REVIEW OF THE LITERATURE

Scope of the Review

The main aim of this literature review is to indicate some of the more recent advances that have occurred in the areas of rumen metabolism and renal physiology. A comprehensive review of the vast quantities of literature on these fields is impossible within the space constraints of this thesis. Consequently only those areas of study in which considerable advances have been made in recent years and of some relevance to this thesis have been detailed.

The Ruminant

Ruminant animals are from the Order *Artiodactyla* and the sub-Order *Ruminantia* . The word ruminant is derived from latin 'ruminare' meaning 'to chew cud'. Ruminants are thus characterised as animals which chew their cud or 'ruminate' at periodic intervals.

Ruminants also possess a characteristic gastrointestinal anatomy. There is an enlarged anterior portion, the reticulorumen, which provides for extensive pregastric fermentation. Microbiota present in the reticulo-rumen possess enzymes which are capable of hydrolysing β-1,4 linkages which bind glucose molecules to form cellulose. Natural ruminant diets are normally very high in cellulose content. The symbiotic relationship between the microbiota present in the rumen and the ruminant is essential for normal digestive function.

The Rumen Fermentation

The rumen environment is well adapted to microbial life. It is essentially an anaerobic ecosystem. The pH range of 5.5 to 7.0 is optimal for a large range of enzyme systems and the temperature is relatively constant at about 40°C. Food supply is fairly continuous and ruminal movements provide adequate mixing of nutrients. Microbiotic waste products are removed via absorption and subsequent passage out of the forestomachs, thus reducing possible inhibition of microbial growth by accumulated toxic waste products.

Carbohydrates

The majority of carbohydrate in natural ruminant feeds is in the form of polymers associated with plant fibres, mainly cellulose and hemicellulose. The rate of degradation of plant fibres depends mostly on the stage of maturity of the plant.

Although the dry matter yield of material may continue to increase with age, the nutritive value of most forage species decreases with maturity (Phillips *et al* 1954, Minson *et al* 1964).

Starch is present in many seeds and tubers and these generally only occur in quantity in ruminant diets under conditions of artificial feeding eg. feedlotting.

The first step of fermentation of the structural carbohydrates is usually the hydrolysis of the β-1,4 linked glucose or xylose units. Generally carbohydrates are fermented to volatile fatty acids (VFA), methane and CO_2. Both intracellular (Leatherwood 1973) and extracellular cellulases (Baldwin and Allison 1983) are found in rumen bacteria and fungi.

The role of protozoa in cellulose degradation is not clear, however atleast some species possess endocellulase (Coleman 1978).

Lignin has a very low digestibility and in many studies is regarded as not being digested at all (Fahey and Jung 1983). Lignin is intimately associated with structural carbohydrates (Himmelsbach and Barton 1980) and this association tends to reduce the microbial attack of plant cell walls (Barton and Akin 1977). Recent studies have revealed that phycomycetous fungi may help to degrade the lignified sclerenchyma of plants and enable further bacterial attack and physical breakdown of plant cell walls (Orpin and Letcher 1979, Bauchop 1981, Akin *et al* 1983).

The bacterial genera *Bacteroides, Ruminococcus, Butyrivibrio* and *Eubacterium* are the major cellulolytic bacteria found in the rumen.

The major route of hexose fermentation is via the Embden-Meyerhoff pathway. Pentoses are generally converted to hexoses and trioses via transketolase and transaldolase enzymes (Baldwin and Allison 1983) however Wallnofer *et al* (1966)

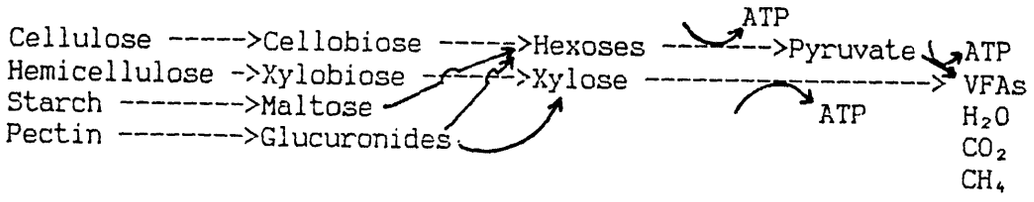


Fig.1. The major pathways of carbohydrate fermentation in the rumen.

suggested that pentose phosphate is converted to α-keto pentose phosphate and cleaved by phosphoketolase.

Pyruvate formed from the fermentation of 5 and 6 carbon sugars is converted to either propionate, lactate or acetate depending on ruminal conditions.

Lactic acid is not normally found in appreciable quantities in rumen fluid derived from animals fed roughage diets. This is explained by Hungate (1982) as being due to the sacrifice of a potential ATP molecule that its production would entail. As rapidly fermenting soluble carbohydrate is normally limiting in the rumen, there is intense microbial competition and an organism capable of producing the most ATP per unit hexose gives the most ATP/unit time. However when the sugar supply suddenly becomes ample eg. the introduction of a starchy grain diet, hexose is converted to lactate by rapidly developing lactacidigenic bacteria, which produce ATP faster than other predominant microbiota.

