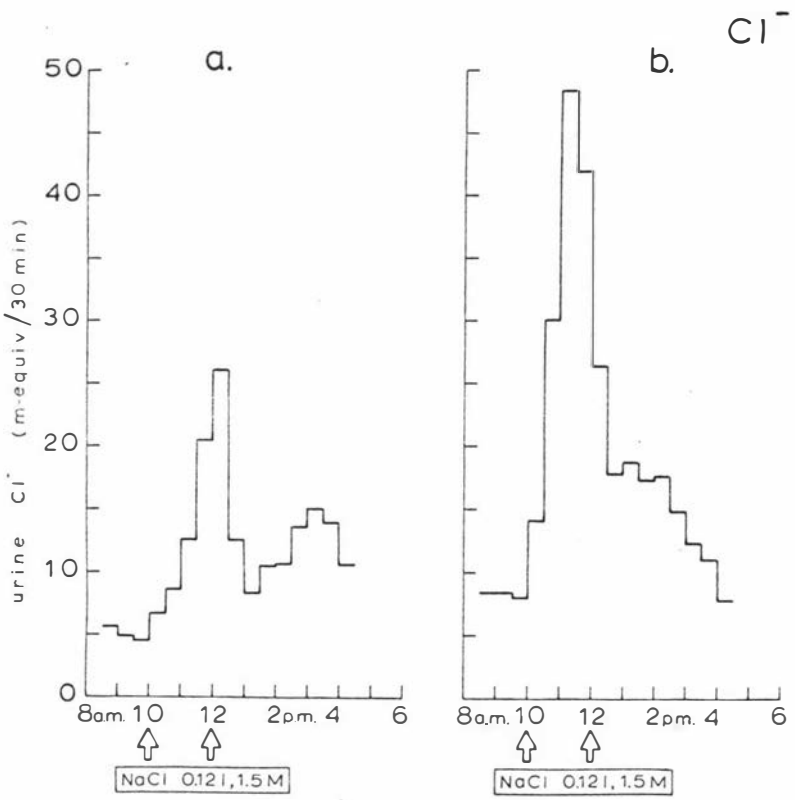

Fig 138. Urine Cl^- excretion following intravenous 1.5M NaCl infusion. Note the peak during or just after the infusion. (a - sheep 2, 14.3.67; b - sheep 15, 13.2.67).

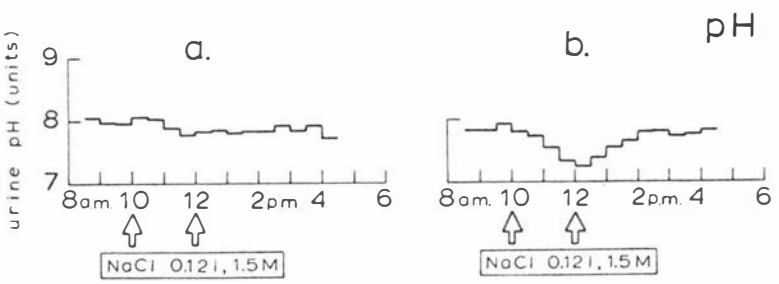

Fig 139. Urine pH following intravenous 1.5M NaCl infusion. Note the fall in pH in b on a day of larger diuresis. (a - sheep 2, 14.3.67; b - sheep 1, 10.1.67).

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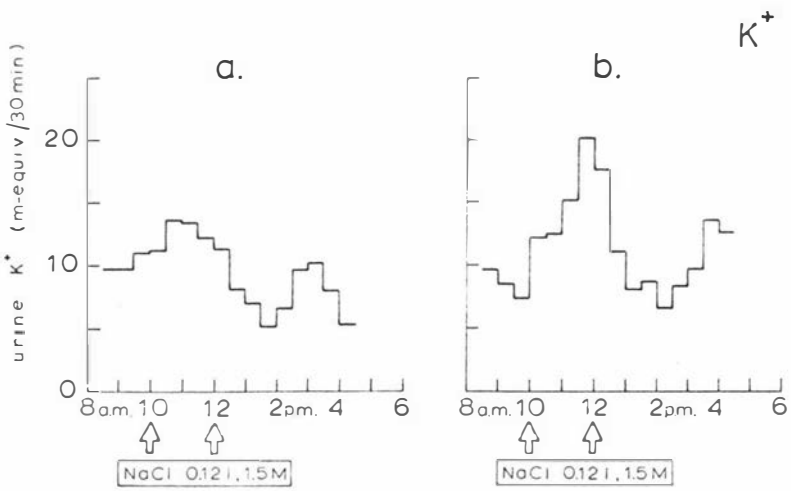

Fig 140. Urine K^+ excretion following intravenous 1.5M NaCl infusion. Note the increased excretion during the infusion and the second peak later; the delay in onset of the kaliuresis when the natriuresis was immediate (a), but no delay when the natriuresis was delayed (b). (a - sheep 15, 13.2.67; b - sheep 2, 14.3.67).

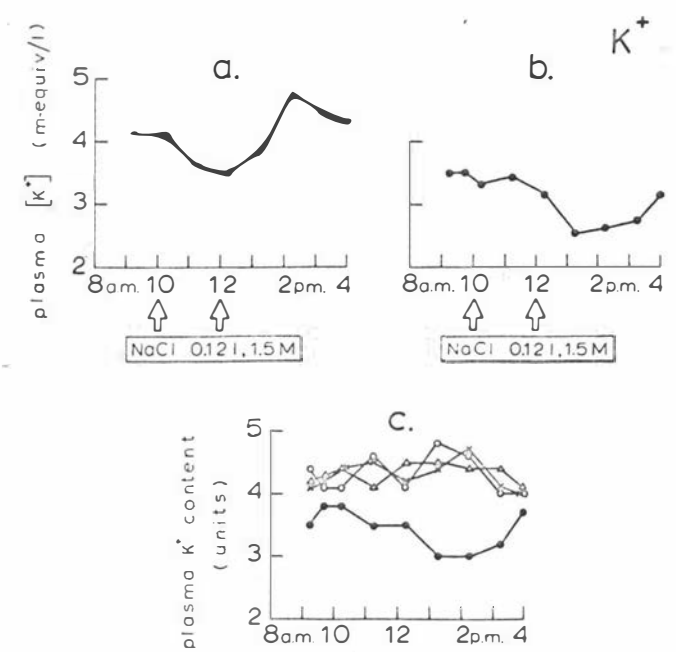

Fig 141. Plasma $[K^+]$ and K^+ content following intravenous 1.5M or 0.9M NaCl infusion. Note the variable changes in $[K^+]$ in a,b: a drop occurring during the infusion in a, in the post-infusion period in b; no trend in K^+ content. (a - sheep 2, 14.3.67; b - sheep 1, 10.1.67; c - ⊙ - sheep 1, 10.1.67; × - sheep 2, 14.3.67; Δ - sheep 13, 13.4.67; O - sheep 15, 13.2.67).

and probably stemmed from a sudden rise in circulating red cell mass. Plasma $[Na^+]$ and $[Cl^-]$ followed the same trends as O.P. (Fig 137a,b).

The fractional Cl^- and Na^+ excretions were very close on all but one day when about 50 m-equiv extra Cl^- was lost, however, the extra K^+ loss was around 50 m-equiv greater than on the other days, and a peak of Cl^- coincided with one for K^+ (compare Fig 138a with Fig 132b, 140b). On all occasions, the Cl^- excretion increased immediately to a peak around 2 hours, then declined noticeably on the 2 days of early natriuresis (Fig 138b) but much less where it was prolonged (Fig 138a).

Urine pH was estimated on 3 occasions; 2 of these, when urine flow increased little, were like controls (Fig 139a), but on one day of large diuresis the pH dropped during the infusion and later increased again (Fig 139b). HCO_3^- excretion was examined on only one day when a small natriuresis was associated with a little increase in HCO_3^-.

K^+ excretion was raised during the infusion then declined markedly over the next 2 hours in all cases, and increased a second time in 3 cases (Fig 140). There was a 30-60 minute delay when the natriuresis commenced immediately (Fig 140a), none when it was delayed (Fig 140b). Compared with an average control decrease of 16%, 68 and 16 m-equiv of extra K^+ were lost with low Na^+, and 17 and 3 m-equiv extra with high Na^+.

