CHAPTER I

INTRODUCTION

INTRODUCTION

Taxonomically, sheep and goats are closely related and some varieties are difficult to distinguish. They are widely distributed in the world, and thrive in a variety of climates. In Australia, for example, most of them live in the arid and semi-arid areas (Squires, 1979; Harrington, 1982) where availability of preferred food and fresh water are frequently limited. In these areas, salt bush (*Atriplex sp.*) and blue bush (*Kochia sp.*) can constitute the main food sources (Wilson, 1966a), and bore water supplied may also contain a significant level of sodium chloride (Pierce, 1957). In the Galapagos Islands, there have been many sightings of goats on beaches apparently drinking from the sea (Dunson, 1970).

Numerous studies on comparisons between sheep and goats in aspects of nutrition and digestion (Jones, Larsen, Javed, Donefer and Grudreau, 1972; Maloiy, Taylor and Clements, 1978; Alam, Poppi and Sykes, 1985; Quick and Dehority, 1986; Dominique, Dellow and Berry, 1991), and water metabolism (Maloiy and Taylor, 1971; MacFarlane and Howard, 1974; Ghosh, Singh, Verma, Saxena and Ranjhan, 1980; Aganga, Umunna, Oyedipe and Okoh, 1989) have been reported. Most of the results indicated that goats have greater efficiency than sheep in digesting a low quality roughage diet, and in using the limited water.

Concerning salt loading, however, investigations comparing sheep and goats carried out up to now are scarce and incomplete, being largely part of the nutritional experiments listed above. The wealth of information available is only for the sheep.

Some effects of high salt intakes by sheep have been determined. Sheep will tolerate about 1.3% sodium chloride (225 mmol/l) in drinking water (Pierce, 1959), and goats about 1.5% (250mmol/l) (Macfarlane and Howard, 1974),

When grazing salt bush and blue bush containing sodium chloride of 3.2 - 8.2% of dry weight with fresh water freely available, the intake of sodium varied between 25 to

97 g/d, and voluntary water intake varied up to 11.3 l/d. Under these conditions, urinary sodium concentration was up to 500 mmol/l (Wilson, 1966a). For sheep grazing salt bush and with free access to fresh water, Macfarlane and Howard (1974) noted that the osmolality of the urine was no higher than 1200 mosmol/l, even though the kidney of sheep can concentrate to three times this value.

Some adverse effects, such as diarrhoea and reduced feed consumption, have been reported when 1.5% salt water was offered to sheep (Pierce, 1959; Wilson, 1966b).

In view of the somewhat restricted information available on comparisons between sheep and goats in their water and salt metabolism, the studies reported in this thesis were designed to investigate the effects of several factors on water and sodium dynamics. These factors included method of salt presentation, animal species and roughage diets, level of salt loads and thermal load.

Two approaches of salt presentation were considered : intraruminal infusion with free access to fresh water (Chapter III), and in drinking water (Chapter V; VI and VII). The experiment reported in Chapter III was a factorial experiment which involved the 2 animal species and 2 levels of salt load being infused intraruminally in addition to no salt infusion. In Chapter V, a comparative study between sheep and goats involved 3 different roughage diets and saline drinking water. In Chapter VI, Australian feral goats were used to investigate the effect of drinking salt water in a hot environment (40°C). The experiment on sheep reported in Chapter VII, was a factorial experiment which involved 2 different temperature regimes (20 and 40°C) and 3 levels of salt load presented in the drinking water.

In addition, it was presumed that some of drinking water containing salt would bypass the reticulo-rumen. An experiment reported in Chapter IV was designed to investigate this possibility and to estimate the extent of the bypass.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The main aim of this review is to provide basic knowledge about the regulation of body fluids. It is a broad area, and a complete review of the voluminous literature is impossible within the space constraints of this thesis.

Concerning the experimental work of this dissertation, it is considered that the review should cover fluid dynamics between compartments in the animal body, between animals and their environments, and in the reticulorumen. The functional aspects of the kidney including regulation and tubular transports are reviewed, even though many of them are outside the scope of the experimental works. In addition, information about sheep and goats used in the experimentation is presented.

2.1. SHEEP AND GOATS : Taxonomy and their origins

The taxonomic position of sheep (*Ovis aries*) and goats (*Capra hircus*) is the subfamily *Caprini*, family *Bovidae*, suborder *Ruminantia* in the order *Artiodactyla*. The first domestication of sheep and goats occurred in about the same place, the dry mountainous regions of southwest and central Asia (Devendra and Coop, 1981), with goats being domesticated some time before sheep.

Most breeds of the present sheep including the Merino are mainly descended from *Ovis musimon* which exists in Europe and western Asia. According to Guthrie (1957), the Merino developed in Spain about 5-6 centuries ago, and the word "Merino" in Spanish means "thick curly hair".

It is generally agreed that most breeds of the present goats are mainly descended from *C. hircus aegagrus* (the bezoar) which spread from Greece to Pakistan (Mason, 1981). According to the horn characteristics, Evans (1985) proposed that the primary

ancestor of Angora goats is the bezoar, while feral "Cashmere" goats are from *C.h. falconery* (50%), *C.h. aegagrus* (40%) and *C.h. ibex* (10%).

Angora goats came originally from the steppe regions of Turkey which are cold in winter, with dry and high temperatures in summer (Mason, 1981; Tuncel and Okuyan, 1988). Compared with the other breeds of goat Angora are smaller, and they do not adapt well to mountainous regions (Tuncel and Okuyan, 1988). According to the importation records, the present Australian Angoras have a French "Cashmere-Angora", Cashmere and Turkish Angora (pre- and post grading up) background (Evans, 1985).

In addition to Angora and Cashmere goats as fibre breeds, there are four recognised breeds of dairy goat in Australia, which are Saanen, Togenburg, British Alpin and Anglo Nubian (Mitchell, 1984). Although no meat breed exists in Australia, the previous mentioned breeds by natural breeding appear to be the ancestors of the recent Australian Feral goats. They have no uniform characteristics. A research project conducted in Condobolin, NSW, has been studying the factors affecting the inheritance of meat characteristics of this feral goat (Mitchell, 1984).

Although there were no Australian indigenous sheep and goats before the advent of Europeans, these ruminants occupy arid and semi arid zones, and they thrive in the harsh environment (climatic, nutritionally and water availability) (Squires, 1981; Hume, 1987).

2.2. BODY FLUIDS AND THEIR DYNAMICS IN MAMMALS

Life seems to have begun in water. Over 70% of the earth's surface is covered by sea water and only approximately 1% by fresh water (Sale, 1985). There have been numerous migrations of animals from water to land in the course of evolution, but only a few animal species have been successful in maintaining themselves entirely free of consumed water. Insects, for instance, have made a successful migration from water,

although some of them still return to water for a part in their life cycle. Birds and mammals return to a watery medium for their embryonic life time. Although they have succeeded in colonising the dry land and the air, the living cells which make up their bodies still contain water as their main constituent, and still have to live surrounded by a watery medium.

In this regard, water is not a passive solvent in which inorganic salt, organic compounds and dissolved gases interact, but water also participates actively in forming the compounds of cells. As well as other components, water in the body is in dynamic condition. Water which makes up the greatest proportion of an animal's body can be distinguished in two major compartments: 1. intracellular water which is an essential part of the protoplasm in which the living processes take place; 2. extracellular water which is the 'milieu interieur' referred to by Claude Bernard. It has also been referred to as 'the sea within us', and the living cell in the body, as a tiny aquatic organism (Bricker, 1975).

Mammalian extracellular fluid provides the cells with a thermostatically controlled and chemically stable environment to protect the cells from dramatic alteration of the external environment, such as changes in climate and food and water supplies. The constancy in chemical and physical properties of extracellular fluid is an essential condition for living cells in which transformation of matter and energy take place.

The fluids that make-up intra- and extracellular compartments are as different chemically as they are different in anatomical location, although they are in dynamic equilibrium with one another.

In most mammalian cells, there is a considerably higher protein concentration compared to extracellular fluid. The main intracellular cation is potassium (100-150 mmol/l) with phosphate as the major anion. The extracellular fluid bathing the cells, is more like a diluted sea water with sodium as the main cation (140-150 mmol/l) and chloride as the main anion (100-110 mmol/l).

The great difference in cation concentration, sodium outside and potassium inside, is found not only in the mammalian body, but also in almost all living cells, plant as well as animals. Although the cell membrane is freely permeable to water, it is only selectively permeable to almost all of the cellular and extracellular constituents. The main cations and many other ions are transferred across the cell membrane by active processes.

2.2.1. Intravascular - interstitial fluid dynamics

Extracellular fluid (ECF) and its distribution into intravascular (≈ 25%) and interstitial (≈ 75%) compartments are normally maintained within a narrow range despite variations in the input of sodium and water. This constancy is a dynamic equilibrium between both compartments of ECF. In accordance with the basic function of the blood circulation as a flow of nutrient, dynamic processes take place mainly in the microcirculation at the capillary level (Schmid-Scoenbein and Ross, 1991; Guyton, 1991).

Ultrastructure of the capillary wall as found in most organs is composed of an extremely thin unicellular layer of endothelial cells, which is surrounded by a basement membrane on the outside. This basement membrane is an exchange barrier only in the sense that it restricts the movement of blood cells, platelets and the largest immune complexes (Zweifach, 1973).

The differences in hydrostatic pressure and colloid osmotic pressure (Starling forces), and concentration gradients of substances between intravascular and interstitial compartments are generally acknowledged as the important factors which determine fluid dynamics between intravascular and interstitial compartments by diffusion, filtration (bulk flow) and osmosis. Additionally, there are some special structures in the endothelial cells which facilitate the processes: 1. intercellular clefts or pores which lie between adjacent endothelial cells; 2. fenestrae or numerous small windows which penetrate directly through the middle of endothelial cells; 3. pinocytotic vesicles. These structures,

however, vary widely in the different organs or tissues either in their number or size (Karnousky, 1968).

There are three types of capillaries in accord with the completeness of the endothelial layer: 1. Capillaries with continuous endothelium and continuous basement membrane, such as in adipose tissue, smooth, skeletal and cardiac muscle, placenta, lung and central nervous system (CNS). In brain tissue, the pores are very tight (blood-brain barrier) and most of the cell cytoplasm is occupied by pinocytotic vesicles. In most tissues, water and small water-soluble substances, such as ions, glucose and amino acids, can readily pass through the pores, but large-water soluble, lipid-insoluble substances, such as protein, are excluded from passage. 2. Capillaries with fenestrated endothelium and with a continuous basement membrane, such as in glomeruli, endocrine glands, salivary glands, pancreas and gastrointestinal tract. The fenestrae are thought to facilitate the rapid diffusion of solutes and water. In glomerular capillaries, the fenestrae may, or may not be, closed by a thin diaphragm, while they are usually closed at other capillaries. 3. Capillaries with discontinuous endothelium (large gaps between adjacent cells) and discontinuous or absent basement membrane, such as the sinusoids of the liver, spleen and bone marrow. These capillaries are the most permeable compared to the other two types as they permit the passage of macromolecules, such as protein (Karnousky, 1968).

In general, lipid soluble substances or non-polar molecules, such as O_2, CO_2, fatty acids and steroid hormones, move freely across the lipid bilayer membranes of the endothelial cells (transmembranous transport). To some extent, water molecules can also diffuse directly across the membrane, but most water together with small water soluble substances such as electrolytes (sodium, potassium, calcium, chloride), glucose, and urea, are transferred through the pores by diffusion and filtration. The pinocytotic vesicles are particularly responsible for the movements of large water soluble molecules, such as protein. The vesicles are moved from the inner to the outer (tissue) side of the endothelial layer by thermal kinetic energy (Berne and Levy, 1993).

Diffusion down concentration gradients is the primary mechanism for exchange of individual solutes except protein. The process continues independently for each solute until there is no longer a concentration gradient between plasma and interstitial fluid. Filtration, on the other hand, is a bulk flow of various constituents moving together with water. This mechanism occurs because of the difference in the hydrostatic and colloid osmotic pressures between plasma and interstitial fluid.

It has been briefly reviewed that water molecules and small water soluble substances can freely exchange between both compartments of ECF either by diffusion or filtration, while proteins are mostly restrained in the intravascular compartment.

In fact, there is only a small amount of solute transferred by filtration, and compared to diffusion, it is considered unimportant in the exchange of nutrients and waste products in cells. However, filtration is extremely important in maintaining the dynamics and distribution of water between both compartments of ECF (Schmid-Schoenbein and Ross, 1991).

In accordance with Starling forces, there are two opposed pressures directing the flow of fluid out of or inward to the capillaries: hydrostatic pressure, and colloid osmotic pressures of the blood and interstitial fluid (Taylor, 1981). In classical theory, it is acknowledged that outward flow of fluid by filtration from capillaries occurs at the beginning to a half length of the capillary, because hydrostatic pressure of the blood, which is due to the pumping action of the heart, exceeds plasma colloid osmotic pressure, which is due to the presence of plasma protein. However in the distal half of the capillary, inward movement of fluid (reabsorption) by osmosis takes place, because hydrostatic pressure of the blood has fallen below the colloid osmotic pressure.

A recent theory, that has received considerable attention, is that filtration occurs throughout the length of all open capillaries, whereas reabsorption occurs throughout the length of all closed capillaries (Sherwood, 1993). According to this theory, when precapillary sphincters are relaxed the hydrostatic pressure of the blood exceeds the

plasma colloid osmotic pressure even at the venous end of the capillary, which promotes filtration throughout the length of the capillary. When the precapillary sphincter is closed, the reduction in blood flow through the capillary reduces the hydrostatic pressure below the plasma osmotic pressure even at the beginning of the capillary, so reabsorption of fluid takes place along the whole capillary.

Whichever mechanism is involved (classical or recent theory), however, the net effect is the same. Under normal circumstances, a protein-poor fluid exits the capillaries by filtration, and most of the filtrate (90%) is ultimately reabsorbed back into the capillary after flowing around the cells (Sherwood, 1993). The remaining 10% of the filtrate is indirectly returned to the blood circulation through the lymphatic system, particularly for the substances of high molecular weight, such as protein.

The fluid dynamics between the two ECF compartments occurs automatically and immediately whenever the balance of forces acting across the capillary wall is changed. Meanwhile reflex mechanisms acting on the heart and blood vessels are also involved. They provide a temporary mechanism to maintain the plasma volume fairly constant. In the process of restoring the plasma volume to an appropriate level, the interstitial fluid volume fluctuates, such as in dehydration or excessive input of fluid. It is much more important to maintain plasma volume constant to ensure that the circulatory system functions effectively. This movement of fluids occurs prior to the action of long-term mechanisms such as thirst and adjustment of urinary output involving neural and hormonal systems, which can compensate more completely for the alterations in whole body fluid.

2.2.2. Interstitial - intracellular fluid dynamics

It is generally acknowledged that the interstitial fluid (ISF) and intracellular fluid (ICF) are in osmotic equilibrium across the lipid bilayer of cell membranes. It means that if the osmolality of one compartment changes, water flows by osmosis across the cell

membrane until osmotic equilibrium is reestablished. For example, when the osmolality of the ISF increases, water flows by osmosis from ICF to ISF until osmotic equilibrium is restored. The changes in hydrostatic pressure in the ISF are also transmitted directly across the cell membranes to the ICF, so there can be no gradient.

Additionally, there is some evidence indicating that the regulation of water permeability in vasopressin-responsive epithelial cells of kidney collecting tubules is the result of facilitated water transporters that include the insertion (exocytosis) and retrieval (endocytosis) of water channels from cell apical plasma membranes (Verkman *et al.*, 1988; Verkman, 1989; Solomon, 1989; Lancer *et al.*, 1990; Kuwahara *et al.*, 1991).

Overall, water can move freely between all compartments of body fluid. Movements between intravascular and interstitial fluid is determined by the differences in hydrostatic pressure (generated by the pumping action of the heart) and colloid osmotic pressure between these compartments. The movements of water between interstitial and intracellular compartments across cell membranes is determined only by osmotic pressure differences in addition to facilitated transporters in specific organs. In contrast to the free movement of water, the movements of ions across the cell membranes are more variable and mostly depend on the presence of specific membrane transporters.

2.2.3. Ruminant - environment fluid dynamics

In accordance with homeostatic concepts, maintaining normal volume and osmolality of body fluid requires the equal input and output of solvent and solute into and from the body. In this regard, water is the solvent of body fluid, while many different solutes determine the osmolality of body fluid. However, since the major extracellular solute is sodium salt, the regulation of volume and osmolality of the ECF is dependent almost exclusively upon the regulation of the input and output balances of water and

sodium. Additionally, since sodium salts are excluded from the cells while water is freely permeable, the volume and osmolality of the ICF will also be influenced by balances of water and sodium in the ECF.

The possible inputs of water and sodium salt into the body compartments are primarily from drinking water and food absorbed from the gastrointestinal tract (GIT) and secondly from metabolic water. Under normal circumstances, these inputs will be balanced by the output through GIT, respiratory tract, body surface and kidney.

Ingested and metabolic water; Although it is mostly considered to be outside of the animal body, the GIT plays an important role in water and electrolyte metabolism. It is the avenue of ingested water and electrolytes absorbed into the blood and contributed to the body compartments. To some extent, it is also an excretory route. An additional water source is the result of cellular metabolism (metabolic water) of nutrients and some body tissues, especially following an extreme dehydration with water restriction (Yagil, Etzion and Ganani, 1979). According to standard values, one gram of protein, carbohydrate and fat produces 0.41, 0.56 and 1.07 ml of metabolic water respectively (Brody, 1945).

However, the relatively large production of metabolic water obtained from fat oxidation led to the erroneous assumption that the hump-fat of the camel was a source of water in times of water deprivation, but the hump remains unaffected during long periods without drinking water (Yagil, Sod-Moriah and Meyerstein, 1974). In addition, when comparing the water per calorie, in terms of oxidative energy, metabolism of lipids offers no advantage over metabolism of carbohydrates, since oxygen used for producing one gram of metabolic water from fat oxidation (1.9 l) is higher than from carbohydrate oxidation (1.5 l) (Schmidt-Nielsen, 1964). Therefore utilizing hump-fat would cost the animals more water lost through respiration to provide the additional oxygen requirements for metabolism, than would be gained from its metabolism .

Under different conditions of temperature (20 and 40° C) and water availability (*ad libitum* and restricted), the partition of water input sources varies between species of ruminants: thus 70-98% of water input is from ingested water (drinking water and food), and 2-30% from metabolic water (Wilson, 1989).