Methanogenic bacteria are poorly understood. The conversion of CO_2 and H_2 to form methane is of nutritional interest because inhibition of this process should lead to increased production of propionate. The ionophorous antibiotics, particularly monensin, have been used successfully to reduce the methane losses and to increase propionate production and feed conversion efficiency (Chalupa 1980).

In addition to the polysaccharides, plant fibres also contain proteins, fats, waxes, organic acids, silica, salts and lignin. The lignin content increases with plant age. For detailed analysis of plant fibres the reader is referred to Waite and Gorrod (1959), Bailey (1964) and Van Soest (1982).

Pasture grasses may vary in lipid content from 5-10 % of the total dry matter (Scott 1971). The major types of dietary lipids in pasture are mono- and di- galactosyldiacylglycerols (Weenink 1961). In cereal grains triglycerides predominate. These are readily hydrolysed in the rumen to form unsaturated fatty acids, which in turn are hydrogenated to form saturated and monoenoic fatty acids. Some protozoa and bacteria possess lipases and hydrogenases. Of the bacteria *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* tend to predominate (Hungate 1966).

N Metabolism

Much of the protein entering the rumen in the diet is subject to microbial degradation. The extent of degradation is generally related to the solubility of the protein in rumen fluid (Wohlt *et al* 1973).

The proteolytic activity in the rumen is mainly associated with the particulate fraction of rumen fluid (Blackburn and Hobson 1960) and is due to both bacteria and protozoa.

The degradation involves two steps. Firstly the protein chain is broken into peptides and amino acids by proteolysis. The amino acids are then subject to substantial deamination, such that free amino N levels in rumen fluid are very low (Allison 1970). The amino acids are generally broken down rather than incorporated intact into microbial cells (Chalupa 1976).

Although both protozoa and bacteria degrade protein the mechanisms are somewhat different. Bacteria hydrolyse the protein outside the cell by enzymes attached to the cell surface (Blackburn and Hobson 1960, Hogan 1976). The resulting peptides and amino acids are then transported intracellularly and the peptides hydrolysed further to amino acids and these amino acids are then either incorporated into bacterial protein or degraded further to VFAs, NH_3, CO_2 and CH_4 which are then released from the cell (Tamminga 1979).

Protozoa engulf both feed particles and bacteria (Coleman 1975), the protein from which may then be hydrolysed within the protozoal cell. Protozoa also play

a role in deamination of amino acids (Coleman 1980).

Most rumen microbes require ammonia for growth and the proteolysis and deamination of proteins provides the bacteria with NH_3, α- keto acids and ATP, all of which are generally beneficial to microbial growth. Many rumen bacteria also require intact amino acids for growth (Hungate 1966).

Non protein N also enters the rumen both in the diet and from endogenous sources (see section on the recycling of N to the reticulorumen). Plants may contain up to 10% of their total N as nucleic acids, which are degraded in the rumen to nucleotides, nucleosides and finally to purine and pyrimidine bases (McAllen and Smith 1973).

Nitrate, which commonly accumulates in plants fertilised with high levels of N fertilisers during overcast weather, is rapidly reduced to nitrite and then to NH_3 (Lewis 1951) in the rumen.

Urea is quantitatively the most important non protein N substance entering the rumen, even though its content in plant material is normally very low. Substantial amounts of endogenous urea enter the rumen, such that sheep may be maintained on N free diets if urea is administered intravenously (Boda *et al* 1976).

Urea is hydrolysed rapidly to NH_3 and CO_2 by bacterial ureases. Less than 0.5 % of rumen bacteria possess urease (Cook 1976), however bacteria adhering to the rumen wall are highly ureolytic with about 10 % of species found in this location possessing urease (Cheng and Costerton 1980).

Consequently sloughing of dead epithelial cells into the rumen fluid accounts for much of the ureolytic activity of the fluid (Mead and Jones 1981). It has been suggested that these adherent flora participate in the regulation of urea secretion across the rumen wall (Cheng and Wallace 1979).

Apart from degrading nitrogenous constituents of the diet and endogenous secretions the microbiota play a very important role in synthesizing amino acids and proteins which may be digested when the organisms pass from the rumen to the lower parts of the digestive tract. Sufficient microbial protein is produced

in the rumen to allow animals to survive on protein free diets and even produce milk yields approaching those of normally fed animals (Virtanen 1966).

As mentioned earlier NH_3 is a requirement for many rumen bacteria and specific amino acids may also be required by some bacteria, but in the mixed population of the whole rumen, one microorganism may produce the required amino acid for another. Incorporation of rumen fluid amino acids into bacteria can be substantial (Nolan 1975). Approximately 80 % of rumen isolates can utilize NH_3 as their sole N source (Bryant and Robinson 1963). NH_3 entering bacteria is captured by glutamic and aspartic acids to form the amide groups of glutamine and asparagine (Boggs 1959), which is followed by the synthesis of alanine and aspartate. Amino groups are then transferred to other carbon skeletons to form other amino acids (Smith 1979). To maximise protein production the available rumen ammonia must be balanced with biologically suitable energy sources.