Changes in plasma $[K^+]$ were variable: in one case plasma $[K^+]$ altered little; in only one was there a marked drop during the infusion (Fig 141a), on the day of largest volume increase. In the post-infusion period, two were higher than before infusion (Fig 141a), one was lower (Fig 141b). On all 4 days the plasma K^+ content was unchanged during the

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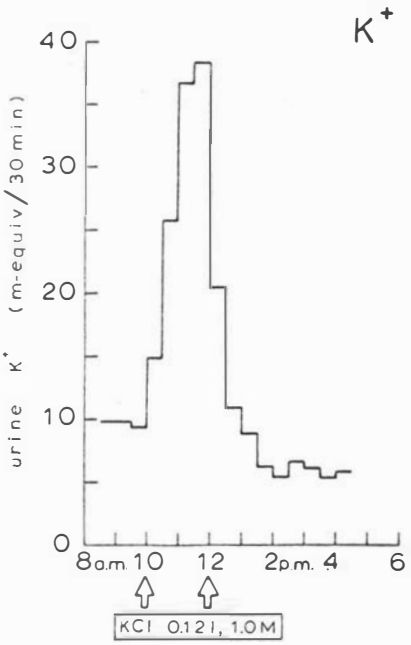


Fig 142. Urine K^+ excretion following intravenous 1.0M KCl infusion. Note the rapid onset of raised K^+ excretion; marked kaliuresis during the infusion and rapid decline after. (Sheep 14, 5.12.66).

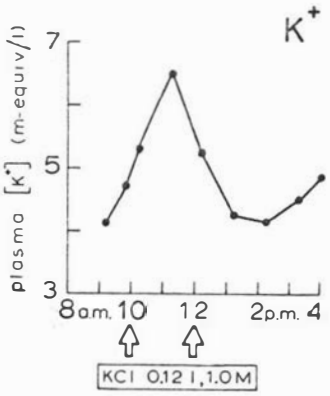
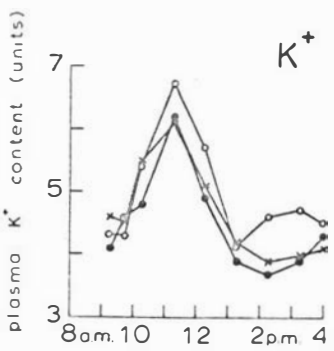


Fig 143. Plasma $[K^+]$ following intravenous 1.0M KCl infusion. Note the similar time course of plasma $[K^+]$ and K^+ excretion. (Sheep 14, 5.12.66).

Fig 144. Plasma K^+ content following intravenous 1.0M KCl infusion.
(O - sheep 2, 16.3.67;
O - sheep 14, 5.12.66;
× - sheep 15, 16.2.67).



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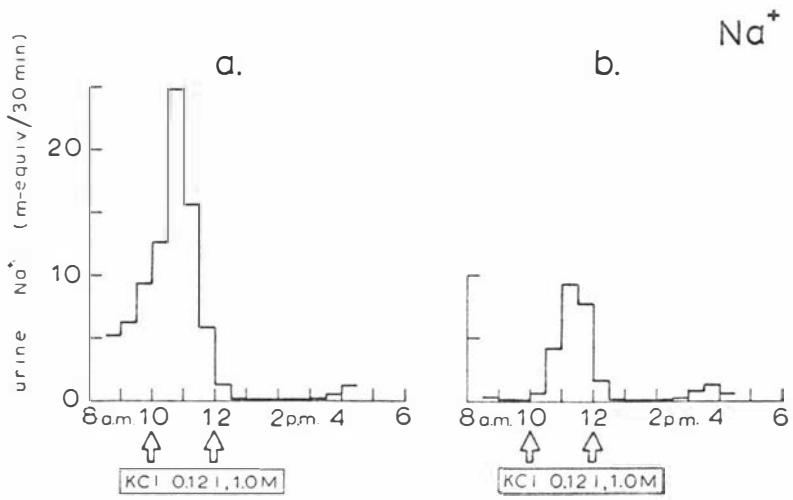

Fig 145. Urine Na^+ excretion following intravenous 1.0M KCl infusion. Note the peak natriuresis, earlier and higher when the preinfusion Na^+ was higher (a) than when it was low (b). (a - sheep 15, 16.2.67; b - sheep 2, 16.3.67).

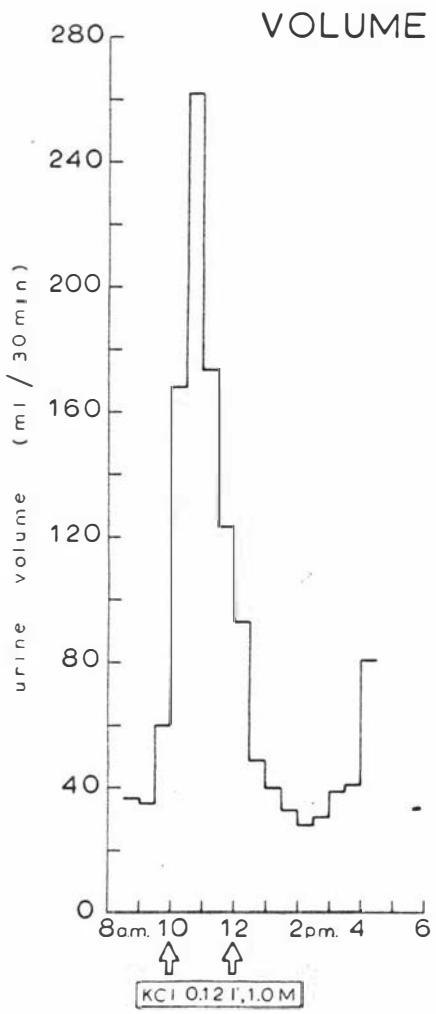

Fig 146. Urine volume following intravenous 1.0M KCl infusion. Note the diuresis during the infusion. (Sheep 15, 16.2.67).

infusion, but afterwards showed a transient increase in 2, and decrease in one (Fig 141c).

(4) 1.0M KCl

A marked kaliuresis commenced within 30 minutes on all 3 days, reached a maximum in the second hour of 32-38 m-equiv/30 min, approximating the rate of infusion plus the basal excretion. When the infusion ended, the decline was rapid, returning to the preinfusion excretion in one hour in 2, and in 2 hours when the complete load was excreted (Fig 142). Allowing a 16% decline in basal excretion, at 6½ hours 28 and 30 m-equiv less and 16 m-equiv more than the load had appeared in the urine.

Excretion of K^+ paralleled plasma $[K^+]$ (Fig 143) and K^+ content (Fig 144). The maximum $[K^+]$, after 1½ hours, of 6.8-7.5 m-equiv/l represented increases of 2.2, 2.6 and 3.1 m-equiv/l, while K^+ content at the same time rose to 132%, 141% and 156%. An hour after the infusion ended, $[K^+]$ and K^+ content were slightly below the initial values, but had returned to them by the end of the observation period. At noon, 32, 40 and 39 m-equiv of K^+ were still retained, the plasma K^+ content was raised to 116%, 137% and 115% respectively, so that approximately 28, 34 and 31 m-equiv of K^+ had left the ECF.

KCl induced a natriuresis beginning in the first 30 minutes. On 2 days when Na^+ excretion was high before the infusion (1.15 and 7.0 m-equiv/30 min), it reached a maximum of 25 and 38 m-equiv/30 min in the second 30 minute sample, then fell considerably over the period of peak kaliuresis (Fig 145a). On the day of low Na^+ (0.17 m-equiv/30 min), the peak natriuresis was only 8.7 m-equiv/30 min in the third sample (Fig 145b).