Losses from the body: Water is continually lost from the animals' body by evaporation from the skin and respiratory tract. Both water and salt are lost in urine and to some extent in the faeces and sweat.

The relative importance of evaporative water losses through the skin and respiratory tract varies among species of mammals, and many changes are responses to changes in temperature and in the state of water availability. In most mammals, evaporation is particularly important in thermoregulation, a function which may lead to a considerable loss of water without a proportional loss of sodium salt. Hence, the loss by evaporation may also influence the relative importance of the different mechanisms regulating the body fluids.

With normal hydration in ruminants, considerable amounts of water are excreted with the faeces, but this loss varies with the species, and in accord with the type of food and its digestibility. However, all ruminants are able to conserve considerable amounts of water particularly by absorption from the caecum and colon. This process is very much more efficient in arid-adapted animals (Maloiy, Taylor and Clements, 1978). It is accompanied by sodium absorption and a possible effect of antidiuretic hormone (Nicholson, 1981).

The ability of the kidney to adjust water and solute excretion makes this organ the most important of all the avenues of water and sodium losses. Unlike the gastrointestinal tract, body surface and respiratory tract, the primary function of the kidney is to conserve or to excrete water and electrolytes in accord with the composition and volume of ECF (plasma), in addition to excretion of nitrogenous wastes and foreign substances.

The regulation is accomplished through a complex mechanism of neural and hormonal feedback systems, which involve the kidney, adrenal gland, hypothalamus, posterior pituitary and cardiovascular system.

2.3. BODY FLUIDS IN THE RUMINANT

2.3.1. Whole body water and its metabolic relevance

In most mammals, about 95% of the weight of the early embryo is water, but this proportion decreases with age to about 75% in the neonate, while deposition of fat and calcium (for the skeleton) increase. A study on sheep, cattle and pigs reported by Kleiber (1975) indicated that during maturation and ageing there is a further decrease in the proportion of water accompanying increases in fat deposition, while the proportion of protein and ash change very little.

A series of dissection studies on indigenous Botswana sheep (112) and goats (145), Owen, Norman, Fisher and Frost (1977, 1978) reported that goats are generally leaner than sheep, and this is primarily associated with less subcutaneous and intramuscular fat, while visceral fat deposition is comparable. Subcutaneous fat in the sheep accumulates at the fastest rate compared with visceral and intramuscular fat (Faurie, Kirton and Jury, 1970).

Panaretto (1963) and Panaretto and Till (1963) used the tritiated water dilution technique and reported that the percentage of fat in a whole body was markedly higher in sheep than in goats, and there was a negative relationship between the percentages of water and fat in the body. Benyamin, Koenig and Becker (1993) used the same technique, and reported a similar conclusion.

A comparative study between tropical and temperate breeds of ruminant species (sheep and cattle) reported by Macfarlane, Howard and Good (1974) indicated that tropical animals have a higher proportion of water compared to the temperate animals, but

tropical animals have a lower water requirement under the same environmental conditions. Swamp buffalo, however, with a higher proportion of body water, consume more water and food compared to Shorthorn x Brahman cattle (Williams and Dudzinski, 1982).

The differences in water and fat content, or body composition as a whole, between sheep and goats may be the cause of the differences in the rates of various physiological processes. Metabolic rates, which relate to oxygen consumption and food intake, differ with species, breed, body weight, age, nutrition and environmental conditions. These differences result not only from alteration in their caloric balance, but in particular from different levels of energy and water turnover.

Most of the body functions can be attributed exponentially to $W^{0.75}$ rather than a direct relationship to W, where W is the live mass of the animals (Brody, 1945; Kleiber, 1975). However, concerning water function, Macfarlane and Howard (1970) suggested the higher exponential value, $W^{0.82}$, since water is involved not only in intermediary metabolism but also for evaporative cooling.

2.3.2. Compartments and measurement

According to Faichney and Boston (1985) body fluid in ruminants may be divided into four compartments: 1. intracellular fluid (ICF), 2. extracellular fluid (ECF), 3. reticulorumen fluid, and 4. post-reticulorumen fluid.

The estimation of total body water (TBW) in ruminants can be made directly or indirectly. The direct method is by slaugthering and drying the carcass in an oven or freeze-drying until no more weight is lost. This method is fundamental, and it is usually made to evaluate the results of indirect methods, although there are some unavoidable errors resulting from some volatile substances other than water (amines and fatty acids) being lost during dehydration. In particular the direct method can not be used to

investigate the dynamics and distribution of water in living animals as affected by altered conditions.

The dilution techniques using tritiated water (TOH) or deuterated water (D_2O) are the most useful indirect methods, since these water isotopes would be expected to distribute almost exactly as H_2O, and the results agree well with the direct method. Several other substances of non water tracers, such as antipyrine, urea, ethanol and N-acetyl-4 amino antipyrine, have also been used for determining TBW. However, they have several undesirable features primarily related to their chemical dissimilarities to water. These properties may cause them to fail to distribute uniformly in TBW or may cause rapid elimination from the TBW. Dilution of isotopic water, therefore, has become the choice for measuring TBW.

The estimation of TBW by the dilution technique using isotopic water normally includes water in the gastrointestinal tract (gut). By using TOH, the proportion of TBW in ruminants is 60-80% (Macfarlane, 1976).

The most important ECF compartments are plasma and interstitial fluid which includes fluid lying between cells and in the lymphatics. Also contained in the ECF compartment are fluid in bone, dense connective tissues (cartilage), intraocular, cerebrospinal and serosal fluid. The two main compartments of ECF have the same electrolyte composition, with Na^+ as the predominant cation and Cl^- and HCO_3^- as the predominant anions. However, the protein content is higher in plasma (6-8%) compared to interstitial fluid (1-3%), because of the low permeability of the capillary endothelium to protein (as well as to lipid). This endothelium is freely permeable to water and to small solutes, such as inorganic ions, glucose and urea.

Accordingly, the estimation of ECF volume by the dilution technique requires markers that can freely cross the capillary endothelium but are excluded from cells. The isotopes of sodium, chloride, bromide and sulphate have been used, but these tracers to some extent enter cells, and therefore tend to overestimate ECF. On the other hand, the

use of inulin, mannitol, raffinose, sucrose and thiocyanate tend to underestimate ECF, since these substances are not easily distributed throughout the entire ECF compartment, in particular penetration of bone and dense tissue fluid. As explained by Edelman and Leibman (1959), the dilution techniques, therefore, would give variations in estimates of ECF volume from approximately 27% (for markers such as inulin which distribute primarily into plasma and interstitial fluid) to as much as 45% of body fluid (not including gut water) for the markers such as sodium isotope. The best estimation is a combination of measurements using a number of markers with analysis of individual tissues to test the extent of their penetration. Macfarlane (1976) indicated that ECF volume is normally 18-22% of body weight, 180-220 ml/kg of body weight or about 300 ml per litre of body fluid.

Since plasma protein is almost exclusively distributed in the vascular compartment, the volume of blood plasma can be estimated using radioisotope (eg. ^{131}I-albumin) or Evans blue dye (T1824) which binds tightly to albumin. A small amount of albumin, in fact, leaks out of the capillaries to the interstitial compartment, but generally it can be ignored.

There are no subtances known to exclusively and uniformly concentrate in ICF without also being present in the ECF. Therefore, the volume of ICF can be estimated only by substraction of the volumes of ECF and gut from TBW.

The whole volume of gut fluid is not easy to measure *in vivo*, but the fluid volume and its flow rate from the reticulorumen can be estimated by dilution techniques. Several soluble markers have been used for this purpose, such as CrEDTA, lithium chloride, phenol red and polyethylene glycol (PEG). Normally, gut fluid volume in ruminants varies from 12 to 20% of body weight which is 2-3 times of that in non-ruminant animals (Macfarlane, 1976). About 65-75% of gut contents (fluid and solid) are in the retriculorumen (Phillis, 1976).

Binnerts, van't Klooster and Frens (1968) proposed non-radioactive CrEDTA as a favourable alternative soluble marker to $Cr^{51}EDTA$ or PEG in the study of rumen fluid dynamics. From a study in sheep, they indicated that 3-5% of the marker was excreted through urination. Godwin and Williams (1986) found 3.6%. The possibility exists that gut absorption of CrEDTA, takes place post-ruminally, since there were no differences in the parameters of rumen fluid dynamics when measured using PEG and CrEDTA (Binnerts *et al.*, 1968).

2.3.3. Fluid dynamics in the reticulorumen

Understanding fluid dynamics in the voluminous compartment of the fore-stomach is fundamental to understanding body fluid physiology in ruminant animals. There is no anatomical barrier between the rumen and reticulum and it is therefore most frequently considered as one compartment, the reticulorumen.

The content of the reticulorumen can be divided into a fluid and a solid phase. The fluid fraction contains water, soluble feed components and nutrient solubilized by degradative processes of the microrganisms. The solid fraction contains undegraded and indigestible materials. The fluid volume of the reticulorumen is determined by the entry of water from ingestion, salivation and a possible trans-epithelial diffusion across the rumen wall.

The transepithelial movement of water is apparently by simple diffusion which is determined by osmotic and hydrostatic pressure differences between these two fluid compartments (von Engelhardt, 1970; Warner and Stacy, 1977). However, available evidence, reported by von Engelhardt (1970), indicates no appreciable net flux of water before, during and after feeding in both sheep and goats.

The total volume of fluid entering the reticulorumen is generally greater than the input of solid matter. Therefore, the fluid containing soluble nutrient may leave the

reticulorumen at a faster rate than that of insoluble nutrients of the solid fraction. Large proportions of bacteria in the reticulorumen are actually associated with the fluid phase (Cheng, Akin and Costerson, 1977). In addition, the amount of microbial protein synthesised per unit of carbohydrate fermented by micro-organisms increases with the elevation of the fluid flow rate (Isaacson, Hinds, Bryant and Owens, 1975).

Dietary, animal and climatic factors may affect either the volume or flow rate or both. Dietary factors, such as level of intake (Eadie, Hyldgaard-Jensen, Mann, Reid and Whitelow, 1970; Grovum and Williams, 1977) and the physico-chemical composition of the diet (Hartnell and Satter, 1979; Hodgson, Thomas and Wilson, 1976; Van Soest, 1975; Faichney, 1983) have been reported to affect the flow rate of rumen fluid.

Flow rates of rumen fluid tend to be faster in young animals (Faichney, 1986), during gestation (Faichney and White, 1988a and b) and lactation (Weston, 1988).

Exposure to heat has been reported to decrease the flow rate of rumen fluid in cattle (Bhattacharya and Hussain, 1974; Warren, Martz, Asay, Hiloderbrand, Payne and Vogt, 1974; Miaron and Christopherson, 1992), while cold exposure of sheep has the opposite effect (Kennedy, Christopherson and Milligan, 1976; Kennedy and Milligan, 1978). These thermal effects are presumed to relate to the decrease (by heat) or the increase (by cold) of reticulorumen motility resulting from alteration in thyroid function (Miller, Swanson, Lyke, Moss and Byrne, 1974; Westra and Christopherson, 1976; Miaron and Christopherson, 1992).

Some evidence indicates that under normal circumstances saliva in ruminants is a major portion of fluid entering the reticulorumen which in turn would affect the outflow of fluid to the omasum. Daily saliva production varies from 6-16 l in sheep (Kay, 1960a), 14-18 l in goats (Kay, 1960b) and 98 to 190 l in cattle (Bailey, 1961). A recent study (Garza and Owens, 1989 cited by Carter and Grovum, 1990) estimated that 92-96% of ruminal fluid in heifers originated from saliva and water flux across the rumen wall, while ingested water constitutes only 5-7.5%.

Increasing plasma or rumen osmolality by drinking salt water or infusion of hypertonic solution into the rumen or blood stream has been found to decrease saliva production in sheep (Warner and Stacy, 1977; Tomas and Potter, 1975; Carr and Titchen, 1978).

A further aspect of fluid flow from the reticulorumen is indicated by the observations by Warner and Stacy (1968), Tomas and Potter (1975) and Mackintosh (1985) that a small proportion of ingested water or saliva may pass directly to the omasum through the closure of the oesophageal groove. Such passage of water could not be detected as outflow from the reticulorumen, omasum or abomasum, but it would affect the flow to the duodenum. Thus the difference between outflow of water from the reticulorumen and duodenal flow may be affected by water bypassing the reticulorumen. In addition omasal absorption and abomasal secretion may also vary.

2.3.4. Endogenous urea in ruminants

In mammals, it is generally known that almost all of the endogenous urea is produced in the liver by the metabolic pathway known as the urea cycle. The enzyme responsible for the final step in the formation of urea is arginase, which hydrolyzes arginine into urea and ornithine. Although arginase is present in many tissues besides the liver, including the kidney (Robinson, Schmidt-Nielsen, 1963; Emmanuel, 1980), these tissues produce only limited amounts of urea from circulating arginine.

The major factor that controls urea production in the liver is the supply of nitrogenous substrate, free ammonium and amino acids (Welser, 1983; Halperin, Chen, Cheema-Dhadli, West and Jungas, 1986). As demonstrated previously by Schimke (1963), the activities of several enzymes of the urea cycle in the liver vary directly with the protein or nitrogen content in the diet.

An acute increase in circulating amino acid concentration can accelerate urea production (Stewart and Walser, 1980).

Data from infusion studies in adult dairy cows indicated that the maximum capacity of the liver to extract NH_3 is approximately 1.80 mmol/min per kg liver weight, and severe signs of intoxication occur when the arterial concentration exceeds 0.8 mmol NH_3/l (Symonds, Mather and Collis, 1981).

Utilization of amino acids for energy metabolism (gluconeogenesis) enhances urea production in which glucagon (Triebwasser and Freedland, 1977; Brebnor, Phillips and Balinsky, 1981), glucocorticoids (Christowitz, Mattheyse and Balinsky, 1981) and catecholamines (Titheradge and Haynes, 1980) may be involved.

In this regard, urea is the waste product that has to be excreted. Contrasting to this necessity is the fact that urea is an important determinant in the urinary concentrating mechanism (see 2.5.5 and 2.5.6). In addition, it is now well established that ruminants can utilise endogenous urea for protein resynthesis, and the process has also been indicated in non-ruminant herbivores such as the horse (Prior, Hinz, Lowe and Visek, 1974), rabbit (Regoeczi, Irons, Koj and McFarlane, 1965; Forsythe and Parker, 1985), rock hyrax (Hume, Rubsamen and von Engelhardt, 1980), ringtail possum (Chilcott and Hume, 1984) and tammar wallaby (Mason, 1984).

In ruminants, this endogenous urea conservation cycle involves: 1. transfer of endogenous urea from blood into the digestive tract directly across the digestive wall and through gland secretions; 2. hydrolysis of urea to ammonia and CO_2 by bacterial urease; 3. reentry of ammonia to the portal system, or the reutilisation of ammonia nitrogen for microbial protein synthesis; 4. digestion and reabsorption of the microbial nitrogen.

Urea is distributed through all body fluids. It enters whenever body fluids are discharged into the gut or between compartments (Egan, Boda and Varady, 1986). The movement of urea across the digestive wall is by diffusion (Houpt and Houpt, 1969), and osmotic and hydrostatic gradients are the determinant factors. In addition, it is also

considered that bacterial urease activity in the rumen epithelium could reduce the diffusion resistance for urea manyfold (Houpt and Houpt, 1969). Ureolytic activity is also found in the caecum and colon of ruminants (Hecker, 1971), and predominantly in the colon for non-ruminants (Jones, Smallwood, Craigie and Rosenoer, 1969). The synthesis and activity of these bacterial ureases can be almost completely abolished by antibiotic (Wilson, Ing, Metcalfe-Gibson and Wrong, 1968; Jones *et al.*, 1969).

Numerous studies on the dynamic aspects of endogenous urea movements in ruminants have been reported (Nolan, 1993; van der Walt, 1993).

Available evidence indicates that the proportion of urea production degraded and reutilised in the digestive tract is determined by various factors, such as diet (nitrogen fermentable carbohydrate and electrolytes) and animal conditions.

A higher recycled proportion of endogenous urea is usually associated with lower N intake, urea production rate and urinary urea-N excretion (Cocimano and Leng, 1967; Ford and Milligan, 1970; Nolan and Stachiw, 1979; Huntington, 1989; Brun-Bellut, Kelly, Mathison and Christopherson, 1991).

Infusion studies in sheep fed a poor-N roughage diet (Godwin and Williams, 1984) indicated that high nitrogen intake (in the form of urea) accompanied with a high intake of sodium or potassium chloride increased urinary urea-N excretion. In their study, plasma urea-N concentration was controlled within the range of 30-40 mg N/100ml when salt loading was introduced compared to a plasma urea level of 68 mg N/100 ml without salt load. Accordingly, these authors suggested that the increased electrolyte content of higher protein roughage diets (such as leguminous hays) aids the increased urinary urea-N excretion by increasing glomerular filtration rate and urine flow rate rather than the diuretic effect of urea alone.

Benlamlih and Pomyers (1989) demonstrated that the recycled proportion of endogenous urea was greater during pregnancy and lactation compared to that non-pregnant-non-lactating sheep. The recycled proportion of urea in Angora goats (fibre

breed), 62%, is higher compared to that in the Boer goat (meat breed), 48% (Cronje, 1992).

Vercoe (1967, 1971) found that water intake was negatively correlated with plasma urea concentration and N balance, and positively correlated with the total urinary N excretion. Restriction or deprivation of water increased N balance in cattle given poor quality roughage (Ikhatura, Ehoche and Umoh, 1985). Urinary N excretion and protein catabolism are also increased with increased rectal temperature in cattle (Vercoe and Frisch, 1970; Vercoe, Frish and Moran, 1972).

2.4. REGULATION OF OSMOLALITY AND VOLUME OF BODY FLUIDS

Regarding the relationship between fluid compartments in the body and the external environment, the control of composition and volume of plasma is central to body fluid regulation.

As sodium salts constitute more than 90% of the osmolality of extracellular fluid (ECF), the osmolality of the plasma is almost exclusively determined by the concentration of sodium which is normally maintained within narrow limits. However, responses to alterations in osmolality or sodium concentration are more attributable to the thirst sensation and antidiuretic hormone (ADH) secretion rather than to the dynamic balance of sodium. Hence, regulation of plasma osmolality or sodium concentration depends on input and output of water into and out of the body.

Under balanced conditions of sodium and water, the osmolality of ECF averages almost 300 mOsm/l with 142 mmol Na^+/l. These values rarely rise or fall more than 1% (Guyton, 1991).