The yield of microbial dry matter in grams per mole of ATP (Y(ATP)) made available from the energy source is however not necessarily constant. The Y(ATP) is generally higher for rapidly growing organisms as maintenance costs are minimal (Isaacson *et al* 1975). Variations in Y(ATP) of from 10 (Bauchop and Elsden 1960) to 25 (Isaacson *et al* 1975) have been recorded. The causes of this variation in yield are not clear, but alterations in rumen turnover rate (dilution rate) have dramatic effects, with faster rates having higher yields (Hobson 1965, Hobson and Summers 1967). Inclusion of fat into the diet has also been shown to give much higher microbial efficiency (Knight *et al* 1978). A lack of certain amino acids, particularly methionine and phenylalanine (Smith 1979), may also limit Y(ATP).

Baldwin and Allison (1983) suggest that much of the discrepancy in Y(ATP) can be accounted for by methodological errors. The most likely correct values for Y(ATP) are within the range of 10-12, whilst some individual microbes may have values as high as 15-23.

Nutritive Value of Microbial Protein

Loosli *et al* (1949) fed sheep a protein free diet with the only source of N being urea. They showed that all the essential amino acids required by non ruminants were contained in the rumen microbial biomass. The essential amino acid profile of microbial protein is not markedly affected by diet and has a relatively high biological value (Bergen *et al* 1967,1968). However the yield of microbial protein may not be sufficient to meet the essential amino acid needs of dairy cattle (Huber and Kung 1981).

The digestibility and net protein utilisation values for protozoa are higher than those for bacteria (Owens and Bergen 1983). However the amount of protozoal protein flowing from the rumen may only be relatively small, due to their apparent death and degradation in the rumen (Leng 1982).

The Rumen Ammonia Pool

Rumen NH_3 has several sources. It may be derived from the degradation of dietary protein and non protein N, and from hydrolysis of endogenous N sources recycled to the rumen, particularly urea. This recycling phenomena will be discussed in a later section.

The ammonia in the rumen has three possible sinks. 1. Microbial capture, 2. washout to the lower digestive tract and 3. absorption through the rumen wall.

Absorption of NH_3

Ammonia appears to be passively absorbed in the non-ionised form (Smith 1975), therefore the absorption tends to increase as the rumen fluid pH increases. The amount of absorption is primarily dependent on the non-ionised NH_3 concentration in the rumen fluid (Leng and Nolan 1984). However it has been suggested that a considerable portion of the absorption occurs in the omasum (Hogan and Weston 1967, Davidovitch *et al* 1977).

Most of the NH_3 absorbed from the gastrointestinal tract is synthesized to urea in the liver via the ornithine cycle. The activity and concentration of the

enzymes involved in this cycle are increased by feeding high protein diets (Payne and Morris 1969). The liver has a limited capacity to synthesise urea (Symonds *et al* 1981). Recent studies have suggested that pH and bicarbonate concentration of the body fluids are important regulators of urea synthesis (Atkinson and Camien 1982).

Recycling of N to the Reticulo-rumen

Urea is recycled to the reticulo-rumen both via saliva and directly across the rumen wall. The quantitative importance of these two routes has been somewhat controversial.

Weston and Hogan (1967) estimated that net endogenous urea transfer was approximately 5 g N/d from the rise in rumen NH_3 following intravenous urea infusion. They found no further increase in rumen NH_3 once plasma urea level had reached 180 mgN/L (6.6 mmol/L). Similar data was obtained by Thornton (1970a) but McIntyre (1971) found that the rumen ammonia levels continued to increase with intravenous urea input.

Measurement of urea return to the rumen of animals given lucerne hay, using isotopic methods, gave values of 1.2g N/d (Nolan and Leng 1972) and 1.1g (Nolan *et al* 1976). MacRae *et al* (1979) found 0.9 g and 1.1 g/d of N was returned for sheep fed *Agrostis-Festuca* and Heather diets respectively. Obara and Shimbayashi (1980) have figures of 1.1-1.8g N/d for two rations containing about 35 % low protein hay and 62 % high energy concentrate.

The mechanism of urea recycling across the rumen wall has been obscure. Gartner (1962) suggested that urea is returned to the rumen by active transport, but the explanation offered by Cheng and Wallace (1979), suggesting that the bacterial population adherent to the rumen wall is highly ureolytic and hence urea is hydrolysed on entry into the rumen, allowing a continual downhill transfer of urea, appears more plausible.

Non-urea N also enters the rumen with up to 10.8 g N of 'endogenous protein' input derived mostly from rumen epithelial sloughing (MacRae and Reeds 1979,

Kennedy and Milligan 1980) and salivary protein (Hogan 1975, Kennedy and Milligan 1980).

The Urea Pool and Plasma Urea Concentration

The entry rate of urea into the body urea pool is determined mostly by the N intake of the animal.

Harmeyer and Martens (1980) took data from the literature and found a highly significant linear relationship between N intake and urea entry rate.

The concentration of urea in plasma is positively related to N intake (Cocimano and Leng 1967, Ide 1975), however McIntyre (1970) found that plasma urea showed a plateauing at about 11 mmol/L (30 mgN/100mL) with higher N intakes.

The plasma urea concentration thus does not always reflect urea entry rate. For example, the rise in plasma urea following starvation of sheep is accompanied by a decline in urea entry rate (Harmeyer *et al* 1973a, 1973b).

Kennedy and Milligan (1978) showed that a reduction in food intake of 50 % caused no change in P_{urea} but reduced the urea entry rate by 39 %. Clearly factors other than P_{urea} concentration control the entry rate of urea. However P_{urea} is a relatively reliable indicator of N utilization of different forages (Egan and Kellaway 1971).