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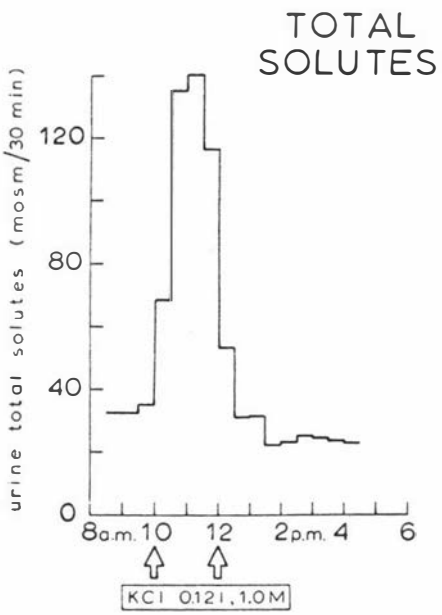
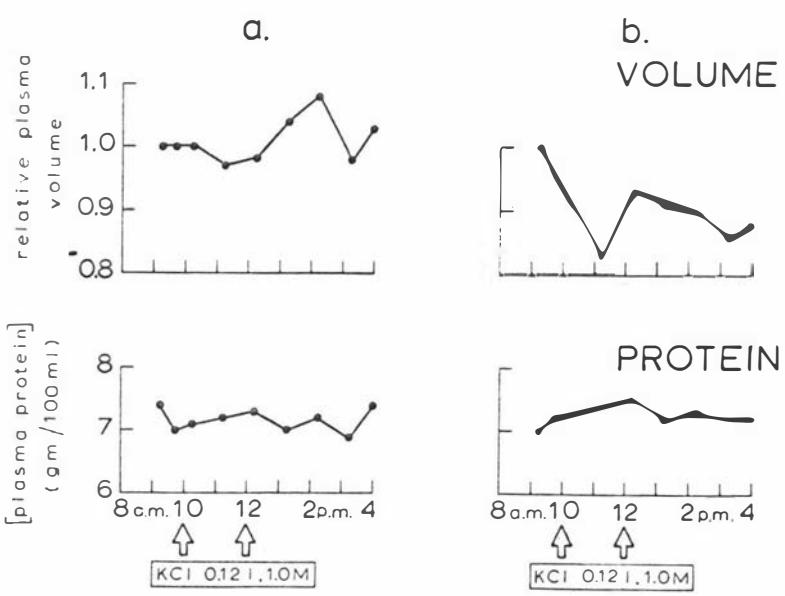


Fig 147. Urine total solute excretion following intravenous 1.0M KCl infusion. Note the peak excretion during the infusion. (Sheep 14, 5.12.66).

Fig 148. Relative plasma volume and plasma protein following intravenous 1.0M KCl infusion. Note the fall in volume during the infusion. (a - sheep 2, 16.3.67; b - sheep 14, 5.12.66).



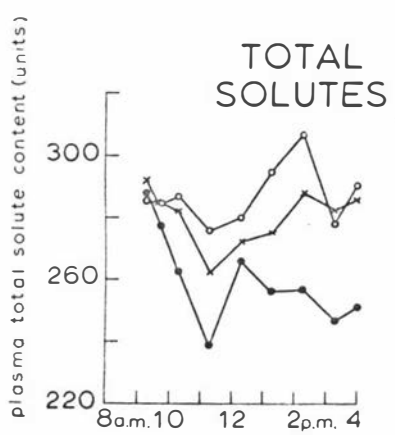


Fig 149. Plasma total solute content following intravenous 1.0M KCl infusion. Note the decrease during the infusion in all. (O - sheep 2, 16.3.67; ⊙ - sheep 14, 5.12.66; × - sheep 15, 16.2.67).

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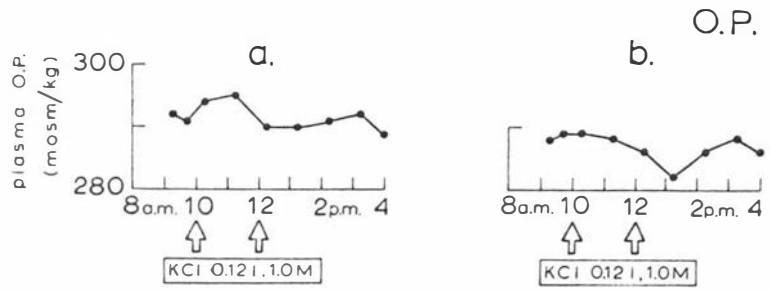

Fig 150. Plasma O.P. following intravenous 1.0M KCl infusion. Note the increased O.P. in a, but the fall in b when Na^+ excretion was greatest. (a - sheep 15, 16.2.67; b - sheep 14, 5.12.66).

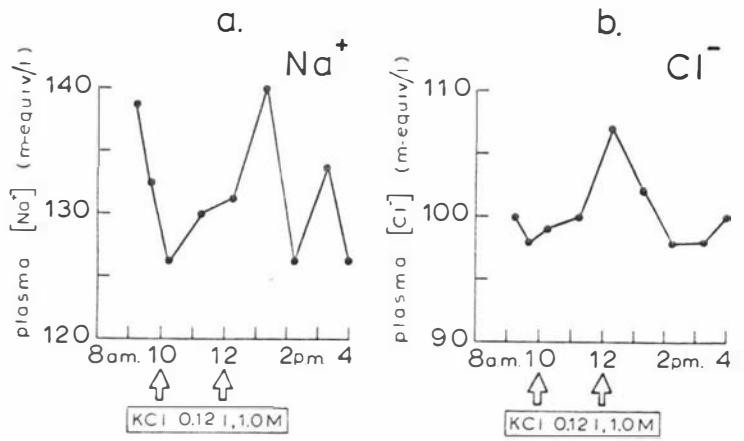

Fig 151. Plasma $[Na^+]$ and $[Cl^-]$ following intravenous 1.0M KCl infusion. Note the decrease in $[Na^+]$ during the infusion and the magnitude of the fluctuations; the peak of $[Cl^-]$ after the infusion. (a - sheep 15, 16.2.67; b - sheep 2, 16.3.67).

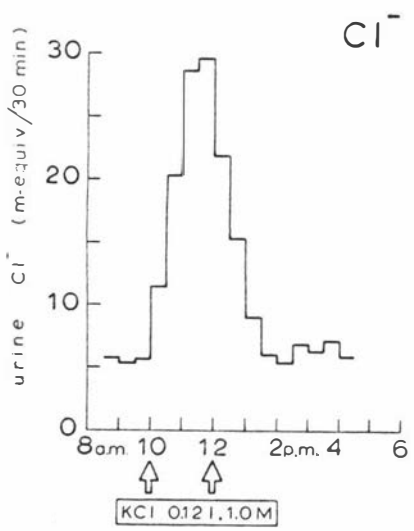

Fig 152. Urine Cl^- excretion following intravenous 1.0M KCl infusion. Note the peak excretion during the infusion. (Sheep 2, 16.3.67).

Over the 6½ hours, the extra Na^+ lost (allowing a 50% decline for high initial rates) was 98, 28 and 25 m-equiv, while at the end of the 2 hour infusion it was 97, 44 and 21 m-equiv.

The urine flow and total solute excretion both increased markedly at the time of natriuresis and kaliuresis (Fig 146, 147). In general, there was a small increase in urine volume and Na^+ excretion late in the post-infusion period (Fig 144a, 146) except on the day of depressed plasma volume and total solute content at that time. The extra water lost over and above that infused was 445, 467 and 555 ml, the latter with the greatest Na^+ loss. During the peak diuresis, the urine osmolality ranged from 420-845 mosm/kg, more commonly 420-650 mosm/kg.

The plasma volume (Fig 148) and total solute content (Fig 149) dropped to a minimum after 1½ hours. On the day when Na^+ loss was least, both were greater after the infusion than before it (Fig 148a); on one day, both increased steadily back to the preinfusion level; after the largest Na^+ loss there was a second decrease (Fig 148b). The greater the natriuresis, the lower the minimum plasma volume - 97%, 90% and 85% of the 9.15 a.m. volume. At the termination of the infusion, the plasma total solute content was depressed to approximately 94%, 96% and 98% i.e. about two-thirds of the total Na^+ loss could be accounted for by ECF Na^+. The $[$plasma protein$]$ increased during the calculated plasma volume shrinkage (Fig 148). The plasma O.P. increased during the reduced volume on 2 days (Fig 150a), more in one than in the other, but fell when Na^+ loss was greatest (Fig 150b).

Plasma $[Na^+]$ did not follow O.P., and on 2 days decreased during the infusion when the volume was reduced (Fig 151a). The fluctuations in $[Na^+]$ were large - many times the difference in individual duplicate

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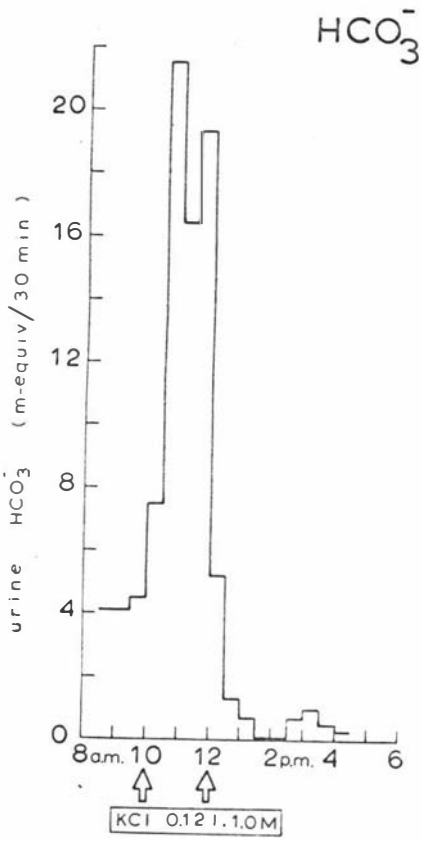

Fig 153. Urine HCO_3^- excretion following intravenous 1.0M KCl infusion. Note the large increase in excretion during the infusion. (Sheep 14, 5.12.66).