About 45% of body sodium is found in ECF, 45% in bone and the remainder in intracellular fluid (ICF). Most of the bone sodium, which resides in the mineral phase of the bone, is non-exchangeable, and is not osmotically active. The exchangeable fraction of bone sodium is mostly present in the fluid phase, but to some extent it may also be available from the mineral phase to ameliorate the osmotic effects of ECF dilution (Edelman and Leibman, 1959; Bland, 1963; Kunin, 1963).

Since the exchangeable sodium is primarily confined in the ECF compartment, its regulation will ultimately determine the volume of the ECF compartment. The fundamental idea is that osmotic activity of total sodium in the ECF can be equated with "water holding capacity", which simply means "more sodium-more water" and "less sodium-less water". Hence, the regulation of the volume depends primarily on the adjustment of the dynamic balance between total input and output of sodium into and out of the body. Internal adjustment for sodium excretion by the kidneys is particularly controlled by the renin-angiotensin-aldosterone mechanism, whereas input of sodium is by salt appetite.

In addition to ADH and aldosterone, available evidence indicates that some other hormones also affect the regulation of sodium excretion through the kidney, including atrial natriuretic peptide (ANP) and prostaglandins.

2.4.1. Thirst sensation and salt appetite

Convincing evidence from studies in rats (Stricker, 1969) and goats (Andersson, 1971) indicated that the hypothalamus plays an important role in the thirst mechanism. The thirst sensitive centre is believed to be located in the anterolateral region of the hypothalamus. Electrical stimulation and injection of a minute amount of hypertonic saline solution into this region in rats and goats elicit drinking behaviour, while its lesion abolishes the normal drinking response to water deprivation or administration of hypertonic saline.

Although there is a possible direct relationship between plasma osmolality and the thirst sensation (and hence water intake), evidence indicates that thirst is regulated by a separate group of osmoreceptors that overlaps with those controlling ADH release. This is indicated by the osmotic threshold of the receptors for stimulating thirst sensation being 2-5 mOsm/l higher than that for ADH release (Robertson, 1977; Robertson, 1984; Phillips, Rolls, Ledingham, Morton and Forsling, 1985). This implies that the sensation of thirst is not apparent until plasma osmolality increases to a level which is sufficient to elevate plasma ADH concentration to 5-6 pg/ml, a concentration resulting in maximal anti-diuresis (Robertson, 1984). From most studies in mammals involving rats, dogs, sheep and primates, the mean concentrations of plasma ADH under normal circumstances are within the range of 1.5-6.0 pg/ml (Bie, 1980).

The importance of the thirst mechanism in water balance is equal to the effects of ADH on increasing conservation of water by the kidney, which then will return plasma osmolality to the normal level (Baylis, 1987). Both mechanisms appear to be initiated by the same afferent stimuli, namely increasing plasma osmolality especially by sodium concentration, and decreasing in circulating volume without appreciable changes in osmolality (such as in hemorrhage). However, the thirst centre responds by controlling the oral intake of water, whereas the ADH releasing center responds by regulating the kidney function for water conservation or excretion. In this manner, through dynamic, coordinated and integrated dual actions of these centres, there is a continuous adjustment of total body water and hence the osmolality of the ECF in the face of various disturbing stimuli.

Concerning a decreased circulating volume, maximal intake of water is preceded by an elevation of plasma angiotensin II as the crucial thirst-eliciting factor rather than diminished reflex stimulation of cardiovascular distension receptors (Andersson, 1978). However, this reflex may affect water intake by changing the osmotic threshold for thirst sensation (Robertson, 1984; Phillips *et al.*, 1985; Mann, Johnson, Ritz and Ganten, 1987).

The mechanism of salt appetite is not well understood. Omnivores, carnivores as well as primates usually obtain an adequate amount of sodium salt from their food and drinking water (Sherwood, 1993). However, sodium-deficient animals, particularly herbivores in mountain areas, have a strong behavioural drive to ingest salt.

It is proposed that the salt appetite mechanism is stimulated by a decreased plasma osmolality or plasma sodium concentration and sodium depletion, and its neural mechanism is analogous to the thirst mechanism (Stricker, 1981, Guyton, 1991).

2.4.2. Antidiuretic Hormone

Antidiuretic hormone (ADH) is a polypeptide synthesised in the supraoptic and paraventricular nuclei in the hypothalamus (Zimmerman and Robinson, 1976; Zimmerman, Nilaver, Hou-You and Sivernan, 1984).

The major stimuli for ADH secretion are hyperosmolality (osmotic regulation) and depletion in systemic circulatory volume (non-osmotic regulation) (Baylis, 1987; Robertson, 1987; Zimmerman, Ma and Nilaver, 1987). In addition to the extreme sensitivity of osmoreceptors in the hypothalamus (Schrier, Berl and Anderson, 1979), the existence of osmoreceptors in the portal vein and liver have also been identified (Schrier *et al.*, 1979; Lautt, 1980; Sawchencko and Friedmen, 1979; Bie, 1980). An inhibiting effect on ADH secretion is induced by stimulation of volume receptors in the left atrium, carotid sinus and aortic arch of the heart (Schrier *et al.*, 1979; Thames and Schmidt, 1979; Sawchenko and Swanson, 1981; Di Bona, 1986)

The interaction between the osmotic and non-osmotic regulation of ADH secretions has been examined using a mathematical model based on a canine subject (Kaushanpous and Stipp, 1982). The result indicated that within normal physiological limits of alterations in blood volume and osmolality, the importance of osmotic and non-osmotic regulation on ADH secretion is additive, but the osmotic regulation is the major determinant for ADH secretion.

Angiotensin II has also been proposed as a mediator in the regulation of ADH secretion (Phillips *et al.*, 1985; Henrich, Walker and Handelman, 1986). Simultaneous intravenous infusion of angiotensin II and hypertonic sodium salt solution caused a more significant increase in ADH secretion than infusion of the hypertonic saline alone. However, Angiotensin II did not affect the final common pathway for ADH secretion, it may only potentiate osmotic regulation of ADH secretion, but have little or no effect on the non-osmotic regulation.

ADH is the primary physiologic determinant of water conservation-excretion through activation of adenylate cyclase (Gapstur, Homma and Dousa, 1988; Abramow, Beauwens and Cogan, 1987).

ADH augments the water permeability of the apical membranes of the cortical and medullary collecting ducts, allowing osmotic equilibration with the interstitium (Kokko, 1987; Sands, Nonoguchi and Knepper, 1987).

ADH also plays an important role in the generation of medullary hypertonicity by increasing the permeability of the inner medullary collecting duct to urea, and at least in some species, by directly promoting NaCl reabsorption in the thick ascending limb, thereby enhancing both urea and NaCl accumulation in the interstitium (Sands *et al.*, 1987; Molony, Reeves, Hebert and Andreoli, 1987). The net effect of these ADH actions is increased renal water reabsorption and an elevation in urine osmolality.

In addition, ADH appears to affect some other processes in the cortical collecting duct, increasing Na reabsorption and secretion of K^+ and H^+, presumably by increasing Na^+ and K^+ specific channels (Schlatter and Schafter, 1987; Field, Stanton and Giebisch, 1984; Bichara, Mercier and Houillier, 1987).

ADH stimulates the production of prostaglandins in a variety of cells within the kidney. The prostaglandins then impair both antidiuretic and vascular actions of ADH (Stokes, 1981; Scharschmidt and Dunn, 1983; Yared, Kon and Ichikawa, 1985). These

findings suggest that prostaglandin production prevents an excessive antidiuretic response.

2.4.3. Aldosterone

The major adrenal hormones are synthesised in different areas of the adrenal cortex: aldosterone in the zona glomerulosa, and glucocorticoid (particularly cortisol), androgen and oestrogen in the zona fasciculata and reticularis.

Aldosterone plays an important role in maintaining volume of body fluid and K^+ balance through its effects on NaCl and K^+ absorption/excretion (Young, McCaa, Pan and Guyton, 1976; Young, 1988).

It is appropriate that angiotensin II (produced inversely with volume, mediated by the renin-angiotensin system) and an elevation in plasma K^+ concentration are the major stimuli for aldosterone secretion. Both stimuli act directly on the zona glomerulosa to promote the conversion of cholesterol to pregnenolone and more importantly, the conversion of corticosterone to aldosterone (Williams and Braley, 1977; Anquilera and Catt, 1978a and b).

The effect of angiotensin II on aldosterone production and secretion appears to be mediated by enhanced phosphatidylinositol turn over (calcium messenger system) (Rasmussen, 1986). An increase in plasma K^+ concentration of as little as 0.1 to 0.2 mmol/l can induce a significant elevation in aldosterone release (Himathongham, Dluhy and Williams, 1975; Young, 1988). The effect is mediated in part by an elevation in

intracellular Ca^{2+} concentration, although this response does not appear to involve alteration in phosphatidylinositol turnover (Rasmussen, 1986).

In addition, aldosterone release can also be stimulated by adrenocorticotropic hormone (ACTH) and hyponatremia (see review by Laragh and Sealey, 1992).

The activity of renin-angiotensin system is inhibited by the presence of atrial natriuretic peptide (ANP) (see review by de Zeeuw, Janseen and De Yong, 1992; Ballermann and Brenner, 1986) and hence angiotensin induced aldosterone secretion.

In the kidney, aldosterone is an important regulator of Na, K and acid base balance. In addition to the kidney, its target tissues include sweat gland, salivary glands, and the colon. In general, aldosterone acts to increase sodium reabsorption and potassium excretion, whereas regulation of hydrogen ion transport is only in the kidney.

The primary sites (target cells) of aldosterone are in the connecting tubule and in the collecting duct (Garg, Kneeper and Burg, 1981; Stanton, 1987), which is mediated by inducing mRNA and rRNA transcription (Funder, Pearce, Smith and Smith, 1988).

Aldosterone promotes the reabsorption of NaCl and the secretion of K^+ in the connecting tubule, in the principal cells of cortical and inner medullary collecting ducts. This occurs by methylation of channel proteins to increase the number of open Na^+ and K^+ specific channels in the apical membrane (Sansom and O'Neil, 1985; Hayhurst and O'Neil, 1988).

In the intercalated cells, aldosterone stimulates the H^+-ATPase pump in the apical membrane, which then increases H^+ secretion (Steinmetz, 1986). This pump activity is also stimulated by acidemia (Stone, Seldin, Kokko and Jacobson, 1983; Batlle, 1986).

2.4.4. Atrial natriuretic peptide

Atrial natriuretic peptide (ANP) is primarily released from atria in response to volume expansion, which is sensed as an increase in atrial stretch. Although both atria appear to contribute, there is suggestive evidence that the right atrium may be quantitatively more important (Edward, Zimmerman and Schwab, 1988; Garcia, Cantin and Thiboult, 1987).

The expansion of extracellular fluid volume induced by sodium chloride loading in dogs results in an appropriate increase in sodium excretion. This natriuretic response cannot be prevented by either high dose administration of aldosterone or experimental reduction in GFR (de Wardener, Mills, Clapham and Hayter, 1961).

A number of studies, reviewed by Olsson, Dahlborn, Karlberg and Eriksson (1991), reveals that ANP infusion into the blood stream evokes natriuresis in all mammals tested, including sheep and goats.

ANP has two major actions. Firstly as a vasodilator, it decreases the systemic blood pressure, and secondly it increases urinary sodium and water excretion (Knepper, Lankford and Terada, 1991; de Zeeuw *et al.*, 1992). The natriuretic and diuretic effect of this peptide may be mediated by alteration in renal and extra renal functions, particularly those known to be important in the homeostasis of volume and blood pressure.

A number of autoradiographic and immunohistochemical studies, reviewed by de Zeeuw *et al.* (1992), reveal that the kidney vascular ANP receptor binding sites are in the renal artery wall, afferent and efferent arterioles of the glomeruli and descending vasa recta. Tubular ANP receptors are in the cortical and inner medullary collecting ducts and distal convoluted tubules.

ANP directly increases the GFR and reduces Na^+ reabsorption in the cortical and inner medullary collecting ducts (Zeidel, Silva, Brenner and Seifter, 1987; van de Stolpe and Jamison, 1988; Nonoguchi, Sands and Knepper, 1989).

The increase in GFR is associated with afferent arteriolar dilatation and efferent arteriolar constriction without an appropriate change in renal blood flow (de Zeeuw *et al.*, 1992). The tubular responses appear to be mediated by cyclic GMP induced closure of the Na^+ specific channel.

Patch-clamp studies conducted on rat inner medullary collecting ducts (Stanton, 1991) revealed that ANP, through its second messenger cGMP, inhibits electrogenic sodium reabsorption by reducing the open channels in the apical membrane.

ANP inhibits aldosterone production *in vitro* and *in vivo* (Chartier, Schiffren, Thilboult and Garcia, 1984; Chartier and Schiffren, 1987; Barrett, Isales, Bollag and McCarthy, 1991). ANP is also known to be a strong inhibitor of renin secretion (Burnett, Granger and Openorth, 1984), and therefore of angiotensin II as well (Harris, Thomas and Morgan, 1987). In addition, ANP inhibits ADH-stimulated water permeability in inner medullary collecting ducts (Nonoguchi, Sands and Knepper, 1988).

2.4.5. Prostaglandins

Renal prostaglandins are produced at a variety of sites including glomerular and vascular endothelium, cortical and medullary collecting ducts and medullary interstitial cells. The tubules primarily produce PGE2, while the glomeruli produce PGE2 and prostacylin (Bonvalet, Pradelles and Farman, 1987; Farman, Fradelles and Bonvalet, 1987).

The production of renal prostaglandins is increased by ADH stimulation, which then inhibits the effect of ADH (Stokes, 1981; Yared, Kon and Ichikawa, 1985). Renal

prostaglandins may also have a natriuretic effect, possibly decreasing Na^+ reabsorption in the thick ascending limb and collecting duct (Kokko, 1981; Patrono and Dunn, 1987).

2.4.6. Pressure natriuresis

It is generally known that expansion in extracellular fluid (ECF) volume resulting from salt loading will directly result in increases in cardiac output, systemic blood pressure and hence renal perfusion pressure. Although within the normal physiological range renal blood flow and glomerular filtration rate are well autoregulated, alteration in renal perfusion pressure results in parallel increases in sodium excretion rate (McDonald and de Wardener, 1965; Selkurt, Womack and Dailey, 1965; Navar, Bell and Burke, 1977).

One possible mechanism of renal perfusion pressure natriuresis is the transmission of the elevated pressure to the post-glomerular vasculature, which in turn achieves a new balance of Starling forces (Earley and Schrier, 1973).

The increase in hydrostatic pressure over the colloid osmotic pressure in the peritubular capillary will reduce the capillary uptake rate of the proximal tubule reabsorbate (Selkurt *et al.*, 1965; Koch, Avnedjian and Bank, 1968), thereby increasing renal interstitial volume and hydrostatic pressure, and reducing its colloid osmotic pressure (Rumrich and Ullrich, 1968). Supporting these findings, there is a significant relationship between renal perfusion pressure and renal interstitial hydrostatic pressure (Ott, Navar and Guyton, 1971).

Under such circumstances, the increase in the hydrostatic pressure of the interstitial fluid leads to a greater back diffusion of sodium from the interstitium to the tubule lumen through the paracellular route (Wilcox, Stersel, Dunckel, Mohrmann and Perfetto, 1984; Granger, Hass and Knox, 1988; Garcia-Estan and Roman, 1989).

In addition to the alterations in peritubular Starling force balance, Early and Friedley (1966) suggested that alteration in medullary hemodynamics may affect sodium transport in the loop of Henle. They proposed that increased medullary blood flow leads to a wash out of medullary interstitial hypertonicity. Studies in rats, reported by Cohen, Marsh and Kayser (1983) and Roman and Smits (1986), elucidated that renal papillary blood flow, measured with a laser Doppler flowmeter, is poorly autoregulated at renal arterial pressures higher than 120 mmHg. Therefore, it is possible that at renal perfusion pressures, which do not exhibit autoregulation of medullary-papillary blood flow, reduction in medullary interstitial solute concentration may contribute to the pressure-natriuresis mechanism.

In addition to hemodynamic mechanisms, pressure natriuresis resulting from an increase in renal perfusion pressure may also involve intrarenal hormones such as the renin-angiotensin system, prostaglandins and kallikrein-kinins.

Since increases in renal perfusion pressure are known to suppress renin secretion, it is possible that part of the increase in the fractional excretion of sodium and water is a response to a decrease in intrarenal formation of angiotensin II (Hall, Guyton, Trippodo, Lohmeier, McCaa and Cowley, 1977; Hall, Guyton, Smith and Coleman, 1980).

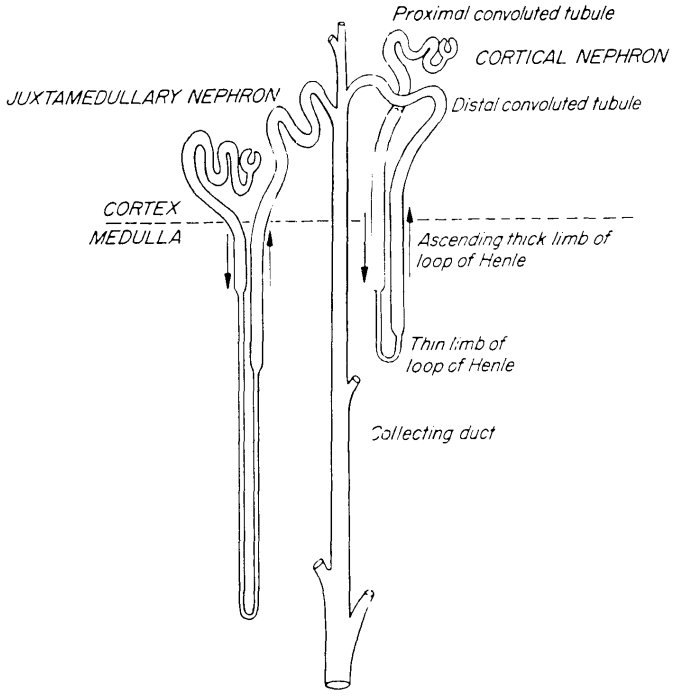
An experiment with dogs (Carmines, Bell, Roman, Work and Navar, 1985) suggested a possibility that intrarenal prostaglandins play a role in the mechanism of pressure natriuresis.

Intrarenal kinins are known to be natriuretic, and its production is stimulated by kallikrein. Several studies have shown that increases in renal perfusion pressure increase kallikrein excretion (Mill and Ward, 1975; Mills, Macfarlane, Ward and Obika, 1976).