Increasing the N content of the diet generally increases the urea pool size and P_{urea}. Harmeyer and Martens (1980) have taken values from the literature and shown a significant linear relationship between P_{urea} and urea pool size.

The urea space is the volume of fluid which would contain the urea pool at the P_{urea} concentration. The urea space has been shown to be constant at approximately 80 % of body weight for a variety of dietary regimes (Gaertner 1961), however Cocimano and Leng (1967) showed a wide variation (20-60% BW) in urea space in animals fed a variety of rations. It appears that urea is excluded from cell water under some circumstances and included in others. During fasting urea is transported from muscle to blood probably by an active process (Havassy

et al 1973). Urea enters human red blood cells by a facilitated diffusion mechanism (Brahm 1982) and hence has a lower concentration in RBC water than in plasma water (Ralls 1943). However dehydration of sheep increases the RBC water urea concentration above that of plasma water (Havassy *et al* 1971). The urea space is also increased in mice with muscular dystrophy compared to control animals (Watanabe *et al* 1981).

Possible Sinks of Plasma Urea

Urea present in the plasma has two major sinks. It is either recycled to the digestive tract or is excreted in the urine.

Faecal losses of N are normally relatively constant, whilst urinary N losses, predominantly urea, vary greatly. If the urinary loss of urea could be diverted to the rumen in animals fed N limiting diets, it is possible that the production of the animal may increase through greater microbial protein production. This has been achieved surgically (Gillette 1967) by transplanting one ureter into the rumen. This procedure reduced the animals requirement for N quite substantially. Diversion of urea in a practical sense has not yet been achieved. Alteration of rumen permeability or increasing salivary flow rate chemically are probably the means by which this may be achieved in the future. Reducing urinary losses of urea, would make available a higher level of urea for return to the rumen. The experimental sections of this thesis detail experiments which were predominately designed to examine which factors determine the excretion of urea in the urine.

Body Fluid Compartments

The total body water of man is normally between 53 and 65 % of the body weight (Holmes *et al* 1956). However the value shows great variability in ruminants because of several factors. The gastrointestinal tract of ruminants may contain relatively large volumes of water, which, although regarded as being outside the body proper, is in continuous flux with the extracellular fluid. The body fat content of sheep also varies greatly, depending on nutritional status and hence the body water content per unit body weight also varies greatly. Panaretto (1963)

has shown that as the total body water content increases, fat content decreases and protein content increases.

The weight of wool carried by the sheep must also be taken into account when total body water measurements are expressed per unit body weight.

The measurement of total body water in ruminants is carried out using tritiated water as the marker of choice as it equilibrates totally with body water. Antipyrine often gives volumes of distribution greater than the weight of the animal (Dale *et al* 1956, Garrett *et al* 1959), whilst urea may show widely differing distribution spaces depending on dietary N intake (Cocimano and Leng 1967).

Values obtained for total body water range from 56 to 75 % of body weight (Macfarlane 1963, Macfarlane *et al* 1967).

The total body water consists of two major compartments; extracellular fluid and intracellular fluid. The extracellular fluid is further subdivided into plasma and interstitial fluid. Many substances have been used for extracellular fluid volume determination, however the true ECV cannot at present be determined, rather an estimate based on the distribution space of various markers is used. The most commonly used markers are as follows, ranked in ascending order of distribution space $Cr^{51}EDTA$, inulin, raffinose, sucrose, mannitol, thiosulphate, radiosulphate, thiocyanate, radiochloride and radiosodium (Ladegaard-Pedersen and Engell 1972, Pitts 1974).

The intracellular water volume is usually determined by difference between total body water and ECV.

The plasma composition and volume is directly controlled by the kidneys. Although it is customary to talk of ECV volume and composition control, it is the plasma volume and composition which is the critical function and directly controlled variable by the kidneys. Indeed ECV is only controlled through plasma changes and the shift of fluids between the interstitial and vascular compartments.

The compliance of the interstitial space is virtually identical to that of the vascular space and hence provided that the balance of Starling's forces at the capillary bed are unaltered, changes in plasma and ECV will be mutual (Gauer

et al 1970).

The Control of the Volume of Extracellular Fluid

In the extracellular fluid, 90 % of the osmotic pressure is due to Na salts, consequently changes in Na metabolism are probably the prime regulators of the ECV.

Changes in the absorption and excretion of water are generally secondary to changes in osmotic pressure and/or Na metabolism. However the control of ECV and Na metabolism can be separated, for example, in renal disease where Na loading increases the ECV and FE_{Na} is increased above that of equivalent Na loading and ECV expansion of normal subjects. This expression of volume regulation is termed the 'magnification phenomenon' (Bricker *et al* 1978).

The Effect of Increased Sodium Intake

An increased Na intake results in an increase in Na excretion such that Na balance becomes restored within a few days. During this restoration period there is a small positive Na balance, resulting in an isotonic increase in ECV. Conversely when Na intake is reduced there is a lag phase of a few days with a small negative Na balance and then a restoration of ECV at a lower level (Reinhardt and Behrenbeck 1967).

Similarly when mineralocorticoids are chronically administered there is an increase in ECV with a prompt natriuresis on cessation of the hormone treatment (Knox *et al* 1980).