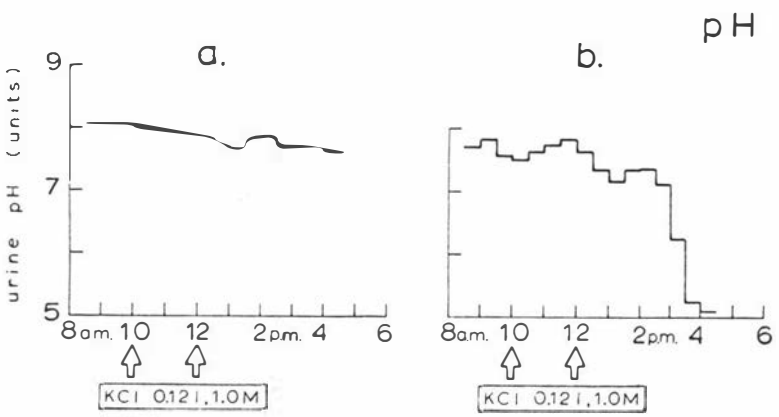

Fig 154. Urine pH following intravenous 1.0M KCl infusion. Note in a urine pH was similar to controls, in b there was a marked fall in pH in the post-infusion period. (a - sheep 2, 16.3.67; b - sheep 15, 16.2.67).

determinations. Nor did plasma $[Cl^-]$ follow O.P., but all 3 showed a peak just after the end of the infusion (Fig 151b).

Cl^- excretion increased considerably in the first 30 minutes and reached a maximum in the second hour (Fig 152). The overall excretion was 53 and 19 m-equiv less and 11 m-equiv more than the infused amount, equivalent to that for excreted K^+ only when Na^+ was low.

HCO_3^- excretion and urine pH were each estimated on 2 days. HCO_3^- increased during the infusion but dropped rapidly afterwards (Fig 153). The extra HCO_3^- loss of 48 and 27 m-equiv during the 2 hour infusion was reduced to 20 and 24 m-equiv overall by later HCO_3^- conservation. On one day, the pH decreased steadily as in the controls (Fig 154a). On the other, it was no higher during the infusion, but dropped precipitously afterwards (Fig 154b) when HCO_3^- excretion was virtually zero.

DISCUSSION

0.15M NaCl

The infusion of 120 ml of 0.15M NaCl represents only a small load of Na^+ and water, and was intended to serve mainly as a comparison for the load of 1.5M NaCl and 1.0M KCl. Nevertheless, the dilute NaCl infusion produced a small natriuresis and diuresis with a little extra K^+ excretion during the infusion, while the plasma composition was almost unchanged. However, it is likely that a small increase in plasma volume and total solute content did result from the infusion since half of the controls showed a decrease, whereas in this group of 4 all increased; the magnitude of the change, however, was similar to the controls. Moreover, a small increase in plasma $[Cl^-]$ frequently followed the infusion.

The more prominent effects were in the excretion of Na^+, Cl^-, water and K^+, which, in many respects, resembled those produced by intraduodenal infusion of 0.15M NaCl. As for the latter, a low preinfusion Na^+ reduced the excretion of Na^+, Cl^- and water, particularly Na^+, but the overall amounts of each were independent. On the days of low excretion, the Na^+ peaks were as small as some seen on control days, yet their consistency of timing suggests they resulted from the infusion. The onset of the natriuresis was immediate on the day of highest initial excretion, but was delayed 60-90 minutes in three. This resembles the case of intraduodenal infusion where there was no delay when high preinfusion Na^+ excretion was observed. The Na^+ and water excretion, where significant, were biphasic with both routes of administration.

There was always some increase in K^+ excretion during the infusion, but the plasma K^+ content was not altered. This probably represents K^+ release from the ICF since it would be unlikely that the kaliuresis was induced by the increase in Na^+ excretion which was only small, was biphasic and was not consistently related in time.

As well as the natriuresis, there would appear to be an active diuresis other than an osmotic one since the urine flow was independent of solute output, and the increase in volume can occur with little or no increase in Na^+ excretion. The stimulus to either the natriuresis or the diuresis is not clear. Isosmotic expansion of the ECF is associated with a natriuresis and depressed proximal tubule fractional Na^+ reabsorption (Cortney et al., 1965; Dirks et al., 1965 and others). It is postulated that a humoral factor is released as a result of stimulation of volume receptors. Most of these studies employ a dose rate many times greater than

do the present experiments, consequently, if this mechanism is involved in the present natriuresis, it is necessary to postulate a very sensitive volume receptor system. The location of the receptors is less likely to be in the arterial circulation than in the low-pressure venous system since only the latter would appear capable of responding to such a low infusion rate. The site of the receptors would be between the jugular vein and the heart in the case of intravenous infusions. Moreover, there was a similar biphasic natriuresis and diuresis after intraduodenal infusion, suggesting that either the same discrete receptors were involved, or else a considerable length of the large veins is sensitive to the stimulus. Perhaps inhibition of the Na-retaining and anti diuretic principles recently demonstrated in heart tissue (Lockett, 1966, 1967; Ilett and Lockett, 1968) or secretion of natriuretic and diuretic substances could be involved.

1.5M NaCl

The infusions of 1.5M NaCl can be compared with those of Potter (1966, 1968) in two breeds of sheep. Although his preinfusion conditions were different and the loads much greater, the effects on urine excretion and plasma composition were qualitatively similar to those observed here, with the exception of K^+.

Potter has shown that the natriuresis of hypertonic NaCl loading results from both increased GFR and reduced fractional tubular Na^+ reabsorption in Dorset Horn sheep, but only from the latter in Merinos. The natriuresis in the Romneys in the present experiments may have involved either or both of these renal changes. In these, a high preinfusion Na^+ excretion was associated with a greater natriuresis during the infusion,

both faster onset and 3-4 times the maximum excretion, with smaller increases in plasma volume and total solute content. The efficiency of elimination of a Na^+ load was increased in Potter's experiments by preconditioning the sheep to Na^+ loading with 1.5% NaCl in the drinking water. In his adapted sheep, the GFR was higher initially and increased to a greater extent during the infusion, an adaptation which would appear to be adequate without Potter's suggested involvement of ADH or an unknown mechanism.

Intravenous hypertonic saline infusion differed from intraruminal or intraduodenal administration in that where the preinfusion excretion was high there was no delay in onset of raised Na^+ excretion, compared with a 1-2 hour delay for infusions into the gut (except on the day on which diarrhoea occurred).

The biphasic natriuresis seen after the small intravenous load of Na^+ was replaced by a considerably greater single peak of Na^+ after the 1.5M NaCl. In this case, plasma volume expansion is a more likely stimulus for the natriuresis since the volume of infusate per se is reinforced by the expansion of plasma volume by water drawn from the cells or the gut by the raised plasma O.P. However, the intraruminal and intraduodenal infusions have shown that the greater the natriuresis the smaller the expansion of plasma volume, so that there appears to be no simple relationship between the two.

Hypertonic NaCl produced more consistent effects on K^+ when infused intravenously than by other routes. On all days, extra K^+ was lost in the urine compared with controls; this was also seen in Dorset Horns (Potter,

1968) although the opposite was observed in Merinos (Potter, 1966). It would appear that more K^+ was lost when Na^+ excretion was low, since 68 and 16 m-equiv of extra K^+ were excreted compared with 17 and 3 m-equiv when there was a greater natriuresis. The immediate rise in K^+ excretion when the natriuresis was delayed may reflect an early release of ICF K^+ in exchange for retained Na^+. This contrasts with the later kaliuresis observed when the natriuresis began at once, in which case it might be expected that the smaller retained load of Na^+ would take longer to displace a significant amount of intracellular K^+. In sheep adapted to saline loading, the urinary K^+ loss was lower (Potter, 1968), which Potter suggested might indicate an adaptive K^+ conservation. It may also reflect the degree of Na^+-K^+ exchange across cell membranes during more rapid Na^+ elimination. The plasma K^+ content did not alter significantly on any day, and on only one day did the plasma $[K^+]$ drop during the infusion. Potter (1966, 1968), using a much larger NaCl load, produced decreasing $[K^+]$ in both breeds of sheep. The extent of plasma volume expansion may determine whether or not the plasma $[K^+]$ falls.