2.5. KIDNEY FUNCTION

2.5.1. Structural-functional unit and renal circulation

In general, it is recognized that the primary function of the kidney is the formation of urine to maintain balance condition of body fluid volume and composition. The processes occur in the nephrons as functional units. Each nephron consists of a glomerulus, which is a tuft of capillaries interposed between two arterioles (afferent and efferent arterioles), and a series of tubules lined by a continuous layer of endothelial cells.



Schematic diagram showing the two general types of renal tubules with their characteristic subdivisions.

The glomerular tuft is composed of: 1. masengium consisting of the mesangial cells which are embedded into the mesangial matrix; 2. the endothelium lining the lumen of the capillaries; 3. the epithelial layer (Bowman's visceral layer or podocytes); 4 glomerular basement membrane (GBM) which separates the endothelial layer from the epithelial layer (Abrahamson, 1987). Accordingly, there are three layers through which

ultrafiltration occurs: the endothelial layer, GBM and the epithelial layer. Further, these three layers are called a glomerular ultrafilter.

The glomeruli, in which ultra-filtration processes occur, are present in the cortex, whereas the tubules in which reabsorption of the filtrate and secretion occur, are in both the cortex and the medulla of the kidney.

In accord with the processes occurring in the tubules, it is therefore considered that the thickness of the medulla relative to the size of the kidney is a structural index of urine concentrating ability in mammals (Browfield and Wunder, 1976). This index, however, accounted for only 59% of the variability among species in their urine concentrating ability (Beuchat, 1990).

The filtered fluid from glomerular tuft enters the Bowman's space, and then into the proximal tubule. The proximal tubule is composed anatomically of an initial convoluted segment (pars convoluta), and it is continued by a straight segment (pars recta) which enters the medulla. The loop of Henle generally includes a thin descending limb, and thin and thick ascending limbs. The hairpin configuration of the loops of Henle plays an important role in the formation of hypertonic urine.

The lengths of the loops of Henle are not uniform (Jacobson, 1981). About 40% of the nephrons in mammals have short loops, which penetrate only the outer medulla or may even turn around in the cortex; these short loops lack a thin ascending limb. The remaining 60% have long loops, which penetrate to the medulla and may extend down to the papilla.

The thick ascending limb also has a cortical segment which returns to the region of the glomerulus in the cortex. The tubule with specialized cells of the macula densa approaches the juxtaglomerular cells of the afferent arteriole from which renin is secreted. After the macula densa, there are three cortical segments: the distal convoluted tubule, the connecting tubule (previously considered as part of the late distal tubule), and the cortical collecting tubule. The connecting tubules of many nephrons form into a single collecting

tubule. Fluid leaving the cortical collecting tubule flows into the medullary collecting tubule and then drains sequentially into the calyces, the renal pelvis, the ureters and the bladder.

The segmental subdivision of the nephron is based upon different permeability and transport characteristics (Jacobson, 1981), and they are named in accord with the standard nomenclature of the kidney (The Renal Commission of IUPS, 1988).

Blood enters the kidney through the renal artery and passes through serial branches (interlobar, arcuate and intralobar) before entering the glomeruli through afferent arterioles. The portion of plasma not filtered leaves the glomeruli through the efferent arterioles, and then enters the post-glomerular capillaries in the cortex (peritubular capillaries) around the convoluted tubules and in the medulla to form the vasa recta capillaries. Through these post-glomerular capillaries the reabsorbed fluid from the tubules is received and returned into the systemic circulation through veins similar to the arteries in name and location. The medullary microcirculation is specialized for its role in facilitating the formation of concentrated or diluted urine (Chow, Porush and Foubert, 1990).

Accordingly, the possible effects of the renal circulation on the urine formation are due to: 1. the rate of glomerular filtration (GFR); and 2. the fluid dynamics between the post-glomerular capillaries and the tubules in the cortex and medulla.

2.5.2. GFR and its regulation

In normal mammals, as much as 20-30% of the plasma that flows through the glomerular capillaries traverses the glomerular ultrafilter and enters the Bowman's space. The filtrate is essentially protein-free fluid containing all crystalloids in the same osmolar concentration as that in the plasma. As in other capillary beds (see 2.4.1), the mechanism of the glomerular ultrafiltration is governed by **the Starling forces,** the permeability of

the membrane (ultrafilter) and to the balance between hydrostatic pressure and colloid osmotic pressure gradients. According to the Starling equation, GFR in a single nephron (SNGFR) is

$$SNGFR = k_f S (P_g - P_i) - (_g - _i)$$

$$= k_f S (P -)$$

where k_f is the hydrostatic permeability of the glomerular ultrafilter, S is the surface area available for filtration, k_f S is the ultrafiltration coefficient (porosity), P and are respectively hydrostatic and colloid osmotic pressure differences. However since the colloid osmotic pressure of protein in the Bowman's space fluid is extremely low, i is negligible.

As blood traverses the glomerular capillary from the afferent to the efferent end, virtually protein-free fluid enters the Bowman's space, causing the protein concentration and, hence colloid osmotic pressure in the glomerular capillary to rise to a maximum value at the efferent end. At this point, exactly offsets the value of P which remains relatively constant along the glomerular capillary. This equality of P and ceases the ultrafiltration process, and is known as "filtration pressure equilibrium" (i.e. P - = 0). In primates, this equilibrium occurs after the ultrafiltration of about 20% of the renal blood flow (RBF) (Maddox, Deen and Brenner, 1974)

In addition, the presence of this equilibrium means that the RBF becomes an important determinant of GFR (Brenner and Humes, 1977). The alteration in RBF (increase or decrease) will be proportionately followed by alteration in GFR.

The ultrafiltration coefficient is also under dynamic control. A variety of vasoactive hormones, such as angiotensin II and noradrenalin, are capable of causing reductions in the coefficient by inducing contraction of glomerular mesangial cells. This results in a decrease in the area available for filtration (Schnermann, Haberle, Davis and Thurau, 1992).

2.5.2.1. Autoregulation

Autoregulation may be defined as the intrinsic property of an organ, such as the kidney, to maintain its blood flow nearly constant despite changes in arterial perfusion pressure (Navar, Marsh, Blantz, Hall, Ploth and Nasjletti, 1981). This implies that regulation of the RBF can occur independently of external influence, such as neural activity and hormones. In addition to match the metabolic requirements of the renal parenchyma as in the other tissues, autoregulation of RBF has an additional responsibility to maintain a constant GFR.

There are two mechanisms which may explain the renal autoregulation: myogenic mechanism and tubuloglomerular mechanism.

Myogenic mechanism:

Since GFR and RBF are maintained in parallel, autoregulation may be mediated by the changes in afferent arteriole resistance (Navar, 1978). The concept of a myogenic mechanism is supported by convincing evidence indicating that changes in the diameter of afferent arterioles inversely result from changes in hydrostatic arterial blood pressure (Gilmore, Cornish, Rogers and Joyner, 1980). This response occurs in the few seconds before any detectable change in the thick ascending limb to stimulate the tubuloglomerular feed back mechanism can occur (Young and Marsh, 1981).

The cellular mechanism by which myogenic auoturegulation occurs is incompletely understood. However, it is generally known that smooth muscle contraction results from an increase in free cytoplasmic calcium and, since sarcoplasmic reticulum in smooth muscle is sparse, transport of calcium from the extracellular compartment may play an important role in the regulation of smooth muscle contraction.

With regard to the myogenic mechanism, a simple hypothesis is that there are stretch receptors in the wall of the afferent arterioles. Lush and Fray (1984) suggested that an increase in renal perfusion pressure will increase the stretch, which in turn results in an

increase in permeability of granular cell membranes to calcium (specific Ca channel), thereby depolarizing the granular cells at the terminal portion of the afferent arterioles (modified smooth muscle cells), and it will inhibit the release of renin. Conversely, a decrease in renal perfusion pressure will produce the opposite effects: it reduces stretch, decreases permeability to calcium, thereby hyperpolarizing the granular cells, and stimulating the release of renin. In this manner, the resistance in the afferent arterioles is controlled by an inverse relationship between renin release and renal perfusion pressure.

In summary, although the precise mechanism of the myogenic mechanism is still uncertain, this mechanism may apparently serve to rapidly buffer the effect of a sudden change in the systemic arterial blood pressure on the GFR. Hence, tubular flow rate remains relatively constant providing a stable delivery of sodium chloride to the macula densa cells. In contrast, the tubuloglomerular mechanism (see below) is apparently to protect against changes in tubular reabsorption, which is a slower process.

Tubuloglomerular feedback mechanism:

Convincing evidence has indicated the existence of the tubuloglomerular feedback (TGF) mechanism for maintaining the constancy of GFR. The mechanism involves a specialized structure within the nephron, which is called the juxtaglomerular apparatus (JGA). This structure is composed of: 1. the macula densa of the thick ascending limb of the Henle's loop; 2. the glomerular mesangial cells which fill the angle between the afferent and efferent arterioles; 3. the granular cells of the afferent arteriole in which renin is produced, stored and released (Barajas, 1970; Blantz and Pelayo, 1984; Schnermann and Briggs, 1986).

Since the anatomical relationship between the macula densa and glomerulus is similar for superficial and deep nephrons, it is presumed that the TGF mechanism exists for all nephrons throughout the kidney. However, available evidence indicates that the sensitivity of the deep nephron is much greater than that of the superficial nephron (Sjoquist, Goransson, Kallskog and Ulfendahl, 1984).

The mechanism seems to be initiated by the macula densa which senses changes in ionic composition of the tubular fluid, particularly Na^+ and/or Cl^- concentration, and this information is transmitted to the vascular structure of the nephron to alter afferent arteriole tone and hence GFR (Briggs and Schnermann, 1987). An increase in renal perfusion pressure leads to a rise in GFR; the increase in fluid flow rate at the point of macula densa and ion delivery will apparently initiate the feedback mechanism responses, which return GFR and flow rate of tubular fluid to normal levels. This response may be mediated by constriction of the afferent arterioles by a decreased capillary permeability (Ichikawa, 1982; Blantz and Pelayo, 1984). This local vascular response may be induced by the renin-angiotensin system (Schnermann and Briggs, 1986).

Numerous studies, reviewed by Schnermann *et al.* (1992), have established that all components of the renin-angiotensin system are present in the JGA.

Perfusion of the Henle's loop with isotonic saline resulted in a two to three fold increase in renin content of the associated JGA. This increased renin content is apparently not due to synthesis of renin, but rather to transformation of renin from an inactive into an active form (Gillies and Morgan, 1978).

A number of studies have examined the predictions of the TGF mechanism at both the whole-kidney and single nephron level. A careful review of these studies (Wright and Briggs, 1979) indicated that the change in systemic or whole kidney hemodynamics are more powerful stimuli for activation of the renin-angiotensin system, and therefore may mask the local effect on the renin-angiotensin system attributable to change in the fluid tubule composition. It is suggested that if the flow through the Henle's loop is altered as a consequence of alterations in systemic or whole kidney hemodynamics (global stimuli), then renin activation and release may be expected to change in the opposite direction to the changes in loop flow rate.

Therefore, an increase in mean renal perfusion pressure will increase GFR and flow through Henle's loop, but it decreases renin activation and release. In contrast, if

flow through Henle's loop is increased while global stimuli are maintained constant, the renin activation and release may be expected to increase. These expected differences may be due to the fact that global stimuli act directly on the granular cells, whereas local stimuli that alter the flow in the Henle's loop, independent of global stimuli, act through the macula densa to alter the release of renin. Accordingly, the activation and release of renin by global and local stimuli are apparently to serve different but related functions in regulating the volume and composition of body fluid by the kidney.

Quantitative examinations in dogs (Finke, Gross, Hackenthal, Huber, and Kirchhheim, 1983) demonstrated that the threshold pressure for renin release is 90 mmHg, and a decreased arterial blood pressure of as little as 1.3 mmHg from 90 mmHg results in a 100% increase in renin release, whereas an increased pressure to 160 mmHg has no effect. Similar findings have also been demonstrated in rats (Conrad, Brink-Johnsen, Gellai and Valtin, 1984). It is suggested that the renin-angiotensin system is primarily activated to prevent a fall in systemic arterial blood pressure below a critical level, and hence to maintain a more normal RBF and GFR.

In addition to the renin-angiotensin system, renal prostaglandins may also play an important role in renal blood flow and renin release (Francisco, Osborn, and Di Bona, 1982; Edwards, 1985). However, despite these important effects of prostaglandins on renal hemodynamics, prostaglandins do not constribute to renal autoregulation and exert only a permissive role in the TGF mechanism (Schnermann, Briggs and Weber, 1984).

From compelling evidence of the myogenic mechanism and the TGF mechanism involving the renin-angiotensin system, it is clearly indicated that no single mechanism can completely explain the autoregulation of RBF and GFR. The myogenic mechanism acts to buffer the changes in RBF and GFR in response to abrupt changes in systemic arterial blood pressure, in particular when the pressure exceeds the normal threshold pressure. The TGF mechanism apparently acts to stabilize GFR in the normal physiological range of systemic arterial blood pressure. The renin-angiotensin system is activated when blood pressure falls below a critical value.

2.5.2.2. Extrinsic regulation

Electrophysiological and ultrastructural studies have established that the kidney is innervated by mixed nerves containing both afferent and efferent fibres (Moss, 1982). The efferent fibres composed of postganglionic sympathetic fibres, enter the kidney together with the renal artery and extend along the artery and terminate on the smooth muscle cells of the afferent and efferent arterioles (Barajar, Powers and Wang, 1984). These nerves exert their effect by releasing norepinephrine onto the adrenergic receptors in the postsynaptic membranes (Insel and Snavely, 1981). There are also dopaminergic fibres innervating both afferent and efferent arterioles (Dinerstein, Jones and Goldberg, 1983).

Available evidence suggests that renal afferents play an important role in coordinating the function of the kidney through centrally mediated renorenal reflexes (Hermansson, Ojteg and Walgast, 1984a and b). However, considering their basal activity, it appears from numerous studies of renal nerve stimulation, reviewed by Maddox, Deen and Brenner (1992), that there is a decrease in GFR and renal blood flow, and an increased renal vascular resistance. Moreover, the evidence indicates that the neurotransmitter involved is noradrenalin.

Catecholamines (noradrenalin and adrenalin) act on 1-adrenergic receptors of the granular cells to stimulate renin release, enhancing angiotensin I production to yield a major arteriolar (afferent and efferent) vasoconstriction (Maddox *et al.*, 1992).

2.5.3. Tubular filtrate transport

There are two distinct routes of transepithelial transport: transcellular and peritubular. All active transport occurs through the transcellular route, whereas the paracellular route is the major route for passive diffusion of ions and large polar non-electrolytes, although it comprises only less than 1% of the epithelial surface area.

Accordingly, since the movement of water in different segments of the tubules occurs entirely by osmotic diffusion, it is estimated that the paracellular route contributes only 5-10% of the transepithelial water transport, and the remaining 90-95% is transported through the transcellular route. Regarding the specific tasks of the kidney, more than 99% of the transported electrolytes are coupled to the primary action of Na,K-ATPase, and there is no ATP-dependent anion pump in the nephron (Kinne and Schwartz, 1978; Katz, 1986; Buckhard and Greger, 1992)

The reabsorption of the filtered load of solutes involves a complex of mechanisms. The major transport function of the kidney tubules may be represented by NaCl transport. It is attributable to the existence of numerous Na^+ coupled transport mechanisms in apical membranes of the epithelial cells along the tubule, which means that the transport of many ions and solutes (such as glucose, amino acids, HCO_3^-, K^+) is intimately related to the transport of Na^+ (Jacobson, 1981; Katz, 1986; Madsen and Tisher, 1986).

The Na^+ transport along the tubule is exclusively driven by the Na,K-ATPase enzyme located in the basolateral membrane of the epithelial cell (Kinne and Schwartz, 1978; Katz, 1982). From measurements made in a number of tissues, this enzyme transports 3 Na^+ ions out of the epithelial cell into the peritubular (interstitial) space and takes up 2 K^+ ions from the peritubular space into the cell (Jorgensen, 1980). This activity results in a favorable electrochemical gradient for passive transport of Na^+ across the apical membrane. In addition, the cells along the tubule use energy, resulting from hydrolysis of ATP into ADP by Na,K-ATPase, in different ways to transport Na^+ and other solutes across the apical membrane.

The activity of Na,K-ATPase is normally determined by intracellular sodium concentration, but this enzyme behaves as an adaptive enzyme (Katz, 1982). The activity of this enzyme is also influenced by the action of several hormones, including aldosterone (Sansom and O'Neil, 1985), thyroid hormones (Capasso and de Santo, 1987), insulin (Nakamura, Emmanouel and Katz, 1983; Baum, 1987), and catecholamines by blocking

the inhibitory effect of vanadate (an endogenous substance that reversibly binds to a phosphate site on the enzyme (Katz, 1982)

2.5.3.1. The proximal tubule

Microanatomically, the proximal tubule is divided into an extensively convoluted part (pars convoluta) followed by a straight part (pars recta), which extends into the outer stripe of medulla. The subdivision into the three subsegments (P1, P2, and P3 or S1, S2 or S3) is based on ultrastructural criteria. The epithelium of the proximal tubule exhibits all characteristics typical for salt absorbing epithelia, and it is most perfect in its most proximal part (P1) (Maunsbach, 1973). In comparison to the other tubular segments, the tight junctions of the proximal tubule are relatively very leaky (Gumbiner, 1987; Maunsbach and Christensen, 1992).

In mammalian kidneys, about 65-70% of the glomerular filtrate is normally reabsorbed in the proximal tubule (Maddox and Gannary, 1987), which is greater than that across any other epithelium in the body including the small intestine (Schafer, Reeves and Andreoli, 1992).

Along the proximal tubule, sodium and the sum-osmolar of non-sodium solutes are reabsorbed in direct proportion to water (iso-osmotic, iso-natric reabsorption), although individual non-sodium solutes may be reabsorbed to a greater (glucose) or lesser (urea) extent than water (Malnic and De Mello-Aries 1970; Velazquez and Wright, 1992).

Based on the alterations in the ratios of the tubular fluid and plasma concentration (TF/P) of solutes, Rector (1983) demonstrated that TF/P for inulin at the end of the proximal tubule increased to a value of 2, indicating that about 50-60% of the filtered water was reabsorbed along the proximal tubule; whereas TF/P for both glucose and amino acids drops to a value of about 0.1 in the first 25% of the proximal tubule (P1), indicating that about 90% of the filtered glucose and amino acids are reabsorbed in this part.