Expansion of the ECV by an intravascular infusion of isotonic fluid results in a prompt increase in the excretion of Na and water (Knox and Haas 1982). During water immersion in man there is an increase in blood volume which is associated with an increase in the excretion of both Na and water (Epstein 1978).

How is the Extracellular Volume Monitored by the Body?

Distension receptors in the carotid sinus, aortic arch and in the atria of the heart monitor the blood volume and exert a reflex inhibitory tonus via the vagal

nerves on the release of ADH from the posterior pituitary gland (Gauer *et al* 1970, Goetz *et al* 1975).

In addition to a direct effect on ADH release, atrial distension also causes a sympathetic modulation of renal haemodynamics (Kahl *et al* 1974).

The control of body water balance depends upon the release of ADH from the neurohypophysis and also upon the ingestion of water driven by the thirst mechanism.

Sodium depletion and ECV contraction activate the renin-angiotensin-aldosterone system. Na depletion also elicits a specific appetite for sodium (Denton 1973).

Extracellular fluid Na balance is thus maintained by both neural and hormonal factors.

The Release of ADH from the Neurohypophysis

There has been much work examining the factors controlling the release of ADH. McKinley *et al* (1978) suggest that there are two major cerebral receptors that influence the rate of ADH secretion. The first is a classical osmoreceptor and is located in an area of the brain not associated with a blood-brain-barrier and the second is a Na detector that monitors cerebrospinal fluid Na concentration. For a detailed review of the factors controlling ADH release the reader is referred to Bie (1980).

The kidneys are probably the major organ controlling the composition of the body fluids. By directly filtering plasma at the renal glomerulus and through plasma exchange with the interstitial fluid compartments the composition of the extracellular fluid is closely monitored.

Glomerular Filtration

The notion that the kidney could be likened to a sieve was perceived well before the classic studies of Bowman and Ludwig in the 19th century. In fact, Malpighi wrote in 1666 *'The ancients conceived of the kidney as a sieve providing a means for separating the urine'* (Brenner 1977).

In mammals about 20-30 % of the large volume of blood plasma entering the renal glomerulus normally passes through the walls of the capillaries.

Using micropuncture techniques in a mutant strain of rats whose glomeruli lie close to the cortical surface, the composition of the glomerular filtrate has been found to be that of a nearly ideal ultrafiltrate, closely resembling plasma water with respect to low molecular weight solutes. For solutes with molecular weights greater than 5000, however the transport across the glomerular capillaries becomes restricted (Pitts 1974).

In mammals the glomerular filter consists of three layers.

1. A thin fenestrated endothelium,
2. a glomerular basement membrane and
3. an epithelial cell layer.

More recently, it has been demonstrated that the glomerulus also has charge selective properties, such that positively charged molecules are filtered with greater facility (Brenner 1978).

The Control of Glomerular Filtration Rate

The glomerular filtration rate of mammals is subject to several controlling variables.

The rate of ultrafiltrate formation is governed by the same driving forces governing fluid movement across other capillary membranes.

This may be expressed as

$$\begin{aligned} J_v &= K_f(\Delta P - \Delta\pi) \\ &= K_f[(P_{GC} - P_T) - (\pi_{GC} - \pi_T)] \end{aligned}$$

where J_v is the local rate of ultrafiltration,

K_f is the effective hydraulic permeability of the capillary wall,

ΔP is the transcapillary hydraulic pressure difference,

$\Delta\pi$ is the colloid osmotic pressure difference and

GC and T are the glomerular capillary and tubule respectively.

The protein concentration of the ultrafiltrate is so small that π_T is considered as equal to zero.

In 1972 Brenner *et al* presented data demonstrating that the colloid osmotic pressure rises along the length of the glomerular capillary (as one would expect with ultrafiltration occurring) to a value equal to the net hydrostatic pressure gradient, ΔP, from capillary to Bowman's space.

ie.

$$J_v = K_f(\Delta P - \pi_{GC})$$
$$\longrightarrow \Delta P - \pi_{GC} = 0$$

As a result of the fact that equilibrium of pressures was achieved within the length of the capillary, nephron filtration rate was highly dependent on changes in plasma flow. This phenomenon is known as 'filtration pressure equilibrium'.

However more recent studies in the hydropenic rat have revealed that there is no universal agreement on the finding of filtration pressure equilibrium (Blantz 1977). In those studies in which filtration pressure equilibrium was not observed the findings can be best explained by reductions in the glomerular permeability coefficient (K_f) in the experimental animals.

The presence or absence of filtration pressure equilibrium markedly influences the degree to which the respective determinants of GFR can affect the filtration rate.

A variety of studies have appeared over recent years that have directly assessed the effects of hormonal agents on filtration forces and permeability surface area properties of the glomerular capillary. Table 1 shows the effects of certain hormonal agents on K_f.