Changes in HCO_3^- excretion (measured on one day only) and urine pH followed the same pattern observed with the other infusions, namely that a small increase in HCO_3^- excretion occurred concurrently with a slight natriuresis, and urine pH declined with rising urine flow.

Illustrated in Fig 134b is the unusual occurrence of a large increase in PCV and [Hb] (16% greater than the previous sample) not accompanied by a rise in [plasma protein]. This would appear to result from adrenaline release and splenic contraction (Turner and Hodgetts, 1959) increasing the red cell mass being used as a marker. English (1966) noted

similar rises of around 12% when sheep were weighed, but the effects of the adrenaline were off in about 20 minutes. The plasma volume estimated an hour later would seem to be a valid one.

1.0M KCl

As previously reported in monogastrics (Winkler and Smith, 1942; Baldwin et al., 1950; Berliner et al., 1951, and others), in cows (Anderson and Pickering, 1962) and in sheep (Keynes and Harrison, 1967), intravenous KCl infusion produced a marked rise in the excretion of K^+, Na^+, Cl^- and water. In monogastrics, and in the present experiments, HCO_3^- excretion was also raised. Of particular interest is the rapidity of K^+ elimination, the magnitude and origin of the natriuresis, and the raised HCO_3^- excretion.

In contrast with the kaliuresis seen after most other infusions, the changing K^+ excretion was associated with both plasma $[K^+]$ and K^+ content. Urine K^+ rose to approximate the rate of administration by the second hour of the infusion, but declined quickly when it was terminated. This rapid K^+ elimination also was seen in cows after 2 hours of infusion by Anderson and Pickering (1962) who found the excretion rate balanced the rate of administration when the infusion was prolonged. In one of the present experiments all infused K^+ had been eliminated by the end of the 6½ hour post-infusion period, however, in the other two, 28 and 30 m-equiv were retained, but not in the ECF.

The extra Na^+ loss was large - 98, 28 and 25 m-equiv overall - and occurred almost entirely during the 2 hour infusion (97, 44 and 21 m-equiv). Approximately two-thirds has been estimated from the drop

in plasma total solute content to come from the ECF, therefore part of the Na^+ appears to be of cell origin, probably in exchange for K^+. On one day, a second decrease in plasma volume and total solutes probably indicated a further redistribution across cell membranes since only 1 m-equiv of Na^+ was excreted.

The stimulus for the K^+-induced natriuresis is not evident; it is obviously not expanded plasma volume since in this case it is contracted. It was suggested by Berliner et al. (1951) and Anderson and Pickering (1962) that in the distal tubule Na^+ may not exchange as efficiently for K^+ as it does for H^+, so that when a load of K^+ is to be excreted extra Na^+ will be lost in the urine. The present observations, however, do not support this explanation since the Na^+ peak preceded that for K^+ except on the day of low preinfusion Na^+ excretion, and the Na^+ loss was very variable for similar K^+ excretion. If the Na^+ loss is not the result of such an interaction, then the kidney is responding to a stimulus whose nature and origin can only be speculative. There may be some means of detecting the passage of Na^+ from the ICF to the ECF, but then it is necessary to explain the large overshoot in Na^+ excretion since the total excreted was three times the intracellular loss. Alternatively, it may be that any intravenous infusion is a stimulus for natriuresis, as suggested for the NaCl infusions of the same volume, but it is noteworthy that many times more Na^+ is excreted after the KCl than after the isotonic NaCl, so that it is necessary to postulate a "sensitising" effect of the infused K^+ on Na^+ excretion. Any working hypothesis obviously requires further experimentation.

An extra 450-550 ml of water were excreted by the kidneys, an effect which is equally as difficult to explain as the natriuresis. The

diuresis is unlikely to be caused by raised GFR since Anderson and Pickering (1962) found no increase with a similar infusion in cows. It could be secondary to solute loss, however the kidney was not operating at its maximal concentrating capacity for the solute being excreted since the urine osmolality was higher after 1.5M NaCl, and after feeding (K^+ was the chief urinary solute). A third possibility is reduced ADH secretion. However, the generally accepted stimuli for this, namely decreased plasma O.P. or increased plasma volume do not pertain in this case: O.P. increased on 2 of 3 days and plasma volume was always reduced. Moreover, the urine never became hypotonic to plasma as commonly occurs with reduced ADH. Finally, there remains the possibility that a diuretic substance was released by the infusion as discussed previously.

After KCl, the urine pH did not rise prominently as it does in monogastrics where the initial pH and $[HCO_3^-]$ are considerably lower. The $[HCO_3^-]$ usually decreased a little when the urine flow increased markedly. HCO_3^- excretion increased during the infusion by 27 and 48 m-equiv on 2 days, particularly when the Na^+ excretion was very high. While this may be the result of an increased filtered load of HCO_3^-, it is more likely due to either reduced H^+ secretion, or excretion of HCO_3^- in association with the increased excretion of cations (with a compensatory increase in H^+ excretion as titratable acidity and NH_4^+). This last suggestion appears worth examining in future work because of the frequent association of raised Na^+ and HCO_3^- excretion observed in the present work, as well as the more commonly reported association of HCO_3^- with K^+ excretion.

CHAPTER 7

GENERAL DISCUSSION

The water and electrolyte shifts and regulatory mechanisms consequent upon feeding or electrolyte administration have already been discussed for each experimental situation in Chapters 2-6. In this section, the contributions of these observations to knowledge of renal regulatory mechanisms and transport across the rumen mucosa will be considered further.

Renal excretion

The urine of ruminants differs in composition from that of carnivores, being alkaline, rich in HCO_3^- and K^+ and low in Na^+. The constitution of the diet appears to be largely responsible for these differences, since ruminant urine can be made to resemble that of monogastrics by suitable manipulation of the diet. For example, acid urine is excreted by cows on feeds of low K^+ but high Ca^{++} and Mg^{++} content (Brouwer, 1952); Forman and Sauer (1962) reported urines of pH 5.8 in sheep fed *ad libitum* Festuca hay of low nitrogen, alkali and alkaline earth content, contrasting with urines of pH 8.3 in sheep fed clover hay.

Although the output of urine of different composition may be entirely due to diet, the possibility exists that the renal controlling mechanisms of ruminants may not be identical with those of species more commonly used for such studies. Unusual responses to administered hormones have indeed been recorded. Kinne *et al.* (1961) reported that aldosterone

failed to raise K^+ excretion in the sheep although Na^+ retention did occur. The ruminant has been described as having an atypical renal response to ADH in that in hydropenic animals it produces an increase in urine flow and electrolyte excretion, particularly K^+ (Kinne et al., 1961; Cross, Thornton and Twedell, 1963; Cross and Thornton, 1966; Macfarlane, Kinne, Walmsley, Siebert and Peter, 1967). Since the sheep appears particularly sensitive to ADH (Macfarlane et al., 1967), it may be that injection of even small doses into an animal producing a high endogenous level results in a pharmacological action of the hormone. Stacy and Brook (1965) and Brook, Radford and Stacy (1968) believe that the action of ADH in physiological amounts in the sheep is no different from that in other species.

In three instances in the present observations the renal response of the sheep appeared at first sight to differ from that of monogastrics. First, the sheep promptly excreted the intravenous load of K^+, in contrast to the poor elimination by carnivores unless subjected to a period of adaptation to K^+ loading (Thatcher and Radike, 1947). It is reasonable to suppose that the sheep were already adapted to handling K^+ loads by the high K^+ content of the diet. Some light has been cast on the mode of this adaptation by Alexander and Levinsky (1968) who showed the enhanced excretory capacity in adapted rats to be associated with chronic hypersecretion of aldosterone. Any role of aldosterone in this phenomenon must be a long-term one since administration of the steroid just prior to the K^+ loading was ineffective. Secondly, the urine pH fell during the diuresis after intraruminal water, 0.15M NaCl or KCl although it usually rises with increasing urine flow in carnivores. However, the typical response in sheep is also seen in alkalotic humans (Barclay et al., 1947; Reid and

Hills, 1965). Eggleton (1946) and Barclay et al. (1947) have shown that, regardless of the initial pH, the urine pH changed towards 6.9 as the flow increased. Finally, the classical diuretic stimuli of reduced plasma osmolality and increased plasma volume did not apply during infusion of water and 0.15M KCl into the rumen. However, similar observations have been made during water loading in man (Cordova and Lococo, 1964), so that this lack of correlation would not appear to be unique to the sheep. It may be concluded that there is no qualitative difference in the kidney regulation of sheep when compared with other species, and that conclusions arising from the present investigation should be generally applicable.