Because of the heterogeneity of distribution of nephrons, the absorption rate of NaCl and fluid in both pars convoluta and pars recta of the juxtamedullary proximal tubule exceed those measured in the corresponding parts of the superficial (cortical) nephron. It is presumably attributed to the fact that the juxtamedullary nephrons receive a larger filtered load than their counterparts in the superficial nephrons, in addition to the higher activity of Na,K-ATPase (Rector, 1983; Hebert, 1992).

Previous findings indicated that transepithelial reabsorption of sodium is a function of the magnitude of the intracellular sodium pool (Katz, Doucet and Morel, 1979; Katz, 1982). The size of this pool is determined by the rates at which sodium enters the epithelial cell by passive or facilitated diffusion and leaves the cell by active transport across the basolateral membrane. Of these two processes, the active transport component at the basolateral membrane appears to be the rate limiting factor.

An experiment in rats indicated that about 60% of the filtered water is reabsorbed by the time the filtrate reaches the end of the proximal convoluted tubule (Giebisch and Windhager, 1964), whereas in the dog and monkeys only 45 and 30% is reabsorbed respectively (Bennett, Brenner and Berliner, 1968; Bennett, Clapp and Berliner, 1967).

In the early proximal convoluted tubule (P1), most of the sodium reabsorption is coupled to that of bicarbonate, glucose and amino acids, and only 10 - 20% of the sodium reabsorption is coupled to that of chloride (Kokko, 1973; Murer, Hopper and Kinne, 1976).

In the late proximal convoluted tubule (P2), convincing evidence indicated that 60% of the NaCl is reabsorbed by an active electroneutral process, where as the remaining 40% is by a passive mechanism presumably through the paracellular route (Berry and Rector, 1980).

In the pars recta of the proximal tubule (P3), the same proportion of sodium transport as in P2 has been estimated (Koppen, 1990). There are three possible electroneutral active transports of sodium chloride: 1. NaCl symporters; 2. parallel action

of Na/H and Cl/HCO_3 antiporters; and 3. parallel action of Na/H and Cl/formate antiporters.

The reabsorption of water through the leaky tight junction (paracellular), resulting from osmotic gradients, provides an additional route for paracellular NaCl transport. In this route, when water moves, NaCl is dragged.

Although there is a parallel relationship between GFR and tubular reabsorption (glomerulo-tubular balance phenomenon), numerous studies, reviewed by Mendez and Brenner (1990), and Wilcox, Baylis and Wingo (1992), demonstrated that alterations in the haemodynamics of the systemic circulation following expansion of extracellular fluid (ECF) or circulating volume, resulting from salt loading, will lead to a disruption of the relationship, resulting in an increased GFR and a decreased fluid reabsorption and natriuresis.

Expansion in ECF or circulating volume by salt loading dilutes plasma protein concentration, hence the plasma colloid osmotic pressure in peritubular capillaries (Brenner, Troy and Daugharty, 1971) and the efficiency of fluid reabsorption decreases.

The increased GFR will lead to a decrease in the tubular transit time, and therefore a reduction in the contact time between the epithelial cells and the filtrate (Dirk, Cirksena and Berliner, 1965). In addition, inhibition of fluid reabsorption may also be attributed to an increased back diffusion of fluid into the lumen caused by an increased permeability of the paracellular route (Rector, 1983; Berry, 1983).

Moreover, the expansion of ECF volume following salt load stimulates the release of atrial natriuretic peptide (ANP) particularly from the right atrium (Garcia, Cantin and Thiboult, 1987; Edward, Zimmermann and Schwab, 1988). The renal tubular action of this peptide is to increase GFR accompanied by a blunting of the tubuloglomerular feedback mechanism, and inhibition of fluid reabsorption in the proximal tubule (Knepper, Lankford and Terada, 1991). The action of ANP on increasing GFR is apparently mediated by dilatation of afferent arterioles and constriction of efferent

arterioles (Weidmann, Hasler and Gnadinger, 1986). Harris, Thomas and Morgan (1987) provided evidence that ANP inhibits angiotensin-stimulated NaCl and fluid reabsorption in the proximal tubule. This action is presumably mediated by increasing cyclic-GMP production (Harris *et al.*, 1987).

At the whole kidney level, diuretic and natriuretic responses following the expansion of ECF volume by salt load, which is mediated by ANP, have also been demonstrated in ruminants (see review by Olsson *et al.*, 1991).

2.5.3.2. The Henle's loop

There are two types of the Henle's loop: short and long loops. A short loop of Henle consists of a thin descending limb (TnDL) and a thick ascending limb (TAL). The terminal portion of the TAL contains the macula densa. A long loop, in addition, has a thin ascending limb (TnAL) before TAL.

Although the exact ratio of short to long loops is species dependent, the majority of loops in mammalian kidneys are of long loop type. In some carnivores, such as dogs, cats and fox, all loops of Henle are of the long type (Kaissling and Kriz, 1992).

Approximately 15-20% of the filtered load of NaCl is reabsorbed in the loop, but the process occurs primarily in the TAL (Hebert, Reeves, Molony and Andreoli, 1987).

The thin limb of the Henle's loop (descending and ascending):

From cytochemical studies, both TnDL and TnAL have extremely low activities of Na,K-ATPase compared with other segments of the tubule (Imai, Taniguchi and Tabei, 1987). This indicates that NaCl is passively reabsorbed, and exclusively depends on permeability and concentration gradients for these ions.

In vitro microperfusion studies, reviewed by Kaissling and Kriz (1992), have revealed significant intranephron and internephron differences between short and long loops and interspecies heterogeneity in Na, Cl, urea and water permeabilities of the

TnDL. This heterogeneity occurs particularly in the upper part of the TnDL of the long loop, whereas the lower part of the TnDL of the long loop in the mammalian species investigated (including rat, hamster, rabbit and *Psammomys*) has high permeability for water, moderate for urea and poor for Na and Cl. These lower part characteristics of the TnDL of the long loop appear to be similar to that of the short loop. In contrast, these four mammalian species have similar characteristics of the TnAL, namely they are highly impermeable to water, highly permeable to Na and Cl, but less permeable to urea.

The permeability differences for Na, Cl, urea and water along the thin limb contribute importantly to the process of countercurrent multiplication (Imai, Taniguchi and Tabei, 1987).

The thick ascending limb

The transition from thin to thick ascending limb (TAL) of the long Henle's loops marks the border between inner and outer medulla, whereas in the short Henle's loops the termination from the thin to thick limb occurs within inner stripe of the outer medulla (Kaissling and Kriz, 1992). A TAL can be subdivided into a medullary TAL and a cortical TAL.

Functionally, the TAL has been characterised as the "diluting segment", since this segment is impermeable to water, the reabsorption of NaCl results in the dilution of the luminal fluid (Burg, 1982).

Reabsorption of Na and Cl in the TAL occurs by a specific electroneutral symporter (1 Na, 2 Cl and 1 K) in the apical membrane, which is driven by the Na,K-ATPase enzyme in the basolateral membrane (Greger, 1985). In the cortical TAL, an additional cotransport mechanism involving parallel Na/H and Cl/HCO_3 antiporters has also been postulated (Greger and Velazquez, 1987). The apical membrane of both the cortical and medullary TAL contain a large number of K channels, which serves to recycle most of the K brought into the cell by Na/K/Cl symporter outback into the luminal fluid.

This recycling of K results in the generation of a lumen-positive transepithelial voltage (Greger, 1985).

It is estimated that approximately 50% of Na reabsorption in the TAL occurs through paracellular routes (Hebert and Andreoli, 1984).

Studies in rabbits revealed that the activity of the Na,K-ATPase in the cortical TAL is significantly lower than in the medullary TAL (Stokes, 1979). However, previous studies, reported by Rocha and Kokko (1973), reveal that medullary TAL can only reduce the sodium concentration of the luminal fluid to about 117 mmol/l, whereas in the cortical TAL the concentration is reduced to 65 mmol/l. These results suggest that the cortical TAL has a lower capacity for NaCl reabsorption, but this segment can generate a larger transepithelial NaCl gradient compared to the medullary TAL.

Taken together, active reabsorption of NaCl along the TAL without osmotic flow of water will lead to the development of hyperosmotic conditions in the surrounding medullary interstitium, whereas tubular fluid emerging from the Henle's loop becomes hypoosmotic. Such a situation induces osmotic reabsorption of water from the adjacent structures, including the TDL and the collecting duct. The tubular fluid in the TDL becomes progressively hyperosmotic and will reach the maximum value at about the loop bend.

There is an inverse relationship between the fractional reabsorption of sodium and water loads by the Henle's loop and the flow rate of fluid into the loop, and hence GFR. This relationship is attributable to the transit time of the fluid through the loop (Landwehr, Klose and Giebisch, 1967).

The responsiveness of the thin and thick limbs to various hormones is determined by the activity of adenylate cyclase. Morel (1981) reported that the TnDL is not responsive to any hormone tested, including antidiuretic hormone (ADH) and aldosterone. The TnAL was found to be only responsive to ADH, but this may be species dependent phenomenon.

2.5.3.3. The distal convoluted tubule

The distal convoluted tubule (DCT) begins a short distance beyond the macula densa with an abrupt increase in epithelial height of interdigitating cells (Kaissling and Kriz, 1992). The nucleus is characteristically situated in the apical cell portion, and the processes of the interdigitating cells are densely furnished with large mitochondria.

Micropuncture studies on this segment have shown that normally 5-7% of the filtered load of NaCl is reabsorbed (Hropot, Fowler, Karlmark and Giebisch, 1985). In accord with the processes occurring in the previous segment, the fluid entering the DCT is always hypoosmotic.

There are two possible mechanisms of Na and Cl transport across the epithelium: Na/Cl symporters and synchronous activity of Na/H and Cl/HCO_3 antiporter (Koeppen, 1990). Regardless of the precise mechanism, it is clear that the primary driving force is the lumen-cell sodium concentration which is maintained constantly by the activity of Na,K-ATPase.

In contrast to its role in NaCl transport, the epithelium of the DCT has low permeability to water except in the presence of ADH (Gross, Imai and Kokko, 1975) and as a result, the DCT contributes as much to the urinary dilution as the TkAL when ADH secretion is low.

2.5.3.4. The connecting tubule

The connecting segment (CT) lies between the DCT and the initial portion of the cortical collecting duct, and shares characteristics of both segments. The CT is composed of two cell types which are 70-80% of principal cell and 20-30% of intercalated cells (Kaissling and Kriz, 1992).

Cytochemical studies in rabbits indicate that the activity of the Na,K-ATPase in the basolateral membrane of the principal cells is high at the beginning of the segment, and it sharply decreases along the segment (Kaissling and Stanton, 1992). In addition, through

specific Na-channels, sodium is partially reabsorped by a thiazide-sensitive Na-Cl cotransporter in the apical membrane (Shimuzu, Yoshitomi, Nakamura and Imai, 1988). However, the CT is water impermeable, and it does not react to increases in cAMP after exposure to ADH (Morel, Chabardes and Imbert, 1976).

The existence of Ca,Mg-ATPase and vitamin D-dependent calcium binding protein has been demonstrated in the principal cells of the CT of the human kidney (Kawashima and Kurokawa, 1986; Borke, Minami, Verna, Penniston and Kumar, 1987)

Apical membranes of the intercalated cells contains a H^+-HCO_3^- transport system by which acid-base balance of the tubular fluid urine may be adjusted (Koeppen and Helman, 1982; Levine and Jacobsen, 1986). There are two types of the intercalated cells: H^+ reab/HCO_3^- secr, and HCO_3^- reab./H^+ secr. In the cortical collecting duct, HCO_3^- secreting cells appear to be more numerous, whereas in the medullary collecting duct the H^+ secreting cells appear to be more prominent (Schwartz and Al-Awquati, 1985). Aldosterone may presumably affect the mechanism by enhancing the activity of H^+-ATPase (Garg and Narang, 1988).

The significance of the intercalated cells in Na reabsorption has not been reported, but available evidence indicate the existence of K-ATPase in the apical membrane (Daucet and Marsy, 1987), and these cells are also able to increase their permeability to water in the response to ADH (Koseki, Yamaguchi, Furusawa and Endou, 1988).

2.5.3.5. The collecting duct

The collecting duct consists of three different segments: the cortical collecting duct and outer and inner medullary collecting ducts. Principal cells are found along the collecting duct, whereas the intercalated cells, depending on species, will disappear before the end of the duct. The ratio between principal cells and intercalated cells differs in the three segments and also among species (Wade, Stanton and Brown, 1992). Normally, the collecting duct (cortex + medulla) reabsorbs about 3% of the filtered sodium (Koeppen, 1990).

In the cortical collecting duct, there are specific channels for Na reabsorption (amiloride-sensitive Na channel) and for K secretion (barium-sensitive K channel) (O'Niel and Hayhurst, 1985; Tago, Schuster and Stokes, 1986).

In the outer stripe portion of the outer medullary collecting duct, Na reabsorption and K secretion continues as in the cortical segment, but to a lesser extent. In the inner portion of the outer medullary collecting duct, however, there are no significant ionic channels (Koeppen, 1990). Therefore, the outer medullary collecting duct contributes less to the high NaCl concentration in the medullary interstitium.

The epithelial cells of the inner medullary collecting duct, in comparison to those in the outer medulla, have functions more like those of the cortical principal cells: they contribute to sodium reabsorption and the production of a concentrated urine (Rocha and Kudo, 1982).

In both the cortical and medullary collecting ducts, aldosterone appears to play a central role in sodium transport, primarily by increasing the number of open Na^+ channels, increasing Na,K ATPase, and opening K^+ channels (Ullrich and Papavassiliou, 1979; Rocha and Kudo, 1982; Sansom, and O'Niel, 1985).

In the cortical collecting duct, during antidiuresis, circulating ADH stimulates Na and Cl reabsorption and K secretion (Schlatter and Schafer, 1987). It has also been established that ADH increases water permeability, but has no effect on urea permeability in this segment (Schafer and Andreoli, 1972; Knepper and Roch-Ramel, 1987).

In the outer medullary collecting ducts, the presence of ADH increases the permeability to water, but they remain impermeable to urea (Knepper and Roch-Ramel, 1987).

Studies on the isolated perfused rat inner medullary collecting duct (Sand, Nonoguchi and Knepper, 1987) revealed that basal permeabilities to water and urea are higher in the terminal two thirds of the inner medullary collecting duct compared to the

previous portions. The presence of ADH increases the permeabilities to both water and urea in this segment (Sand *et al.*, 1987).

2.5.4. Renal pelvis, ureter and bladder

The renal pelvis, ureter and bladder in mammals have generally been considered to serve only as temporary storages (pelvis and bladder) and conduit (ureter) of the urine excreted by the kidney.

Available evidence, however, supports the view that the mammalian renal pelvis plays an important role in the urinary concentrating mechanism.

The papillary tip of the renal pelvis is covered by a single layer epithelium of the same type as that of the inner medullary collecting duct (Pfeiffer, 1968; Silverblatt, 1974), and is derived from the same embryological source (Langman, 1969). This part of the pelvis has little or no supportive connective tissue separating it from the underlying capillary endothelial cells or tubular epithelium (Pfeiffer, 1968). Moreover, intercellular space of this pelvic epithelium in the rat are found to be wide during antidiuresis and narrow during water diuresis, suggesting that the permeability of the epithelium might be influenced by the presence of ADH (Bonventre, Karnovsky and Lechene, 1978). In addition, available evidence indicates that urea may diffuse from pelvic urine to the papilla interstitium, depending on urea concentration (positive relationship) and the flow rate of urine (negative relationship) (Gertz, Schmidt-Nielsen and Pagel, 1966; Bargman, Leonard, McNeely, Robertson and Jamison, 1988).

The epithelium lining the lower part of the pelvis, however, is a transitional type similar to the epithelium lining the ureter and bladder. It is characterised by a thick asymmetrical membrane, and keratinised. This type of epithelium may possibly act as a barrier to water and solute penetration (Schmidt-Nielsen, 1977).

Micropuncture and microperfusion studies of the ureter of rats with a moderate diuresis demonstrated that 7, 3.4 and 3.5% of urea, potassium and creatinine respectively were reabsorbed from the ureter, whereas 9.2% of sodium was secreted into it (Walser, Yagil and Jamison, 1988).

Englund (1956) demonstrated that the bladder of dogs is not a perfect barrier for the movement of water and ions (^{24}Na, ^{32}P, ^{42}K and ^{82}Br), which moved across the wall in both directions. A similar result was also reported by Hlad, Nelson and Holmes (1956) using ^{22}Na, ^{36}Cl, ^{42}K and ^{32}P. The evidence suggests that there are several factors affecting the net movement across the bladder wall, including pH (Hlad *et al.*, 1956), flow rate of urine (Levinsky and Berliner, 1959) and concentration gradient between plasma and urine (Rapoport, Nicholson and Yendt, 1960).

The previous section reported that the accumulation of urea in the medullary interstitium is primarily supplied by reabsorption from the inner medullary collecting duct. Together with the accumulation of NaCl, the reabsorbed urea contributes to the hyperosmolality of the medullary interstitium, which is an important factor determining urinary concentrating ability. Information regarding the permeability of the renal pelvis and ureter to urea suggests an important contribution to the concentrating mechanism of the kidney, which then serves in the regulation of body volume homeostasis.

2.5.5. Urinary concentrating mechanism

Various concepts have been introduced to explain the urinary concentrating mechanism. The most fully developed hypothesis was independently but almost simultaneously proposed by Stephenson (1972) and Kokko and Rector (1972), who called the phenomenon the "passive mechanism" in the inner medulla. The principle of this mechanism is based on a previous idea, introduced by Kuhn and Ryffel (1942 reviewed by Jamison and Kriz, 1982). According to the Stephenson/Kokko-Rector model, the counter-current mechanism is made possible by mixing of urea and sodium

choride in the inner medullary interstitium, creating at any level of the inner medulla a total osmolality higher than that in the descending thin limb as well as in the collecting duct.

According to the permeability characteristics of both tubular segments, water is absorbed from their lumens, thereby concentrating the descending limb fluid (osmotic equilibration) as well as the final process of urine formation in the collecting duct.

Resuming the previous section (2.5.3, Tubular filtrate transport), sodium chloride is delivered from thin and thick ascending limbs, whereas urea is delivered from the last two thirds of the inner medullary collecting duct. The total osmolality in tissue slices from dog's kidney (Culrich and Jaravech, 1956 cited by Marsh and Knepper, 1992) indicated that urea contributes approximately one-third of the osmolality, whereas the remainder was mainly NaCl.