Probably the most studied of these hormones is angiotensin II. It causes noted reductions in renal plasma flow because of both afferent and efferent arteriolar resistance increases and a large reduction in the glomerular permeability coefficient (K_f). Angiotensin II specific receptors within the glomerulus that cause contraction of mesangial cells have been demonstrated (Ausiello *et al* 1980). So

Table 1. The effects of various humoral agents on glomerular function

Hormone	*Glomerular Effects*
Angiotensin II	$\uparrow K_f \downarrow$ERPF $\uparrow \Delta P$
Prostaglandins E_1,I_2	$\uparrow K_f \uparrow$ ERPF
ADH	$\uparrow K_f \pm$ERPF
Acetylcholine	$\uparrow K_f \uparrow$ ERPF
Bradykinin	$\uparrow K_f \uparrow$ ERPF
PTH	$\uparrow K_f$ —
Histamine	$\uparrow K_f \uparrow$ ERPF

(adapted from Blantz 1980)

the mechanism of angiotensin reduction of K_f may be as follows:

1. Capillary constriction causing decreased surface area,
2. Decreased capillary wall permeability to water and small solutes,
3. Diversion of glomerular flow to shorter capillary segments.

The effects of all the above hormones, except for ADH, can be prevented by giving saralasin, an angiotensin antagonist, suggesting that most hormones affecting glomerular permeability act via the action of angiotensin II on K_f.

Tubuloglomerular Feedback

Changes in systemic arterial blood pressure cause little effect on the GFR, because of the phenomenon known as tubuloglomerular or distal tubular feedback.

It has been known for some time that each distal tubule is closely apposed to the vascular pole of the glomerulus of the same nephron.

The distal tubular feedback hypothesis proposes that a structure in the distal tubule, presumably the juxtaglomerular apparatus or macula densa area, is capable of sensing a physico-chemical component of the fluid emerging from the ascending loop of Henlé and initiating a sequence of events that culminates in the local adjustment of vascular resistance and glomerular filtration rate. The negative feedback function subserved by this system requires that increases in

flow into the distal tubule lead to decreases in GFR.

The precise nature of the intraluminal component in the distal tubular fluid that is monitored is at present uncertain.

Early studies suggested sodium (Thurau 1964), then in the late 1970s after the demonstration of active chloride transport from the ascending thick limb of Henlé (Burg and Green 1973), chloride became a strong contender (Schnermann et al 1976). However during perfusion of tubules with chloride free solutions feedback responses could still be elicited (Bell *et al* 1981). One study showed that urea or mannitol microperfusion can cause decreases in GFR. Some studies suggest that transmembrane potential may play a role, but the bulk of the evidence still rests on Na^+ and/or Cl^- delivery.

The mediator which links the macula densa to the glomerulus has also been a contentious issue. The juxtaglomerular apparatus renin content is directly related to the NaCl delivery to the distal tubule, however renin secretion rate is not related to the distal tubular NaCl concentration (Wright and Briggs 1979).

Atleast one important mechanism regulating renin release operates through some type of macula densa feedback mechanism. Since the release of renin leads to the formation of angiotensin II, some investigators have attempted to integrate the feedback mechanism for the control of GFR with the feedback mechanism for the control of renin release.

Burghardt and his associates (1982) have proposed that since the endothelial cells of the glomerular vasculature contain angiotensin converting enzyme, the release of renin in response to increased delivery of NaCl would result in the subsequent local formation of angiotensin II.

Another approach has revealed that the renin concentration in blood taken from efferent arterioles was lower than in the blood from the renal artery or renal vein. It was suggested that renin was released into the tissues where it may act directly on the vascular elements.

Most of the studies on the feedback phenomena support angiotensin II as the mediating agent. Those studies which don't support angiotensin II were

only concerned with changes in vasoconstriction and have not allowed for the glomerular angiotensin II receptors which alter the glomerular permeability.

Nervous Activity and GFR

In normal man and dogs the renal nerves have little influence on the GFR. Artificial stimulation of the renal nerves produces substantial decreases in GFR and renal blood flow, with an increase in filtration fraction, indicating participation of efferent arteriolar constriction. After maintained stimulation for 2-4 days both the GFR and the renal blood flow returned to normal (DiBona 1977). Sympathetic stimulation using adrenalin produces constriction of the efferent arterioles, with larger amounts causing constriction of both afferent and efferent vessels.

Wise and Ganong (1972) stimulated 15 points in the medulla oblongata of dogs with one innervated and one denervated kidney. A substantial decrease in GFR was noted only in the innervated kidney.

The Intrarenal Distribution of GFR

It is well known that the kidney is not a population of homogeneous nephrons, but rather consists of various subgroups of nephrons depending on species.

Most of the data obtained on SNGFR is applicable specifically to the superficial cortical nephrons and need not necessarily relate to the whole kidney population.

It has been demonstrated in both rats and dogs that SNGFR of the outer cortical nephrons is lower than the inner cortical nephrons (Lameire *et al* 1977). Reddy *et al* (1981) using radioactive microspheres have shown that the outer cortex of the sheep kidney has a substantially higher blood flow than the inner cortex, although this difference was attributed to the size and density of glomeruli in these two regions.

Early work cited by Lameire *et al* (1977) suggested that a true redistribution of SNGFR in response to high levels of dietary salt occurs, such that the superficial SNGFR increase and the deeper nephrons decrease. However later studies

have shown that the redistribution is simply a proportionately greater increase in superficial SNGFR compared to juxtamedullary SNGFR. Conversely sodium restriction has the opposite effect.

Proximal Tubule

The proximal tubule may be divided into two segments; a convoluted portion and a straight portion (pars recta).