K^+ balance is obviously closely regulated, although little is known of the homeostatic mechanisms involved. Urinary K^+ has been said to be influenced by cell $[K^+]$ (Mudge et al., 1950; Landwehr et al., 1966), by Na^+ excretion (Berliner et al., 1951; Davidson et al., 1958; Walker et al., 1961; Malnic et al., 1966a,b) and by competition for secretion with H^+ (Berliner et al., 1951; Maren, 1954; Orloff and Davidson, 1959). Attempts were made to relate the changes in K^+ excretion in the present experiments to possible determinants of the kaliuresis including changes in plasma $[K^+]$, plasma K^+ content, and predicted changes in cell $[K^+]$ or K^+ content. If the K^+ content of either ECF or ICF is the kaliuretic determinant, this would necessitate the added monitoring of volume coupled with the integration of concentration and volume changes.

Although the association between urinary and plasma K^+ has not been favoured in the literature, the correlations between these were considered in the present experiments since the latter could not be excluded

as the kaliuretic factor after K^+ loading. The experiments involving substantial addition of K^+ into the ECF support the hypothesis that either plasma $[K^+]$ or K^+ content is the determinant of the kaliuresis since, following intraruminal 0.15M KCl, and during 1.0M KCl intravenous infusion, K^+ excretion paralleled both plasma $[K^+]$ and K^+ content. On the other hand, following all other infusions, there was no consistent relationship between plasma $[K^+]$ and K^+ excretion, and, in fact, when large loads of NaCl were introduced into the gut, the resultant kaliuresis was associated with a consistent decrease in plasma $[K^+]$. The hypothesis that ECF K^+ content might be the kaliuretic factor received more support since the kaliuresis after intraruminal infusion of both NaCl solutions and the later phase of the kaliuresis after intraruminal 1.5M KCl were associated with increased plasma K^+ content. However, this association was not borne out by the other infusions, and again the kaliuresis after the intraduodenal 1.5M NaCl occurred with a large decrease in K^+ content.

Changes in cell $[K^+]$ and K^+ content would seem the next logical consideration as causes of increased K^+ excretion. Although cell K^+ was not measured in these experiments, movement in and out of the cell may be inferred from changes in ECF K^+ content and urine losses. Following the intravenous KCl infusion, when calculation of K^+ penetration into cells is possible, by $3\frac{1}{2}$ hours after cessation of the kaliuresis 28 and 30 m-equiv of K^+ were retained outside the ECF on 2 days, presumably elevating the cell K^+ content. It would thus seem that one of the plasma parameters is the kaliuretic determinant in this case, although cell $[K^+]$ also may have returned to the initial level after water movement into the ICF had reached equilibrium. Raised cell $[K^+]$, probably also involved in the kaliuresis

after 0.15M KCl intraruminally, would be favoured by the large increase in the ECF K^+ content and the increase in plasma O.P. Similarly, after the intraruminal infusion of 1.5M KCl, although the ECF K^+ content rise was only small initially, the rise in plasma O.P. was steady and in itself would tend to raise cell $[K^+]$ by dehydration. In the case of infusions other than KCl, it is harder to predict what would happen to cell $[K^+]$, since on one hand the K^+ is apparently displaced from the cell, and on the other water may be drawn out of the cells, particularly after hypertonic infusions. After isotonic NaCl infusions, it seems unlikely that the kaliuresis could be ascribed to either increased cell K^+ content or concentration. It would certainly seem that cell K^+ content can be ruled out as a determinant of kaliuresis after these other infusions since movement of K^+ would seem to be out of cells. The possibility remains, however, that it is not the $[K^+]$ of body cells in general which determines K^+ excretion, but the $[K^+]$ of specialised cells such as those of the renal distal tubule, a possibility which would be difficult to determine experimentally.

From the above discussion it would appear that there is no single obvious controller of K^+ excretion in all situations. There is no reason, however, why several factors might not determine the rate of K^+ excretion, the predominant one depending upon the physiological status of the body with respect to body K^+ content, adaptation to K^+ loading, acid-base balance, availability of other cations and state of hydration.

The effects of the major determinants of K^+ excretion may be modulated by interactions with transport of other ions across the renal tubule. K^+ excretion may be influenced by the acid load to be excreted

and the amount of Na^+ reaching the distal tubule. However, increased K^+ excretion associated with increased Na^+ excretion, or reduced acid loss may not always result from such a renal tubular interaction, and in two instances in the present experiments such would appear to be the case. In most instances after infusion of either NaCl or KCl, there was both a natriuresis and a kaliuresis, however, frequently the peak of Na^+ excretion did not coincide with that for K^+. Moreover, when KCl was infused intravenously, the natriuresis clearly preceded the peak kaliuresis, and was declining rapidly during the latter. Such observations make it difficult to conclude that the increased Na^+ excretion was related to the kaliuresis at the renal level. The second case is the depressed K^+ excretion during feeding in the sheep, along with increased acid excretion and Na^+ retention. Stacy and Brook (1964) supported Berliner's hypothesis that competition with H^+ for secretion could cause lowered K^+ excretion. However, as discussed in Chapter 2, the evidence from the present experiments is against this competition being the sole factor, since there was a separation in time of the minimum K^+ excretion and urine pH, and kaliuresis forced by acetazolamide occurred at the expense of plasma K^+.

H^+ secretion by the kidney results in reabsorption of the luminal HCO_3^-, followed by elimination as titratable acidity and NH_4^+. In ruminants, the acid load is small, HCO_3^- is incompletely reabsorbed, the excretion of titratable acid and ammonia is low, and the amount of HCO_3^- being excreted reflects the H^+ secretion. In carnivores, HCO_3^- excretion in the urine is insignificant, and titratable acidity and NH_4^+ reflect the larger acid secretion. In the sheep, during a single daily feed, acid excretion usually increased to such an extent that $[HCO_3^-]$ fell to very low levels

and the urine became acid; on a few days, however, the urine remained alkaline with higher $[HCO_3^-]$.

While it is generally believed that HCO_3^- reabsorption is in the form of CO_2, Reid and Hills (1965) calculated that some HCO_3^- reabsorption as such must occur in alkalotic man. Further, they proposed that the extent of direct HCO_3^- reabsorption was dependent upon the luminal $[HCO_3^-]$, and thus decreased with rising urine flow. If their hypothesis is correct, altered HCO_3^- excretion may not reflect altered H^+ secretion only.

The normally alkaline urine of sheep gives the opportunity to relate changes in HCO_3^- excretion to that of excreted cations. In carnivores, the virtual absence of urinary HCO_3^- leaves fixed anion such as Cl^- to accompany Na^+ and K^+. In sheep, however, HCO_3^- not only acts as an absorber of H^+ but also as an anion associated with Na^+ and K^+. After several electrolyte infusions in the sheep, raised HCO_3^- excretion accompanied not only increased K^+ excretion, which might be expected on the basis of K^+-H^+ competition, but also accompanied increased Na^+ excretion. If part of the excreted HCO_3^- is associated with cations in this way, should available HCO_3^- be limiting, it may force excretion of acid in other forms (titratable acid and NH_4^+). This could be determined experimentally. The converse situation may be involved in the raised excretion of Na^+ and K^+ along with that of HCO_3^- during water diuresis in sheep, and in alkalotic man. Reid and Hills (1965) suggested that part of the increased cation loss may be secondary to reduced reabsorption when HCO_3^- reabsorption is reduced by the lowered luminal $[HCO_3^-]$.

The rate of excretion of both Na^+ and water depends upon the net

effect of glomerular filtration and tubular reabsorption. In the present series of electrolyte infusions, since GFR was not measured, it is impossible to determine how much of a diuresis or natriuresis was due to increased filtration or to decreased reabsorption. To achieve the higher increases in excretory rate, it seems likely that both mechanisms were contributing.

In the case of water, decreased reabsorption is classically attributed to reduced ADH secretion, which in turn results from a fall in plasma osmolality or increased plasma volume. Neither of these stimuli was observed before or during the diuresis following some infusions. For example, after the intraruminal water infusions, plasma volume and O.P. changes were no greater than in the controls; this has also been reported with water loading in man (Cordova and Lococo, 1964). Furthermore, an intense diuresis followed intraruminal administration of 0.15M KCl, although the plasma volume decreased and the O.P. increased initially. Observations such as these suggest that a component of the diuretic mechanism is sensitive to a stimulus other than generalised changes in plasma volume or osmolality. A localized stimulation of a receptor close to the site of absorption (and to the site of administration in some experiments) may be the means of sensing small changes in O.P. or increases in ECF or plasma volume. In fact, an osmoreceptor in the portal circulation has been demonstrated by Haberich and coworkers (Haberich, Aziz and Nowacki, 1964, 1965, 1966; Aziz, Nowacki and Haberich, 1966; Nowacki, Aziz and Haberich, 1966; Haberich, Aziz and Ohm, 1967; Haberich, 1968). They believe that the ingestion of water stimulates this receptor, and that the hydration of the liver buffers systemic O.P. changes so that the hypothalamic receptors

only come into play with high rates of absorption.