Hypertonic reabsorption of NaCl from ascending limbs results in hypotonicity of tubular fluid at the end of the thick ascending limb with urea as the main solute. This hypotonicity of tubular fluid at the end of the thick limb is achieved regardless of whether the kidney produces a dilute urine or a concentrated urine. This hypotonicity is maintained throughout the distal convoluted tubule and the connecting tubule, although fractional content of electrolytes, urea and H^+ ion are largely changed by the distal tubule and connecting tubules. Under the influence of ADH, reabsorption of water and urea from collecting duct increases. In the absence of ADH, the hypotonic fluid that leaves the thick ascending limb largely represents the final urine. An additional dilution effect, however, may presumably come from active transport of NaCl along the collecting ducts (Marsh. 1966, Jamison and Lacy, 1972; Sonnenberg, 1974).

In summary, the key function of the urinary concentration mechanism is the hypotonic reabsorption of NaCl by the thick ascending limb in the outer medulla. Diluting capability of the kidney is established by the function of the thick ascending limb and it can be enhanced by active reabsorption of solute from the collecting duct (ADH - lack). Under ADH stimulation, on the other hand, reabsorption of urea from the collecting duct

may be a key component in the concentrating mechanism, and hence hyperosmolality of the medullary interstitium. The macula densa, which is located within the terminal of the cortical thick ascending limb, appears to control the dilution function of this segment. As has been pointed out by Knepper and Burg (1983), the sodium delivered to the distal convoluted tubule is obviously buffered against rapid short-term alterations by various mechanisms, including glomerulo-tubular balance and the tubulo glomerular feedback mechanism.

2.5.6. Urea recycling

The existence of a medullary recycling of urea has been elucidated in many studies. Micropuncture studies in hamster (Mars, 1970); rats (Lassiter, Mylle and Gottchalk, 1965) have revealed that urea concentration in the fluids collected from the loop bend and the early distal convoluted tubule are much higher than that which is possible by escape from the proximal tubule reabsorption. This suggests that urea is effectively recirculated within the inner medulla. An *in vivo* study in rats and hamsters reported by Imai, Hayashi and Araki (1984) conformed to these micropuncture results.

This recirculation involves urea reabsorption from the inner medullary collecting duct and its subsequent diffusion/secretion into descending limbs that is, pars recta of the proximal tubules, thin descending limb, thin ascending limb, and vasa recta (Knepper and Roch-Ramel, 1987).

Ullrich, Kramer and Boylan (1962 cited by Jamison and Kriz, 1982) estimated that approximately 80% of the filtered load of urea in the inner medullary collecting duct is reabsorbed; 50% of it is recirculated by way of the pars recta of the proximal tubules, descending and ascending thin limbs of Henle's loop, whereas the 30% remaining is trapped in the vasa recta counter current diffusion of urea.

Urea diffusion into the descending limb appears to be an additional solution for osmotic equilibration of this tubular segment. However, Kokko (1970) suggested that the osmotic equilibration of this segment occurs 96% by water extraction and only 4% by solute entry.

By assuming the endothelium of descending vasa recta is impermeable to plasma protein, it was calculated that osmotic equilibration in descending vasa recta occurs mainly by solute (NaCl and urea) entry (88%) rather than water extraction (Jamison and Kriz, 1982).

Reabsorbed water from the collecting duct and Henle's descending limb is removed from the medulla and returned to the general circulation through the ascending vasa recta (Jamison and Maffly, 1976).

CHAPTER III

EFFECTS OF INTRARUMINAL SODIUM CHLORIDE INFUSION ON DIGESTION, WATER AND SALT METABOLISM IN MERINO SHEEP AND ANGORA GOATS

SUMMARY

The experiment was conducted to compare the effects of intraruminal NaCl (salt) infusion on rumen dynamics and metabolism of water and salt in sheep and goats. Twelve mature Merino ewes and eleven mature Angora does were used. They were fed a mixed roughage diets consisting 400 g of wheaten chaff and 400 g of lucerne chaff daily at 2 h intervals. Fresh water was offered freely. Two levels of salt infusion, 25 and 50 mmol/kg W/d in 500 ml water, were infused intraruminally as well as a control with no salt infusion.

During the control regime, the goats showed significantly higher intakes of organic matter (OM) and nitrogen (N), estimated metabolic water production and apparent sodium digestibility, but faecal moisture content from the goats was lower than from the sheep.

Most of the differences between sheep and goats were obtained in the responses to the high level of salt infusion.

The results showed that salt loads decrease the intakes of food by the sheep and goats to the same extent. Water intakes by the sheep or goats increased with increased input of salt, which in turn resulted in increases in the out-flow rate of rumen fluid. The increased water intake by the sheep with the high level of salt was significantly higher than by the goats. This caused the faster flow rate of rumen fluid in the sheep than in the goats. This latter situation resulted in the lower digestibility of OM and N in the sheep than in the goats.

Rumen fluid volume (ml/kg $W^{0.82}$) increased with increased input of salt in both the sheep and goats. At any salt level the volume in the sheep was higher than that in the goats. However, when it was expressed as a percentage of body weight, there were no differences between the species.

Apparent sodium digestibility and apparent water absorption by the sheep and goats were increased by the salt loads to similar levels.

Tritiated body water space (TBWSp) (ml/kg $W^{0.82}$) in the sheep was only slightly increased with the increased input of salt to the high level, but in the goats it increased markedly. These different responses were presumed to be species differences in water intake, kidney function and estimated metabolic water production.

Water turnover rate (WTOR) (ml/kg $W^{0.82}$/d) in the sheep and goats substantially increased with increased salt input, due primarily to water intake. The increase in the sheep with the high level of salt was greater than in the goats. These differences were also reflected in water losses (ml/kg $W^{0.82}$/d) through urination, defaecation and evaporation (insensible loss).

The relative importance (% WTOR) of the kidney route of water loss was increased by salt loads, and there were no significant differences between the species. The increase was from 30-35 in control to 60-65% of WTOR at the high salt input. On the other hand, the proportion of water lost through faeces and insensible loss decreased with salt loads in both species. The decrease in faecal water loss from the goats was greater than from the sheep.

Concentration of sodium in the urine faecal water from the sheep and goats increased markedly with increased input of salt, and on the high level the concentrations were significantly higher in the urine and faeces from the goats compared to that from the sheep.

The sheep and goats showed a similar pattern in N balances as responses to salt loads. The results showed that salt loads decreased nitrogen retention by increasing urinary nitrogen excretion (% digestible N) following natriuresis and diuresis.

The findings suggested that under the condition of continuous intraruminal salt infusion with free access to fresh water, some species differences between sheep and goats did occur. In addition some similarities were shown in the way they eliminate the excessive salt. However, there are some interesting aspects for further experiments, in relation to natural conditions in which the animals face salt load from food or drinking water in addition to thermal loads or water limitation.

INTRODUCTION

Although it was recognised that sheep and goats, in general, have different characteristics in their taste preferences (Goatcher and Church, 1970), food utilization (Devendra, 1978; Talkamp and Brouwer, 1993) and water metabolism (Macfarlane and Howard, 1974), most information regarding the effect of salt load on water and salt metabolism are from studies on sheep. Studies on sheep (Godwin and Williams, 1986) have shown that high salt loads via continuous intraruminal infusion, can have profound effects on gut

dynamics and digestibility. The effects of salt loading on rumen fluid dynamics and its consequent effects on nutrition of goats have not been studied.

It is, therefore, to obtain information on these aspects in goats, the comparative studies reported herein were designed to avoid different salty taste preferences between sheep and goats which was by intraruminal infusion of salt solution with free access to fresh water.

It is hypothesised that both the sheep and goats would have similar responses to salt infusion as those found by Godwin and Williams, (1986).

MATERIALS AND METHODS

Design of the experiment

There were 2 levels of NaCl loading, 25 and 50 mmol/kg body weight per day which were continuously infused intraruminally in 500 ml of H_2O per day, as well as a control (low salt) with no salt infusion. The experiment started with the low salt loading consisting of 4 sheep and 4 goats, and was followed by medium salt loading with 6 sheep and 7 goats, and then the high salt loading with 6 sheep and 4 goats. Henceforth, the term salt intake refers to both the dietary and infused salt. The experiment was carried out in a thermoneutral environment (20-25°C and 40-50% rh). The treatments, by necessity, were supplied sequentially to allow adaptation time to the increasing levels of the salt infused.

Animals and management

Merino ewes aged approximately 3 years and Angora does aged 2-3 years were used. At the commencement of the experiment, their body weights ranged from 32.5 to 43.1 kg for the sheep and from 24.8 to 42.0 kg for the goats. At the end of the experiment, body weights ranged from 32.2 to 43.9 kg and from 22.8 to 38.6 for the sheep and the goats respectively. The weights were monitored a day prior to and after every period of salt loading.

All animals were orally dosed with Ranide anthelminthic (Merk, Sharp and Dohme, Granville, NSW, Australia), and vaccinated with Glanvac-6 (Commonwealth Serum Laboratories, Victoria, Australia) subcutaneously shortly after being housed.

They were fitted with permanent rumen cannulae (Godwin and Chaffey, 1988). The horns of the goats were shortened under anaesthetic immediately before rumen cannulation. The experiment was started 2 months after cannulation, and the animals were freshly shorn prior to the experiments.

All the animals were fed the same diet consisting of 400 g of lucerne chaff and 400 g of wheaten chaff daily and tap water containing 2.1 mmol/l sodium was offered freely. In the metabolism crates the animals were fed automatically (Nicol and Corbett,1971) with one twelfth of their daily ration at 2 h intervals.

Antibiotic was intramuscularly injected in the animals to avoid bladder infection caused by catheterization. In the controls, a dose (1 ml/kg body weight) of terramycin (Pfizer Agricare Pty. Ltd., NSW, Australia) was administered a day prior to catheterization of the bladder. This proved inadequate, and therefore in the medium and the high levels of salt loading, terramycin (1ml/10kg body

weight/3 days) and penicillin-streptomycin (2ml/50 kg body weight/day) (Norbrook Laboratories, U.K.) were injected a day prior to catheterization and then daily until the catheters were removed.

Protocol of the experiment

There were three consecutive treatments of salt loading each of 14 d, which were low salt (no salt infusion), followed by intraruminal infusion of sodium chloride solution to treat the animals with 25 and 50 mmol sodium chloride per kg body weight per day. The first 7 d were an adjustment period to the treatments followed by a 6 d collection period to measure digestibility, dynamics of rumen fluid, glomerular filtration rate (GFR) and water turn-over rate (WTOR) simultaneously. Tritiated body water space (TBWSp) was measured on the last day of the salt loading periods.

Techniques of the experiment

a. Catheterising the jugular vein and bladder; One jugular vein of the animals was catheterised using a sterilised single lumen polyethylene tube (ID = 1.0 mm, OD = 1.5 mm) (Dural Plastic & Engineering, NSW, Australia) which was inserted through a 13G sterilised needle.

The bladder catheters were hydrogel coated foley catheters (Bard Limited, West Sussex, U.K.) and the balloon was inflated with 10 ml of diluted betadine solution (F H Faulding & Co Limited, Thebarton, South Australia). The catheter was inserted into the bladder using a vaginal speculum, and a guitar string in the lumen of the catheter to direct it into the bladder. In some cases, Xylocaine

2 % Jelly (Astra Pharmaceuticals Pty. Ltd., Australia), an anaesthetic lubricant, was used to relax the urethral orifice muscle.

b. Urine and faeces collection; Daily urine and faeces were collected separately during the collection period. The urine was directly drained from the bladder through the catheter into a 2 l urine bag (Urogard, Terumo Corp., Japan) to which was added 15 ml of 6 N H_2SO_4 to acidify the urine. Daily urine outputs of each animal in every treatment were weighed, and a 10% sample obtained and pooled in a sealed polyethylene container. On the last day of each treatment period, a 20% subsample was taken and frozen until analysed. A separate 20 ml sample of daily urine output of each animal was taken and frozen for determination of GFR.

Daily faecal outputs were measured and a 10% subsample was directly dried in an oven at 80°C for 48 h to obtain dry matter and water content of the daily faeces. The daily dried samples were pooled in a sealed plastic bag for later analysis.

c. Dynamics of rumen fluid; Flow rate and volume of rumen fluid were estimated from the disappearance of CrEDTA (a water soluble marker) infused intraruminally as a single dose (Faichney, 1993). The marker was prepared using the procedures of Binnerts *et al.* (1968). One hundred ml (1681.4 µg Cr/ml) of the marker was administered intraruminally into each animal. Fifty ml rumen fluid samples were obtained at 0, 2, 4, 6, 8, 10, 12 and 24 h after dosing. The fluid samples were acidified with 1 ml of 6 N H_2SO_4, and frozen. They were later thawed and centrifuged at 3500 rpm for 15 min. The supernatant was decanted and re-frozen until analysed for Cr concentration.

In accord with the model proposed by Faichney (1993), the flow rate of rumen fluid was estimated as the slope of a curve of the marker disappearance calculated by linear regression analysis. The natural logarithms of Cr concentrations were regressed against sampling times, and rumen fluid volume was obtained by extrapolation of the regression line at zero time.

d. Estimation of WTOR and TBWSp; WTOR and TBWSp were estimated separately using the tritiated water (TOH) dilution technique. TOH (5 Ci/l, Amersham International, Bucks, U.K.) was diluted in isotonic sterile saline solution (0.9 % w/v) to a final activity of about 50 μCi per ml and stored at 4°C. There were two isotope infusions. The first was to estimate WTOR and the second to determine TBWSp. The volume of the labelled solution was approximately 5 ml per animal, the actual volume being calculated from weights of the syringe before and after infusion. The dose ranged between 5 and 10 μCi/kg body weight as a single infusion.

A 10 ml blood sample was taken immediately before infusion and labelled as a zero sample. In WTOR determinations, further blood samples were taken at 24 h intervals for 5 days. Animals were fed and watered normally. In TBWSp determination, blood samples were taken at 2, 4, 5, 6, 7, 8, and 9 h post infusion and the animals were without water or food or salt infusion for 12 h prior to infusion of the isotope. Blood samples were centrifuged, and plasma was separated and frozen until tritium was assayed.

WTOR and TBWSp were calculated according to the procedures of Holleman, White and Luick (1982). The turn-over rate was the slope (k) of the regression line obtained by plotting the natural logarithmic values of the disintegration counts of the tritium in the

plasma samples from the first isotope infusion against sampling times. TBWSp was estimated from the total radioactivity of the tritium infused, corrected by the zero sample radioactivity relative to the diluted radioactivity in plasma sample at equilibrium time.

TBWSp = Standard Counts (dis/min) x dose (ml) - zero sample counts (dis/min)

Equilibrium sample counts

The WTOR in absolute value per unit time was calculated as the product of TBWSp and k, i.e. **WTOR** = k x TBWSp

e. Glomerular filtration rate (GFR); The GFR was measured by using endogenous creatinine (Godwin, 1987) which was determined from daily urine and daily plasma samples.

Balance calculation

Apparent digestibilities of dry matter, organic matter, nitrogen and sodium, and apparent water absorption were calculated according to McDonald, Edwards and Greenhalgh (1988). Nutrient intakes and excretions were expressed in term of metabolic body weight ($kg^{0.75}$) (Kleiber, 1962) to allow the comparison of data between animals of different body weights of the different species.

Total ingested water (TIW) was calculated from the expression :

TIW = DW + FW

where DW is drinking water and FW is free water in food which was calculated as feed intake minus dry matter intake. Evaporative loss from drinking vessels was monitored.

All the experimental animals were assumed to be in water equilibrium. Therefore, water influx was equivalent to water outflux and total flux of water can be determined as tritiated water turn-over rate (WTOR). Metabolic water and evaporative water loss were estimated according to Taylor (1970). Metabolic water was calculated as the differences between WTOR and TIW, and evaporative water losses (insensible water loss from the skin and by respiration) was estimated as the differences between WTOR and the sum of urinary and faecal water losses (sensible water loss).

Water flux (TIW, water losses and WTOR) was expressed in term of body weight to the 0.82 power (Macfarlane, 1965). This author calculated the 0.82 power from water flux rate measurements of some ruminant species, including sheep and goats.

Analytical techniques

Dry matter of food (lucerne and wheaten chaff) and faeces were determined by drying samples in an oven for 48 h at 80° C, cooling in a dessicator for 8 h and weighing. Samples were then finely ground and stored in sealed polyethylene containers until analysed. The organic matter of the food and faeces was determined by ashing for 2 h at 300-400° C followed by 24 h at 600° C.

The ground samples of food, faeces and urine were digested by the method of Cresser and Parsons (1979) and total nitrogen was estimated with an auto-analyser (Technicon Instrument Corp, New York), and sodium concentration by atomic absorption spectrophotometry (SB 900, GBC Scientific Equipment, Melbourne).

Plasma and urine creatinine were measured with a Cobas Bio centrifugal analyser (Hoffman-La Roche, Switzerland). Plasma electrolyte (Na, K and Ca) concentrations were measured using an AVL 988-3 electrolyte analyser (Graz, Austria) and plasma osmolality with a vapour pressure osmometer (Wescor 5100B, UT, U.S.A.).

The radioactivity of tritium in the plasma was determined directly by mixing 0.5 ml of plasma, 0.5 ml H_2O and 10 ml Ultima Gold scintillation fluid (Canberra-Packard Pty.Ltd., Victoria, Australia) in a 20 ml scintillation vial and then counting in a Packard "Tri-Carb" Liquid Scintillation Spectrometer for 10 min..

Statistical analysis

Data were analysed according to an analysis of variance of " Unequal frequencies of 2 x 3 Factorial experiment" (Systat Statistical Package, 1990). Factor A was animal species, and factor B was salt levels. Mean values among the treatments of each parameter were compared according to Newman-Keuls procedures (Winer, 1971).

RESULTS

Body weight and intakes of sodium, water and feed (Table 3.1)

There were 3 levels of sodium intake (infusion plus dietary): low, medium and high with about 5, 50 and 100 mmol/kg $W^{0.82}$ per d respectively. The intakes were not significantly different between sheep and goats.

Intakes of water by sheep and goats increased significantly with increased sodium intake, but it was only on the high level of salt that the sheep drank significantly more water than the goats.

Organic matter and total nitrogen intakes of the sheep and goats were decreased significantly by salt load, but there were no significant differences between medium and high levels in both species, or between sheep and goats with medium or high level.

Digestion, absorption and dynamics of rumen fluid (Table 3.2 and Figure 3.1)

Apparent digestibility of organic matter in the sheep was gradually decreased with increased input of salt. By comparison, digestibility in the goats was not significantly different with different inputs of salt. With the high level, the goats had significantly higher digestibility than the sheep. The responses in apparent digestibility of nitrogen were similar.