The superficial nephrons have longer pars recta and shorter convoluted portions than the deep nephrons (Jacobson and Seldin 1977). Tubular cell morphology also changes along the proximal tubule, such that it may be further subdivided on cell size, staining characteristics, brush border depth and organelle diversity (Maunsbach 1964, Maunsbach 1966, Tisher *et al* 1969).

Because of their greater accessibility the superficial proximal convolutions have been studied more than the pars recta and/or juxtamedullary nephrons.

The proximal tubules normally reabsorb about 50-60% of the glomerular filtrate, with the convoluted portions having a much greater net volume reabsorption than the pars recta (Schafer *et al* 1974, Kawamura *et al* 1975).

The tubular epithelia consists of a simple cuboidal epithelium with an apical brush border of microvilli. The cells in the pars convoluta interdigitate extensively whereas the cells in the pars recta interdigitate much less (Foote and Grafflin 1942, Maunsbach 1973).

The proximal tubular epithelium has a very low electrical resistance and its permeability to Na^+, K^+ and Cl^- is much higher than that of the peritubular cell membranes. The major determinants of the epithelial permeability are the paracellular pathways (Fromter *et al* 1971); see Fig.2.

Procedures which alter the ECV generally alter the permeability of the proximal tubule by changing the extent of the paracellular movement of ions. This is shown in Fig.3.

During ECV expansion the paracellular space is dilated and the tight junctions widened (Bentzel 1972). When the tubular lumen is made hyperosmotic

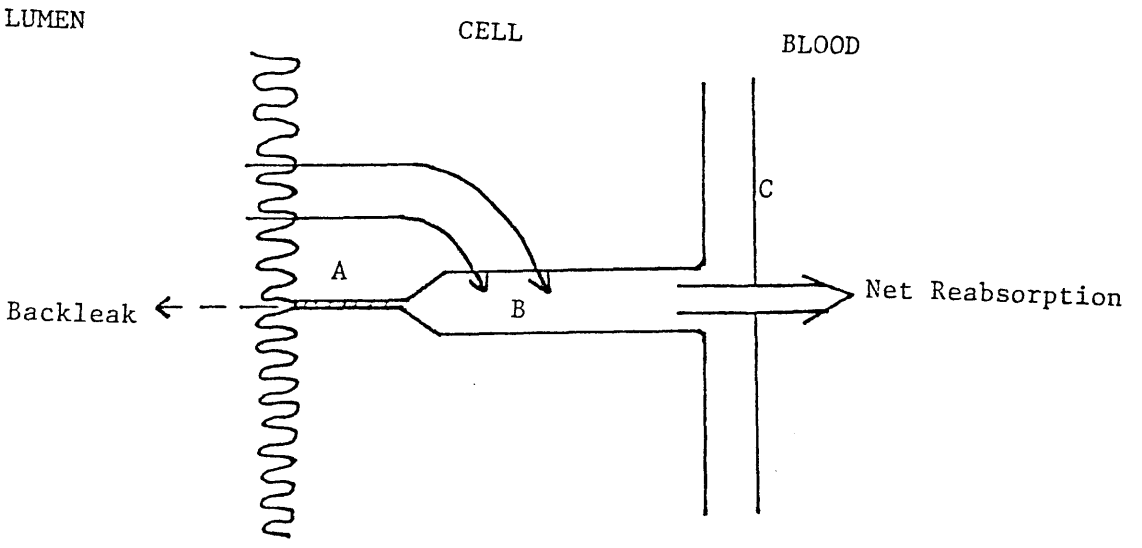


Fig.2. Model representing the role of the paracellular pathway in reabsorption in the proximal tubule. A – *refers to the tight junction between cells,* B – *refers to the intercellular space and* C – *is the basement membrane. Arrows indicate the movement of solutes and water.*

with urea or mannitol the paracellular pathways and the tight junctions also widen (Rawlins *et al* 1975).

The reabsorption from the proximal tubule is iso-osmotic and isonatric (Walker *et al* 1941, Berry and Rector 1980) ie., the fluid remaining at the end of the pars recta is isotonic although greatly reduced in volume.

The movement of Na^+ into the paracellular space consists of both active and passive components. The precise mechanism(s) of NaCl reabsorption by the proximal tubule have not been completely defined.

Na^+ is actively transported across the proximal tubule epithelium by the operation of Na-K-ATPase which is located in the basal lateral membranes. However Na^+ ions must firstly gain access to this enzyme.

About 50% of the Na^+ reabsorbed by the kidney is transported by a process involving Na-K-ATPase, about 20% is related to the reabsorption of HCO_3 and