In the case of Na^+, decreased reabsorption has been attributed to a natriuretic factor acting in the proximal tubule (Cortney et al., 1965; Cirksena et al., 1965; Dirks et al., 1965; Watson, 1966; Landwehr et al., 1967), or over a longer period of time, to reduced aldosterone secretion leading to decreased distal tubular reabsorption. The proximal mechanism is assumed to be stimulated by ECF volume expansion such as results from massive saline loading. However, after large Na^+ loads the magnitude of the increase in plasma volume was inversely related to the extent of the natriuresis; after small Na^+ loads (0.15M NaCl intravenously or intraduodenally) a small increase in plasma volume was associated with a natriuresis, and a larger increase in volume with complete Na^+ retention. Since the natriuretic stimulus does not appear to be the systemic increase in ECF volume, it may arise from a local receptor mechanism analogous to that for ADH in the portal circulation.

Some properties of the natriuretic mechanism are apparent from the present experiments: it appears to be sensitive to the initial Na^+ status (as reflected in the delay when the preinfusion Na^+ excretion was low) and to the source or rate of acquisition of Na^+. When large Na^+ loads were infused into the gut, Na^+ excretion, but not absorption, was delayed; with intravenous Na^+, the delay was dependent on the preinfusion excretion being low; when KCl was given, excretion of the Na^+ displaced from the cells commenced without delay.

The small diuresis seen after the intravenous and intraduodenal isotonic saline infusions seemed to be closely related to the natriuresis.

The time course of the two was similar, and low preinfusion Na^+ excretion was associated with inhibition of both. Like the natriuresis, the more intense the diuresis, the smaller was the plasma volume expansion after the 3 litre intraruminal infusions i.e. the diuresis was greatest after water, next after KCl, and least after NaCl, but the plasma volume changes were in the reverse order. Thus, the natriuresis and the diuresis may share the same receptor site, or the one efferent system may have two efferent effects. The common receptor may be one already shown to be associated with ADH control, such as the portal system receptor, or perhaps the left atrial receptors of Gauer (Henry et al., 1956; Gauer and Henry, 1963). Alternatively, an independent antidiuretic substance similar to or identical with the antidiuretic-antinatriuretic substance isolated by Ilett and Lockett (1968) from heart tissue may be involved.

The site or nature of any such receptor is purely conjectural. It would more likely be located in the low pressure side of the circulation since it must be sensitive enough to respond to an intravenous 0.15M NaCl infusion rate of as little as 1 ml/min. From the similar response to infusion into the duodenum or into the jugular vein, it is suggested that the receptor would be close to the heart, or else related to the stretch of the walls of the capacitance vessels over a considerable length.

Several aspects of the control of renal excretion of water and electrolytes have been discussed because present mechanisms fail to explain satisfactorily observations in these experiments. Further experimentation should show whether or not hitherto undescribed components do play a significant role in these regulatory processes. In particular, the following questions need to be answered. Is HCO_3^- excretion in the sheep coupled to

Na^+ and K^+ excretion, and if so, is there a redistribution of the forms in which H^+ is excreted in order to maintain acid-base balance? What type of receptor(s) can detect gains of ECF Na^+ and water, and what is the mechanism of the resultant natriuresis and diuresis? Is there one, or are there several determinants of K^+ excretion, and how important is the intracellular K^+ in this respect?

Transruminal water and electrolyte transfer

The characteristics of net transport of water and electrolytes across the rumen mucosa have usually been studied in simplified systems - *in vivo* in the isolated, emptied and washed rumen, or rumen pouch, filled with a simple solution, or *in vitro* across stripped sheets of rumen epithelium. Such experiments have established that Na^+ is actively absorbed from rumen contents, that K^+, Cl^- and HCO_3^- move according to the electrochemical gradient, and that the rumen mucosa is water permeable. Although these observations are probably qualitatively correct, there has not been accurate quantitative estimation of net transfers in physiological conditions.

Extrapolation from the experiments described above to a real physiological situation has not been possible since the particular conditions and procedures employed may alter the rate, or even the nature of the transport. It is known that VFA absorption can be depressed by starvation (Armstrong *et al.*, 1957), cannulation of the rumen (Masson and Phillipson, 1951), excessive washing out, and leaving the rumen filled with saline overnight (Ash and Dobson, 1963). Although water and electrolyte transfers need not be effected by procedures which alter VFA transport, it is likely that maintenance of intact rumen epithelial metabolism may be necessary for

exhibition of the normal transport properties. This is indicated by the need for the sheep to be fed before a sample of rumen epithelium is taken for study in vitro, and for the tissues to be bathed by VFA- containing solution to obtain a stable preparation (Ferreira et al., 1966).

Estimates have been made of the rate of transruminal net water movement by measuring the rumen fluid volume and the dilution rate, and comparing these with the salivary flow, either measured or assumed (Hydén, 1961; Engelhardt, 1963; Stacy and Warner, 1966; Ternouth, 1967). In practice, rumen volume estimations using marker dilution techniques contain inaccuracies because experiments are frequently performed under conditions when there is a discontinuity in the marker concentration/time curve. As a result, there is considerable variation in the results obtained by different workers. Hydén (1961) calculated the net absorption of water from the rumen of sheep unfed for 2-12 hours to be of the order of 0.15 l/hr. During feeding the rumen fluid volume increases; Stacy and Warner (1966) believed that salivary inflow accounted almost entirely for the extra fluid, but Ternouth (1967) estimated that 20% entered across the rumen wall.

Some reported observations, taken together with findings in the present experiments, seem to indicate that, with the exception of Na^+, little net transfer of water and electrolyte occurs across the rumen wall. This was illustrated for K^+ by the striking difference in the time course and total amount of K^+ absorption and excretion following intraruminal infusion of 0.15M and 1.5M KCl. When a high concentration of K^+ was built up in the rumen by 1.5M KCl infusion, absorption and onset of excretion of extra K^+ began immediately, suggesting that a small amount of transruminal K^+ movement had taken place. When the K^+ load was given in a larger volume, the onset

of absorption was delayed, but over the 6½ hour observation period 50% more K^+ was excreted. This may be explained by increased reticulo-ruminal outflow to more distal regions of the gut caused by the larger increase in rumen volume (Ash, 1962). The overall greater K^+ absorption would suggest that the rumen K^+ permeability is not high, or at least considerably lower than that of the small intestine.

In contrast, the rate of Na^+ uptake from the rumen appeared to be appreciable, and to differ very little with the two concentrations of NaCl infused, perhaps being a little greater from the 1.5M solution. Na^+ absorption is similar in the two cases although reticular outflow would be expected to be increased far more by the larger volume (0.15M) than by the smaller volume (1.5M). Infusion of 1.5M NaCl into the rumen and into the duodenum resulted in a qualitatively similar picture, and a slightly greater uptake after the intraruminal infusions than from the range of intestinal infusions. It would therefore seem likely that the rate of Na^+ absorption from the rumen does not differ greatly from that in the intestine.

Several observations suggest that the rumen wall exhibits little net water transfer. The prolonged hypotonicity of rumen contents in fasted animals (Engelhardt, 1963; Warner and Stacy, 1965; Ternouth, 1967) could arise from continuing Na^+ absorption in excess of water. The depressed rumen O.P. following intraruminal infusion of water rises only slowly and at an almost constant rate (Warner and Stacy, 1965), whereas if the water flowed along the osmotic gradient created across the rumen wall the rise in O.P. would be expected to be greatest initially, then slowing as the gradient was reduced. The observed steady increase could be the result of the hypotonic outflow to the omasum being replaced by a fairly uniform inflow of

hypertonic or isotonic saliva. Further support for the relatively low water permeability of the rumen is the failure of Stacy and Warner (1966) to detect transruminal water influx when hypertonic solutions were infused into the rumen. In the present experiments, there was no evidence that water entered the rumen after the hypertonic infusions: in no case did the relative plasma volume decrease after the infusion as it might be expected to do if the rumen were highly water permeable, particularly after the 1.5M KCl which was only slowly absorbed.