The apparent digestibility of sodium in the sheep and goats was increased by salt loads. However, there were no significant differences between medium and high levels either in the sheep or goats, or between sheep and goats on medium or high level. With low level, the digestibility was higher in the goats than in the sheep.

Rumen fluid volume (ml/kg $W^{0.82}$) in the sheep or goats increased with increased sodium intake, and the volume in the sheep was significantly higher than in the goats with any level input of salt. However, there were no significant differences in the volume between sheep and goats when it was expressed as percentage of body weight.

Flow rates of rumen fluid (ml/kg $W^{0.82}$/h) in the sheep or goats increased significantly with increased salt intakes, but the increased intake of sodium from medium to high level by the goats did not significantly alter the flow rates. With medium and high levels, the flow rates in the sheep were significantly faster than in the goats, but there were no significant differences between them on the low level..

Distribution and dynamics of body fluid (Table 3.3)

Tritiated body water space (TBWSp) (ml/kg $W^{0.82}$) in the sheep did not significantly increase with increased sodium intake. Rumen fluid volume as a proportion of TBWSp (%TBWSp) was not significantly different with different input of salt intake, or between sheep and goats.

Water turn-over rate (ml/kg $W^{0.82}$/d) (WTOR) increased significantly with increased sodium intake in both the sheep and goats. There were no significant differences between sheep and goats on low and medium levels, but with the high level the sheep values were significantly higher compared to the goat values.

Urinary, faecal and insensible water losses (ml/kg $W^{0.82}$/d) from the sheep and goats were increased by salt loads, except for faecal water loss in the goats which remained relatively constant. With the low or medium level of salt input there were no significant differences between sheep and goats in losses by those routes. With

high level, however, the losses through those routes from the sheep were significantly higher than from the goats.

When water loss was expressed as a proportion of WTOR (%WTOR), the results indicated that urinary loss from the sheep and goats were significantly increased by salt loads, but faecal and insensible losses decreased.

Moisture contents (%) in the faeces from the sheep increased significantly by salt loads, and they were significantly higher compared to those from the goats with any level of sodium intakes. There was no significant effect of salt load on moisture contents in the faeces from the goats.

Glomerular filtration rate (ml/min) (GFR) in the sheep and goats were increased by salt load, but there were no significant differences between medium and high levels. The differences between the sheep and goats were only on high level, GFR being lower in the goats.

Sodium and nitrogen balances, and plasma electrolytes (Table 3.4)

Sodium concentration (mmol/l) in the urine and faecal water from the sheep or goats increased with increased input of salt. There were no significant differences between sheep and goats with low or medium level of sodium intake, but with high level, the concentration was higher from the goats than from the sheep.

The proportion of sodium intake excreted through the urine from the sheep or goats increased significantly by salt loads, while the faecal sodium proportion decreased. Positive balances were about 2.9 and 1% of the intake in the sheep with medium and high levels respectively, while in the goats with corresponding levels it was between 8 and 1%. Some of these positive balances which are high in the goats may have been partly due to small losses due to rumen cannula leakage during the infusions.

Total nitrogen intake (mmol/kg $W^{.75}$/d) by the sheep with low sodium intake was significantly lower than the intake by the goats, but with medium and high levels, there were no significant differences in the intakes by both species.

The excretion of nitrogen through urine by the sheep and goats were not significantly different, but the excretions from both species decreased with the salt loads, which due to a decrease in the intake. As a proportion of total digestible nitrogen, the excretion from the sheep with medium and high levels exceeded the digestible nitrogen by 4 and 10% respectively, and they were significantly higher than the excretion from the goats. Faecal N losses were also increased with increasing salt intake. This effect was much greater in the sheep than the goats. Consequently, there were negative balances of nitrogen in the sheep with increased input of salt from medium to high level. The goats showed a similar trend.

Plasma sodium concentration (mmol/l) in the sheep and goats did not significantly alter with increased sodium intake, and there were no significant differences between sheep and goats at any

level of sodium intake. Plasma potassium concentration in the sheep, however, was increased by salt loads, while it did not significantly alter within the goats.

Table 3.1. Mean values of body weights, salt treatments and intakes of organic matter, water and sodium (mean ± se)

PARAMETERS	Animal	Salt treatment		
		low	medium	high
	n =	4 S & 4 G	6 S & 7 G	6 S & 4 G
Body weight (kg)	SHEEP	34.4 ± 1.5 a	36.9 ± 3.2 a	36.7 ± 4.0 a
	GOAT	25.8 ± 1.0 a	32.6 ± 3.8 b	32.4 ± 4.0 b
	S vs G	**	*	*
Salt treatment (mmol/kgW/d)	SHEEP	0	25	50
	GOAT	0	25	50
	S vs G			
Organic matter intake ($g/kgW^{0.75}/d$)	SHEEP	51.7 ± 1.5 a	39.7 ± 3.0 b	43.6 ± 3.6 b
	GOAT	58.4 ± 1.9 a	42.7 ± 4.2 b	45.2 ± 2.9 b
	S vs G	**	ns	ns
N-intake ($mg/kgW^{0.75}/d$)	SHEEP	1065.5 ± 28.0 a	767.9 ± 59.4 b	840.6 ± 70.4 b
	GOAT	1164.6 ± 44.2 a	824.5 ± 137.9 b	871.8 ± 56.3 b
	S vs G	**	ns	ns
Ingested water ($ml/kgW^{0.82}/d$)	SHEEP	107.2 ± 15.9 a	217.7 ± 26.4 b	333.9 ± 43.6 c
	GOAT	109.7 ± 12.3 a	204.6 ± 45.2 b	277.1 ± 40.9 c
	S vs G	ns	ns	**
Sodium intake ($mmol/kgW^{0.82}/d$)	SHEEP	5.1 ± 0.7 a	50.3 ± 0.5 b	99.3 ± 1.5 c
	GOAT	5.4 ± 0.8 a	50.4 ± 1.1 b	98.7 ± 1.8 c
	S vs G	ns	ns	ns

Means values on the same row with different letters are significantly different ($P<0.05$)
Mean values in the same cell (S vs G) :
* = differ significantly at level $P < 0.05$
** = differ significantly at level $P < 0.01$
ns = not significantly different

Table 3.2. Mean values of digestibility and absorption, rumen fluid dynamics

Parameters	Animal	SALT TREATMENTS		
		low	medium	high
	n =	4 S & 4 G	6 S & 7 G	6 S & 4 G
Digestibility and absorption				
Apparent organic matter digestibility (%)	Sheep	66.1 ± 0.2 a	59.4 ± 2.2 b	48.3 ± 8.7 c
	Goat	63.8 ± 0.5 a	58.2 ± 3.2 a	60.6 ± 2.4 a
	S vs G	ns	ns	* *
Apparent Nitrogen digestibility (%)	Sheep	67.1 ± 3.5 a	62.3 ± 1.9 b	51.7 ± 2.5 c
	Goat	67.7 ± 5.4 a	62.0 ± 4.9 a	64.3 ± 1.4 a
	S vs G	ns	ns	* *
Apparent Sodium digestibility (%)	Sheep	69.7 ± 4.5 a	85.6 ± 0.8 b	85.7 ± 1.5 b
	Goat	75.9 ± 2.7 a	88.1 ± 1.6 b	87.0 ± 1.4 b
	S vs G	* *	ns	ns
Apparent Water absorption (%)	Sheep	79.1 ± 1.5 a	87.2 ± 1.8 b	91.2 ± 2.6 c
	Goat	79.2 ± 3.3 a	89.8 ± 3.5 b	92.4 ± 1.4 b
	S vs G	ns	ns	ns
Rumen fluid dynamics				
Rumen fluid volume ($ml/kgW^{0.82}$)	Sheep	263.8 ± 8.9 a	290.6 ± 9.8 b	305.6 ± 15.6 c
	Goat	250.2 ± 6.6 a	269.9 ± 9.4 b	282.1 ± 4.5 c
	S vs G	*	* *	* *
Rumen fluid volume (% W)	Sheep	14.0 ± 0.8 a	15.2 ± 0.7 b	15.5 ± 0.9 b
	Goat	14.0 ± 0.7 a	14.4 ± 0.8 ab	15.1 ± 0.5 b
	S vs G	ns	ns	ns
Flow rate of rumen fluid (% rum.vol/h)	Sheep	8.7 ± 0.2 a	10.6 ± 0.4 b	11.7 ± 0.5 c
	Goat	9.1 ± 0.3 a	10.3 ± 0.5 b	10.3 ± 0.3 b
	S vs G	ns	ns	* *
Flow rate of rumen fluid ($ml/kgW^{0.82}/h$)	Sheep	22.9 ± 1.7 a	30.8 ± 1.3 b	35.7 ± 2.1 c
	Goat	22.8 ± 0.7 a	27.8 ± 1.9 b	29.0 ± 1.7 b
	S vs G	ns	*	* *

Means values on the same row with different letters are significantly different ($P<0.05$)
Mean values in the same cell (S vs G) :
* = differ significantly at level $P < 0.05$
** = differ significantly at level $P < 0.01$
ns = not significantly different

Figure 3.1. Rumen Fluid Dynamics

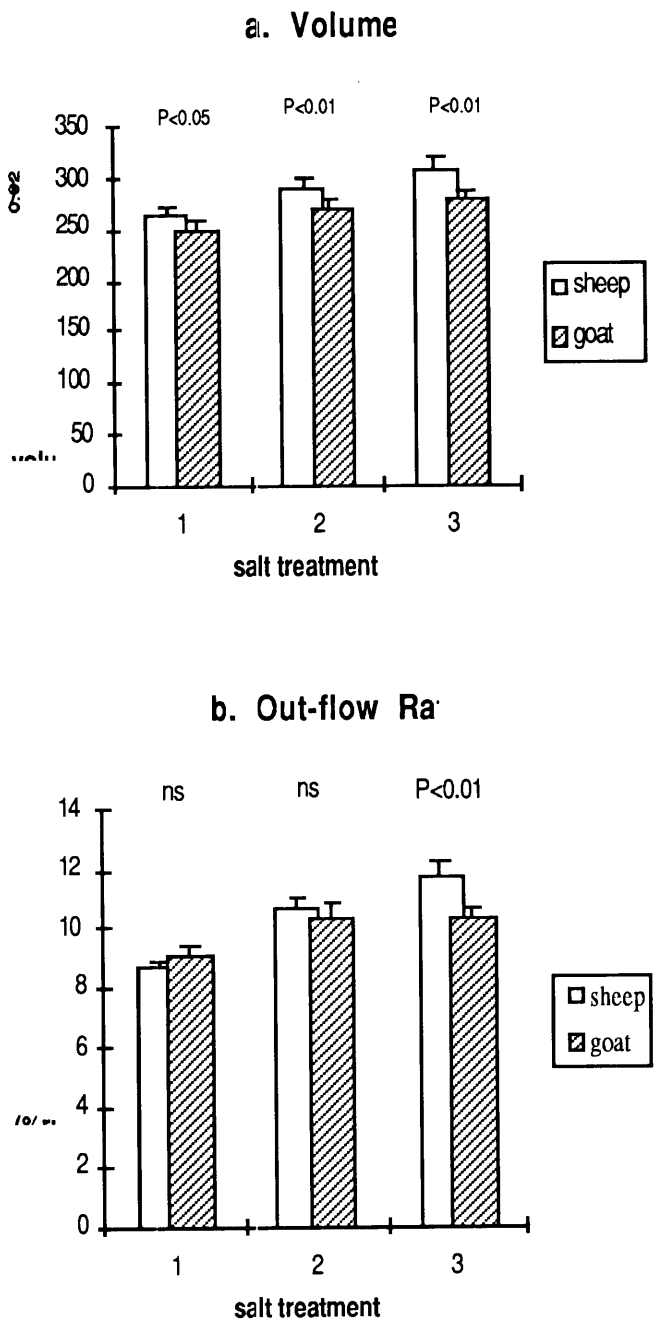


Table 3.3. Mean values of distribution and dynamics of body fluid

Parameters	Animal	SALT TREATMENTS		
		low	medium	high
	n =	4 S & 4 G	6 S & 7 G	6 S & 4 G
Tritiated body water space (TBWSp) ($ml/kgW^{0.82}$)	Sheep	1416.4 ± 142.1 a	1436.0 ± 130.5 a	1438.9 ± 100.0 a
	Goat	1351.5 ± 117.2 a	1454.7 ± 130.5 b	1459.0 ± 120.0 b
	S vs G	*	ns	ns
Tritiated body water space (% W)	Sheep	74.9 ± 2.2 a	75.1 ± 3.2 a	75.3 ± 2.1 a
	Goat	75.3 ± 2.4 a	77.8 ± 4.1 ab	78.3 ± 2.2 b
	S vs G	ns	*	*
Rumen fluid volume (% TBWSp)	Sheep	18.6 ± 0.9 a	21.0 ± 1.2 a	20.6 ± 1.7 a
	Goat	18.5 ± 0.8 a	19.8 ± 2.2 a	19.7 ± 1.7 a
	S vs G	ns	ns	ns
Water turn-over rate (WTOR) ($ml/kgW^{0.82}/d$)	Sheep	122.3 ± 16.2 a	234.3 ± 26.1 b	349.8 ± 42.9 c
	Goat	120.1 ± 10.1 a	221.1 ± 42.3 b	298.0 ± 36.0 c
	S vs G	ns	ns	* *
Est.metabolic water ($ml/kgW^{0.82}/d$)	Sheep	15.1 ± 0.6 a	16.5 ± 2.9 a	15.9 ± 3.6 a
	Goat	10.4 ± 2.6 a	16.5 ± 5.6 ab	20.9 ± 7.0 b
	S vs G	* *	ns	*
Glomerular filtration rate (ml/min)	Sheep	57.7 ± 0.7 a	71.0 ± 5.5 b	75.8 ± 2.9 b
	Goat	53.8 ± 2.4 a	67.6 ± 4.9 b	70.4 ± 3.0 b
	S vs G	ns	ns	*
Urinary water loss ($ml/kgW^{0.82}/d$)	Sheep	41.5 ± 12.0 a	133.9 ± 16.2 b	215.6 ± 18.1 c
	Goat	42.8 ± 6.5 a	133.8 ± 20.0 b	192.6 ± 20.1 c
	S vs G	ns	ns	*
Urinary water loss (% WTOR)	Sheep	33.9 ± 5.2 a	57.1 ± 4.7 b	61.6 ± 3.6 b
	Goat	35.6 ± 3.7 a	60.5 ± 7.3 b	64.6 ± 7.7 b
	S vs G	ns	ns	ns
Faecal water loss ($ml/kgW^{0.82}/d$)	Sheep	22.4 ± 3.0 a	23.7 ± 9.1 ab	29.3 ± 8.2 b
	Goat	22.5 ± 2.3 a	21.1 ± 2.2 a	20.8 ± 3.0 a
	S vs G	ns	ns	* *
Faecal water loss (% WTOR)	Sheep	18.3 ± 1.1 a	11.9 ± 1.8 b	8.4 ± 2.5 c
	Goat	18.9 ± 2.7 a	9.9 ± 2.1 b	7.1 ± 1.2 c
	S vs G	ns	*	*
Faecal moisture (%)	Sheep	59.0 ± 1.6 a	64.5 ± 3.1 b	65.5 ± 4.8 b
	Goat	55.5 ± 1.6 a	55.9 ± 3.4 a	58.6 ± 2.1 a
	S vs G	* *	* *	* *
Insensible water loss ($ml/kgW^{0.82}/d$)	Sheep	58.4 ± 2.9 a	72.4 ± 15.4 a	104.9 ± 12.9 b
	Goat	54.8 ± 7.1 a	66.9 ± 7.1 a	84.6 ± 28.6 b
	S vs G	ns	ns	* *
Insensible water loss (% WTOR)	Sheep	48.2 ± 4.9 a	30.8 ± 4.8 b	30.0 ± 3.2 b
	Goat	45.6 ± 3.7 a	31.1 ± 5.9 b	28.6 ± 8.7 b
	S vs G	ns	ns	ns

Means values on the same row with different letters are significantly different ($P<0.05$)
Mean values in the same cell (S vs G) :
* = differ significantly at level $P < 0.05$;
** = differ significantly at level $P < 0.01$; ns = not significantly different

Table 3.4. Mean values of sodium and nitrogen balances (intake and excretion), and sodium and potassium concentrations in plasma