The absorption and excretion of water after the 3 litre intraruminal infusions is compatible with only a small amount of direct absorption across the rumen wall and a large fraction from the intestine. The total fluid absorbed from the 3 types of infusion was similar (around 75% of the volume administered) although the proportion appearing in the urine varied with the infused solute. Increasing the rumen volume raises the omasal outflow in proportion to the volume added (Ash, 1962), although the composition of this digesta would vary after the different infusions. The time course of fluid absorption appears to be similar after water and 0.15M KCl, the two most easily compared, yet the water infusion would halve the rumen O.P. while the 0.15M infusions would raise it slightly. These observations suggest that, rather than water movement taking place along osmotic gradients, water is absorbed relatively independently of these gradients; this form of water movement is most readily visualized in the intestine where absorption follows equilibration to isotonicity in the proximal segments.

During a single daily feed the rumen contents also become hypertonic to plasma and the rumen fluid volume increases rapidly (Reid, 1965; Warner and Stacy, 1965; Stacy and Warner, 1966; Ternouth, 1967). The

latter two authors have attempted to partition this gain of fluid into that entering in saliva and that from direct transruminal flow, but use methods and assumptions which preclude accuracy. The present feeding experiments suggest that the relative contributions of salivary water and direct diffusion may vary with the rate of eating. In all cases, the plasma volume shrank over the first 15 minutes of feeding; where the fall was greatest, the O.P. also decreased, but where it was less, the O.P. increased (Fig 61). These observations were interpreted as involving the simultaneous secretion of a hypotonic (diffusion) and a hypertonic component (saliva), the former making a relatively greater contribution when the plasma volume falls least. Since the saliva is only slightly hypertonic to plasma, the volume of saliva would probably be many times the volume of transruminal water.

The degree of permeability of the rumen to substances which move passively along their electrochemical gradient could be of major significance in preserving an almost constant internal environment in the ruminant. If the rumen were highly permeable, allowing rapid equilibration of rumen contents with the ECF, then rapid feed intake, drinking large volumes of water, or, under experimental conditions, administration into the rumen of large infusions, would quickly produce extensive changes in the ECF composition. Further, the permeability characteristics of the rumen mucosa may have affected the development or not of receptor organs in the rumen itself. It is possible that there are receptors involved not only in the co-ordination of rumen movements, salivary flow and abomasal secretion, i.e. regulating digestive functions, but also in controlling overall body composition. Such organs could be involved in hunger, satiation, thirst, salt appetite and perhaps even in the state of hydration or salt repletion. The possibility

of their being a reality is suggested by observations such as the correlation of feed and water intake, both in quantity and pattern, and the rapid metering of the water drunk by thirsty ruminants (Schmidt-Nielsen, Schmidt-Nielsen, Houpt and Jarnum, 1956; Bott et al., 1965). Should the rumen be highly permeable, it might be advantageous to have sensory receptors in the rumen. If, on the other hand, the rumen wall were relatively impermeable, changes in rumen composition would present no immediate threat to homeostasis in the body itself, and absorption from the gut could be very similar to that in monogastric species.

It is clear that further experimentation is needed in several areas. The extent of net transport across the rumen wall in the intact organ filled with digesta of varying composition remains to be determined directly and reliably. A step in this direction would be to bypass intestinal absorption by means of re-entrant intestinal cannulae and thus study the relative contribution of the rumen to absorption of an intraruminal load.

The non-uniformity of composition in different regions of the reticulo-rumen, the result of inadequate mixing, may play a significant role. On one hand, net transruminal fluxes of water and electrolytes may vary in the different areas both in rate and direction. On the other hand, specific receptor organs sensitive to the electrolyte or solute concentration may be located only in certain regions. The dorsal rumen frequently contains a raft of dry material, while, by contrast, Reid (personal communication) has found in cows that ingested water initially causes greater dilution of ventral rumen contents than those in other areas. The different environment in these two sites would mean that one might be more suitable for location of a specific type of receptor. Perhaps valuable

information is lost by attempting to obtain an average ruminal concentration of solute by averaging values or pooling samples from different regions.

However, if there are no such ruminal receptors, nor is the rumen functionally very permeable, then the maintenance of the enlarged water and electrolyte content of the digestive tract may not present as great a load on overall homeostasis as may be supposed, and the regulatory mechanisms of the monogastric would also be adequate in the ruminant.

SUMMARY

The relationship in sheep between water and electrolytes in gut fluids, especially in the rumen, and in the body fluid compartments has been studied by following the changes in urine excretion and blood composition associated with a single daily feed of 2-3 hours, during *ad libitum* feeding and following water, NaCl and KCl infusion into the rumen, duodenum and jugular vein. The following conclusions have been reached:

Associated with a single daily feed were changes which have been interpreted as reflecting an initial net secretory phase, predominantly of saliva, followed by a net absorptive phase. During the former, there was an antidiuresis, urinary acidification, retention of Na^+, K^+, HCO_3^- and urea, reduced plasma volume and raised tonicity. During the absorptive phase, there was raised excretion of water and all electrolytes, a return of the urine to an alkaline pH, and an expansion of plasma volume.

The most prominent effect of removal of the drinking water during feeding was the reduced feed intake.

Acetazolamide administration during feeding produced in the sheep the typical effects of renal carbonic anhydrase inhibition, with the exception of a prominent reduction of Cl^- excretion.

Sheep feeding *ad libitum* showed smaller fluctuations in urine and blood composition than did sheep feeding once daily, but diurnal and feeding components appeared to be involved.

The electrolyte infusions indicated that the rumen absorption of K^+ and water is lower than from the intestine, but that Na^+ uptake from the two areas is similar.

Apart from any differences in gut absorption, the sheep appeared to handle

loads of water and electrolytes by mechanisms common to other species. However, responses to certain infusions support the existence of a natriuretic and diuretic mechanism similar to that proposed by Lockett, as well as the operation of receptors sensitive to local changes rather than to systemic hypotonicity and hypervolaemia, such as have been identified in the portal circulation by Haberich and coworkers.

Appendix 1. History of infusions for each sheep, described in detail in Chapters 4 - 6.

Sheep	Date	Infusion	Route*	Volume (litres)	Concentration (M)
1	25. 7.66	NaCl	R	3.0	0.15
	26. 7.66	NaCl	R	0.3	1.5
	22. 8.66	water	R	3.0	
	24. 8.66	control			
	12. 9.66	KCl	R	3.0	0.15
	14. 9.66	NaCl	R	0.3	1.5
	10. 1.67	NaCl	V	0.12	1.5
	12. 1.67	NaCl	V	0.12	0.15
2	1. 8.66	control			
	2. 8.66	NaCl	R	0.3	1.5
	30. 8.66	NaCl	R	3.0	0.15
	1. 9.66	water	R	3.0	
	19. 9.66	KCl	R	3.0	0.15
	21. 9.66	NaCl	R	0.3	1.5
	22.11.66	water	R	3.0	
	24.11.66	NaCl	R	3.0	0.15
	12.12.66	NaCl	V	0.127	0.15
	13. 2.67	(Na+K)Cl	R	3.0	0.3
	14. 3.67	NaCl	V	0.12	1.5
	16. 3.67	KCl	V	0.12	1.0
	30. 3.67	KCl	R	0.3	1.5
3	18. 7.66	water	R	3.0	
	20. 7.66	control			
	16. 8.66	NaCl	R	3.0	0.15
12	27. 2.67	KCl	R	3.0	0.15
	3. 4.67	NaCl	D	0.2	0.15
	5. 4.67	NaCl	D	0.2	1.5
	17. 4.67	control			
	19. 4.67	KCl	R	0.3	1.5
13	20. 2.67	KCl	R	3.0	0.15
	20. 3.67	NaCl	D	0.2	0.15
	22. 3.67	NaCl	D	0.2	1.5
	11. 4.67	NaCl	V	0.12	0.09
	13. 4.67	NaCl	V	0.12	0.9
	24. 4.67	KCl	R	0.3	1.5
	1. 5.67	control			

Sheep	Date	Infusion	Route*	Volume (litres)	Concentration (M)
14	3.10.66	NaCl	D	0.15	1.5
	18.10.66	control			
	20.10.66	NaCl	D	0.2	1.5
	3.11.66	NaCl	D	0.2	1.5
	16.11.66	NaCl	D	0.2	0.15
	5.12.66	KCl	V	0.12	1.0
15	31. 1.67	NaCl	D	0.3	1.5
	2. 2.67	NaCl	D	0.3	0.15
	13. 2.67	NaCl	V	0.12	1.5
	16. 2.67	KCl	V	0.12	1.0
	6. 3.67	NaCl	V	0.12	0.15

* R = intraruminal
D = intraduodenal
V = intravenous

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