Parameters	Animal	SALT TREATMENTS		
		low	medium	high
	n =	4 S & 4 G	6 S & 7 G	6 S & 4 G
Sodium balance				
Sodium intake (mmol/l ing.water)	Sheep	45.9 ± 6.2 a	238.1 ± 26.6 b	301.9 ± 41.5 c
	Goat	49.7 ± 3.8 a	258.6 ± 66.7 b	357.7 ± 59.6 c
	S vs G	ns	ns	ns
Urinary sodium (mmol/l)	Sheep	84.8 ± 23.7 a	312.1 ± 43.1 b	389.1 ± 54.7 c
	Goat	95.9 ± 12.5 a	348.0 ± 67.7 b	442.4 ± 80.2 c
	S vs G	ns	ns	*
Urinary sodium (% Na-intake)	Sheep	68.5 ± 4.4 a	82.7 ± 8.9 b	84.6 ± 7.8 b
	Goat	74.7 ± 2.5 a	79.7 ± 4.3 ab	85.9 ± 6.7 b
	S vs G	* *	ns	ns
Faecal sodium (mmol/l fae.water)	Sheep	66.0 ± 21.9 a	268.6 ± 38.7 b	514.5 ± 81.6 c
	Goat	58.2 ± 26.8 a	327.5 ± 73.5 b	612.3 ± 77.9 c
	S vs G	ns	ns	*
Faecal sodium (% Na-intake)	Sheep	30.3 ± 4.5 a	14.4 ± 3.8 b	14.4 ± 2.5 b
	Goat	24.1 ± 3.7 a	12.0 ± 2.6 b	13.0 ± 2.7 b
	S vs G	* *	ns	ns
Na balance (mmol/d)	Sheep	+ 4.6 ± 2.0 a	+ 52.9 ± 9.9 b	+ 48.1 ± 5.8 b
	Goat	+ 4.2 ± 1.5 a	+ 39.2 ± 10.1 b	+ 31.2 ± 9.9 b
	S vs G	ns	*	* *
Nitrogen balance				
Total N intake (mmol/kg $W^{0.75}$/d)	Sheep	76.1 ± 2.0 a	54.9 ± 1.9 b	60.1 ± 5.0 b
	Goat	83.2 ± 3.2 a	58.9 ± 2.7 b	62.2 ± 4.0 b
	S vs G	* *	ns	ns
Total urinary N (mmol/kg $W^{0.75}$/d)	Sheep	47.3 ± 3.3 a	35.6 ± 3.6 b	34.2 ± 7.6 b
	Goat	50.1 ± 6.4 a	35.3 ± 5.3 b	39.7 ± 2.5 b
	S vs G	ns	ns	ns
Total urinary N (% digestible N)	Sheep	92.6 ± 2.6 a	104.0 ± 0.1 b	110.3 ± 2.3 b
	Goat	93.6 ± 3.8 a	97.2 ± 5.8 b	99.5 ± 0.5 b
	S vs G	ns	* *	* *
Total faecal N (mmol/kg $W^{0.75}$/d)	Sheep	25.1 ± 3.0 a	20.6 ± 2.2 b	28.9 ± 6.4 c
	Goat	25.5 ± 4.2 a	22.4 ± 4.2 b	22.2 ± 2.0 b
	S vs G	ns	ns	*
Total faecal N (% N-intake)	Sheep	32.9 ± 3.5 a	37.7 ± 2.0 b	48.3 ± 10.5 c
	Goat	32.3 ± 5.4 a	38.0 ± 2.7 b	35.7 ± 8.4 b
	S vs G	ns	ns	* *
N-balance (mmol/kg $W^{0.75}$/d)	Sheep	+ 3.30 ± 1.2 a	- 1.4 ± 0.1 b	- 3.1 ± 0.3 c
	Goat	+ 3.30 ± 1.9 a	+ 1.2 ± 1.6 a	+ 0.3 ± 0.2 b
	S vs G	ns	* *	* *
Plasma electrolytes (sodium and potassium)				
Sodium (mmol/l)	Sheep	149.6 ± 4.0 a	151.2 ± 5.5 a	151.3 ± 2.4 a
	Goat	147.9 ± 3.4 a	149.7 ± 0.9 a	150.1 ± 1.0 a
	S vs G	ns	ns	ns
Potassium (mmol/l)	Sheep	4.1 ± 0.1 a	5.1 ± 0.5 b	5.4 ± 0.3 b
	Goat	4.5 ± 0.2 a	4.5 ± 0.3 a	4.7 ± 0.2 a
	S vs G	*	* *	* *

Means values on the same row with different letters are significantly different (P<0.05)
Mean values in the same cell (S vs G) :
* = differ significantly at level P < 0.05; ** = differ significantly at level P < 0.01
ns = not significantly different

DISCUSSION

Intake and digestion

Comparing Merino ewes and Angora does during the control period when no salt was infused suggested that for most of the parameters measured there was no differences between the species. The digestibility of organic matter and nitrogen were not markedly different between the species (Table 3.2). However, the intake of organic matter (g/kg $W^{0.75}$/d) and nitrogen (mg/kg $W^{0.75}$/d) by the goats were significantly higher than by the sheep.

Some earlier comparable studies of digestion in Merino sheep and Angora goats fed about the same quality roughage (Doyle and Egan, 1980; Gamble and Mackintosh, 1982) indicated similar results. Other studies with good quality roughages have also indicated no differences in overall digestibility between sheep and goats (Elhag, 1972; Antoniou and Hadjipanayiotou, 1985; Alam, Poppi and Sykes, 1985; Quick and Dehority, 1986). Studies with poor quality roughages, however, showed that goats had better digestibility than sheep (Gihad, 1977; Antoniou and Hadjipanayiotou, 1985; Alam *et al.* 1985). The results of the present study probably resulted from the lack of difference in the outflow rates of rumen fluid between the sheep and goats (Table 3.2). This result suggests that in the control condition with no salt infusion, sheep and goats had equal digestive rates to provide nutrients.

Continuous infusion of salt solution directly into the rumen would have resulted in some changes in the rumen environment. Although no measurement of rumen osmolality was made in the present work, a previous study in the same laboratory using the same experimental technique, Godwin and Williams (1986) reported that at the rates of salt infusion of 1000 mmol per d (≈ 50 mmol/kg $W^{0.82}$/d) and 2000 mmol per d (≈ 100 mmol/kg $W^{0.82}$/d), rumen osmolality in the sheep increased from 284 in control to 296 and 312 mosmol per kg respectively. Similar results have also been demonstrated in sheep by Warner and Stacy (1977) and Barrio, Bapat and Forbes (1991). Moreover, from experiments on sheep accustomed to drinking 1.3% salt water, Potter, Walker and Forrest (1972) reported an increase in osmotic pressure within the rumen, which was

normally maintained hypotonic to the blood (Warner and Stacy, 1972). In addition Bergen (1972), Carter and Grovum (1988) and Barrio *et al.* (1991) demonstrated that increasing osmotic pressure resulted in a decrease in food intake.

In agreement with these previous studies, the increase in rumen osmolality is apparently, primarily an effect of continuous salt infusion, which in turn increases water intake and decreases food intake in the animals. The responses in the long term of imposing salt load on animals are presumably a complex mechanism under the influences of neural and hormonal controls. There were no further decreases in food intake either by the sheep or goats with increased input of salt from 50 to 100 mmol/kg $W^{0.82}$/d (Table 3.1), while further increases in water intake appeared to be markedly greater by the sheep than the goats. Presumably, these different effects are derived from species differences in the responses to the amount of salt being loaded, since at all level of salt load fresh water was freely offered.

There was an expected effect of the continuous salt infusion into the rumen of increasing flow rate of rumen fluid (Godwin and Williams, 1986). The present study with sheep showed that the flow rate increased with increased salt infusion, while the outflow rate in the goats with medium and high levels of salt infusion were not different. Under steady state conditions, without salt load, Silanikove (1992) suggested that saliva is the major determinant of dilution rate of rumen fluid. However, salivation is reduced with increases in water intake (Tomas and Potter, 1975) and rumen osmolality (Warner and Stacy, 1977). The hypertonic condition of rumen fluid resulting from salt infusion may possibly increase net-influx of fluid from blood to the rumen pool (Warner and Stacy, 1972). Therefore, in addition to the increased water intake as the main reason for the increased outflow rate (Godwin and Williams, 1986), a possible increase in net-flux of fluid into the rumen from the blood may also occur.

The reduced digestibility of organic matter and nitrogen in the sheep (Table 3) may be related to the following factors: First, increasing outflow rate of rumen fluid (Table 4) resulting from the increased intake of water may have decreased the time available for microbial digestion. Hemsley *et al.* (1975 a,b) indicated that increasing outflow rate of

rumen fluid resulting from oral salt loading, reduced microbial degradation of organic matter and protein, but increased the availability of digestible nutrients to the intestine. In the present experiment, the diet offered was solely roughage in which the nutrient availability for the animal depended mostly on rumen fermentation prior to the intestinal digestion. Second, there is the possibility that the raised osmotic pressure of rumen fluid (Godwin and Williams, 1986) depressed cellulose degradation (Bergen, 1972; Bennink *et al.* 1978). *In vitro* studies indicated that growth rates of rumen micro-organisms were inhibited when tonicity of the culture medium is increased beyond the osmotic pressure of plasma (Carter and Grovum, 1991). Based upon measurements of DNA and polysaccharide, Potter et al. (1972) found that microbial populations in the rumen of sheep drinking 1.3 % NaCl solution were smaller than when the drinking water was fresh, but rumen metabolic activity was not different.

Apparent digestibility of organic matter and nitrogen in the goats, however, was not significantly affected by the salt load. There was an increased dilution rate of rumen fluid in the goats but it was much less than in the sheep. The higher water intake of the sheep compared to the goats with the high level of salt infusion would have promoted a faster outflow rate of rumen fluid. It would have also caused a dilution of the bacterial population with a resultant poorer digestibility, particularly of crude fibre (Gihad, 1976, Hemsley, Hogan and Weston, 1975).

Apparent absorption of water and sodium (Table 3.3 & 3.4) in the sheep and the goats was significantly increased by salt load and there were no significant differences of mean values between sheep and goats at any level of salt load. There is an interdependence of water and sodium transport in the rumen (Dobson *et al.* 1970; Warner and Stacy, 1972), in the omasum (Engelhardt and Hauffe, 1975), in the large intestine (Smith, 1969; Hecker and Grovum, 1971) and in the small intestine (van Weerden, 1961; Care *et al.* 1967; Parson, 1967). In the present study, the apparent absorption of water and sodium correlated significantly ($r = + 0.90$; $P < 0.0001$).

Distribution and dynamics of tritiated body water space

Determination of total body water (TBW) and water turn-over rates (WTOR) were based on tritiated water dilution technique described by Macfarlane, Howard and Good (1972). In TBW determination, fasting has been thought necessary to reduce some variability caused by a substantial contribution of gastrointestinal water. Panaretto (1963) used a 2-day period without food or water intake, but later Panaretto (1968) reduced it to 18 h. Searle (1970) used 24 h, Macfarlane (1975), Trigg *et al.* (1978) and Aganga *et al.* (1989) used an 18 h fasting period. However, from previous observations in this laboratory, fasting for such a period would have resulted in 1-3 days before returning feed intake to pre-fasting levels. A similar effect of fasting has also been reported by Massart-Leen and Peeters (1985) and Dahlborn (1987) in lactating goats. In the present experiment, therefore, the tritiated body water spaces were determined after tritiated WTOR determinations, at the end of each experimental period.

There were possibilities of overestimating TBWSp and WTOR in the present study. Firstly, there may have been invalid assumptions (Sheng and Huggin, 1979; Nagy and Costa, 1980). King, Nyamora, Stenley-Price and Heath (1978) found that tritiated water space overestimated the actual total body body water determined by desiccation by about 15% and therefore they used a correction factor of 0.85 in the calculation of these parameters. Sheng *et al.* (1979) in a review showed that the overestimates of tritiated body water ranged between 3 and 15 %. Nagy and Costa (1980) suggested that in most situations water flux rates measured with tritiated water can be expected to be within ± 10% of the actual flux rates. Overestimates are usually explained as being due to a decline in the specific activity of tritium in blood during the equilibration time because of the loss of isotope before equilibration, the dilution of tritium by metabolic water production, and isotope incorporation into body compounds (Nagy and Costa, 1980). These possible errors were unavoidable.

Secondly, an overestimate result can presumably result from non-purification of plasma water. In most previous investigations, radioactivity of tritium in plasma was determined from plasma water obtained by the vacuum sublimation method (Vaughan and

Boling, 1961) or from non-protein plasma obtained by the dioxane precipitation method (Springell and Wright, 1976). In the present study, however, radioactivity of tritium was counted directly from plasma. The results could presumably be overestimates, since solid contents and color of the plasma may reduce the count. However, a later evaluation on this possible error showed that the "Ultima Gold" scintillation fluid used in the present study minimized the error to a negligible degree (Appendix 1). Therefore, no correction was made for TBWSp and WTOR. El Hadi and Hassan (1982) used the same procedure but with a different scintillation fluid.

The results indicated that tritiated body water space (TBWSp) in the sheep were not markedly different with different levels of salt load, although the trend was to an increase. In the goats, however, TBWSp was increased markedly by salt loads. Possibly, this response occurred in the first few days of salt infusion, and it was then maintained during the salt loading (Hladky and Rink, 1986). The different responses in TBWSp between sheep and goats appeared to be a species differences which may be ascribed to one or both factors discussed below.

Firstly, concerning species differences between sheep and goats, the results (Table 3.3) indicated that estimated metabolic water (EMW) in the goats (ml/kg $W^{0.82}$/d) increased with increased salt infusion, while in the sheep it was relatively constant. Under the condition of no salt infusion, EMW in the goats was lower than in the sheep, but on high levels of salt infusion the goats had significantly higher EMW compared to the sheep. The level of metabolic water production is attributable to the level of nutrient oxidation of dietary origin in addition to body tissues. Therefore, there is a possibility that the increasing TBWSp in the goats with high salt load resulted from a replacement of oxidized body tissues with water. On the other hand, the higher water intake by the sheep compared to the goats had probably fulfilled the increased requirement for water to eliminate the excessive salt without additional production of metabolic water.

Secondly, concerning kidney function, glomerular filtration rate (GFR) in both species increased with salt loads, but the sheep GFRs were significantly higher than those for the goats when the high level of salt was imposed. The increase in GFR would lead to

an increased filtered load of salt within the kidney which in turn can result in a decreased percentage of sodium reabsorption, although the absolute amount of reabsorbed sodium is increased. (Potter, 1963; Tomas, Jones, Potter and Langford, 1973). In such a situation, the sheep consumed more water which balanced the requirement to excrete the excessive salt. The goats with the same level of salt load, however, consumed less water. Because of the lower GFR in goats, these animals showed greater body water space than sheep given the same salt load despite the lower intake of water. These animals also had higher sodium concentrations in the urine (Table 3.4) which appears to be a physiological adjustment in goats to maintain osmotic balance. In both species, sodium concentrations in the plasma were maintained relatively constant and they were not significantly different (Table 3.4).

In both species, however, continuous salt infusion with free access to fresh water resulted in natriuresis and diuresis. Possibly, the mechanism was under the influence of natriuretic factor which inhibits antidiuretic hormone secretion and the renin-angiotensin-aldosterone mechanism (Sealey, Kirshman and Laragh, 1969)

Water turn-over rates (WTOR) (ml/kg $W^{0.82}$/d) in the sheep and goats increased with increase salt load (Table 3.3). A similar result has been reported by Macfarlane, Howard and Siebert (1967) for sheep grazing salt-bush which provided relatively high levels of salt in the food. In the present study, the faster turn-over rates in the sheep and goats resulted from the extra water intake and the extra water losses through urination, defaecation and evaporation (insensible loss). However, faecal water loss from the goats was not significantly altered by salt loads. With the high level of salt load, the water losses (urination, defaecation and evaporation) from the sheep were markedly higher than those from the goats. This together with their lower water intake led to a higher WTOR in the sheep.

The relative importance of each route (% WTOR), however, indicated that urinary loss from the sheep and goats increased with salt loads, while faecal and insensible losses decreased. Accordingly, the urinary loss was the main route of elimination of excessive salt.

The differences between sheep and goats in the proportions of water lost through faecal, urinary or insensible routes were quite small although significantly different. Concerning faecal water loss, Maloiy and Taylor (1971) showed that at 22° C and when water was available *ad libitum* both sheep and goats lost about 20% of their total water loss through their faeces. Faecal water loss expressed as ml/kg $W^{0.82}$/d was lower in the goats than the sheep, but this was mostly a reflection of the lower intake in the goats (Table 3.1)

The increases in the estimated insensible water losses (ml/kg $W^{0.82}$/d) from both the sheep and goats (Table 3.3) were probably the result of a calorigenic effect of salt. Arieli, Naim, Benyamin and Pasternak (1989) showed that an additional intake of 1 g of salt by sheep fed salt bush or a salt treated ration lead to an increase in heat production of 5 kJ. This was caused mainly by extra heat production from the digestive tract and kidneys.

Sodium and nitrogen balances

There are three routes for excreting salt, namely urine, faeces and sweat. Apparent digestibility of sodium was increased by salt loads which was shown by the decreased proportion of the sodium intake in the faeces. Michell and Moss (1987) suggested that, on 'sodium intake above 60 mmol/d, urinary excretion becomes predominant, but below 30 mmol/d sodium excretion was primarily faecal'. Similarly, McSweeney, Cross, Wholohan and Murphy (1988) found that faecal and urinary Na excretion were non-linearly increased with increased Na intake, and up to the level of 0.75 g/d Na intake daily (32.6 mmol/d) the faecal Na excretion was higher than the urinary excretion. In the present experiment, the control animals consumed 70 mmol sodium daily.

Positive balances of sodium in both the sheep and goats were measured. In addition to cumulative errors in measurement, there is the possibility of the excretion of some excess of absorbed sodium through the skin. Stacy, Brook and Short (1963) showed a high correlation between sweating and the presence of salt on the skin of the sheep; a result which implies that suint, a water soluble component of fleece, was derived from sweat glands. Suint was produced at rate 0.5 - 1 g daily (Stacy *et al.* 1963) which

contained 1.3 % Na and 26 % K (Farnworth, 1956). Pierce *et al.* (1967) suggested that more NaCl was lost in the suint from sheep accustomed to 1.3 % saline water than when offered fresh water.

The decreases in urinary N excretions (mmol/kg $W^{0.75}$/d) (Table 3.4) were mainly due to the lower intake of nitrogen by the animals, and lower digestibility of nitrogen in the sheep with salt loads. However, the sheep and goats showed a similar pattern in the urinary excretion of nitrogen relative to the digestible nitrogen (% digestible N), which was increased by salt loads. This then lead to a decreased nitrogen retention. Godwin and Williams (1984) revealed that the generally increased electrolyte content of high protein roughage diet aids urinary urea excretion by increasing GFR, and urine flow rate by an osmotic diuretic effect. Infusing sodium chloride intraruminally results in an increased urea and total urinary N excretion (Godwin and William, 1984).

In a number of experiments of sodium chloride supplementation to sheep (Nelson, MacVicar, Archer and Meiske, 1955; Davlin and Robert, 1963; Moseley and Jones, 1974; Moseley, 1980) in which salt consumption varied from 150 to 450 mmol per d it was shown that a decreased nitrogen retention was associated with increased salt intake. Possibly, the decrease in nitrogen retention is attributable to differences in kidney function with lower circulating ADH due to the ANP mechanism mentioned previously. In the absence of ADH, collecting tubules are relatively impermeable to water and urea (Nora, Laiken and Fanastil, 1984). Therefore, it is clear from the results presented that salt loads decrease nitrogen retention by increasing urinary N excretion following natriuresis and diuresis.

In this experiment the goats maintained a slight positive nitrogen balance, but the sheep went into negative nitrogen balance with salt loading. The effect appears to be largely due to increases in both faecal and urinary losses of N in the sheep. The effect was similar to that noted by Godwin and Williams (1986) and is due largely to the increased throughput of digesta and the consequent reduction in N digestibility. Goats appear to have a lesser effect of salt on the turnover of foregut contents and hence are not

as greatly affected in terms of digestibility of N than sheep. The increased turnover of rumen contents is probably related to the increased water intake of the animals. This increased water intake of the sheep, particularly with the high salt intake, results in a greater urinary flow rate and wash out of N into the urine.

Clearly the results have shown that as hypothesised the responses of the sheep were similar to those found by Godwin and Williams, (1986). However, the responses of the goats were not of as greater magnitude as that of the sheep and remainded relatively similar between the medium and the high treatments.