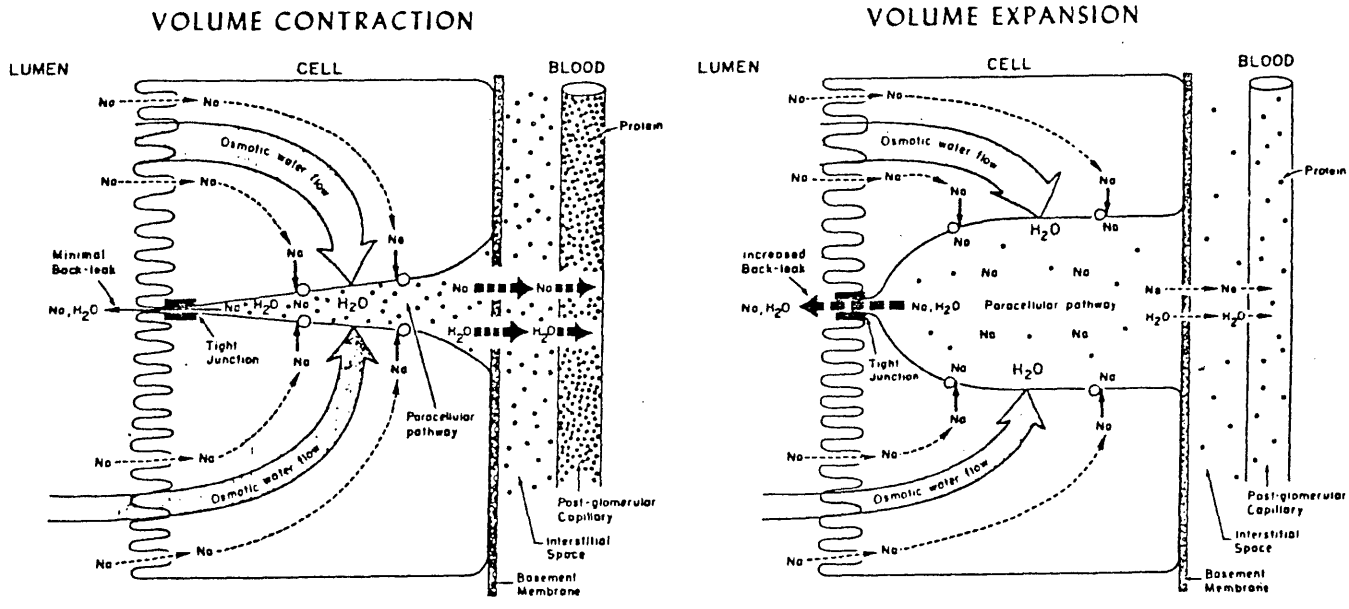


Fig.3. Schematic model of how peritubular protein concentration controls proximal tubular reabsorption, by controlling backleak. (Reproduced from Jacobsen and Seldin 1977).

the remainder by a temperature sensitive mechanism also requiring metabolic energy (Besaarab *et al* 1976).

In the initial portion of the proximal tubule there is preferential reabsorption of organic solutes and the sodium salts of bicarbonate, acetate, phosphate, citrate and lactate. These compounds are transported into the cells by specific carrier complexes which combine with Na^+ ions. The Na^+ concentration of the tubular cells is then controlled by the Na-K-ATPase located on the basolateral membrane. The organic solutes diffuse out of the cells across the basolateral membrane or are metabolised.

This co-transport of Na^+ involves three types. a) With neutral organic solutes such as some amino acids and sugars. This transport generates a lumen negative electrical potential difference (Burg *et al* 1976). Thus Na^+ may then diffuse back into the lumen or Cl^- may diffuse into the cell. b) As sodium salts of phosphate, acetate, citrate and lactate. This is considered a neutral process.

c) The Na^+-H^+ antiport. A direct Na^+-H^+ ion exchange occurs across the brush border (Murer *et al* 1976).

The active absorption of organic solutes, including amino acids and sugars, from the early proximal tubule renders the later sections of this tubule with a higher concentration of NaCl than plasma.

In this segment Na^+ is absorbed passively across the luminal membrane and then pumped out through the basolateral membrane by Na-K-ATPase (Kawamura *et al* 1975).

Rector *et al* (1966) proposed that passive reabsorption of NaCl occurs in the rat kidney and subsequent work has shown that about 1/3 of the Na^+ reabsorption is passive, probably of a diffusive nature via the paracellular pathway (Andreoli *et al* 1979).

Descending Loop of Henlé

The pars recta gradually becomes the thin descending limb of Henlé at the junction of the inner and outer stripe of the outer medulla.

The ultrastructure of the thin limbs of Henlé differ markedly from other segments. The cells are thin and virtually devoid of mitochondria (Jamison and Kriz 1982).

Short loop epithelia show cellular interdigitation and very shallow intercellular occluding junctions. Long loops in the outer medulla have a similar epithelium to the short loops except for the presence of microvilli. In the inner medulla the long loops exhibit a transition epithelia with no interdigitation and with intercellular tight junctions with long apical-basal lengths (Schwartz and Venkatachalam 1974).

The functional significance of these differences is unknown (Jamison and Kriz 1982).

Not all segments of the descending limb of Henlé have been studied, but those which have are permeable to water and virtually impermeable to Na^+ and urea (Kokko 1970). It has been suggested that the permeability to water is pressure

sensitive (Stoner and Roch-Ramel 1979). However in their study, perfusion reservoir height and not luminal pressure was measured. The movement of solutes out of the descending thin limb is passive and the epithelia appears to be incapable of active transport.

Ascending Thin Limb of Henlé's Loop

The diameters of the ascending thin limb are larger than those of the descending thin limb (Koepsell *et al* 1972). The epithelium is characterised by very flat but strikingly interdigitating cells (Schwartz *et al* 1979, Schiller *et al* 1980). The paracellular pathway consists of shallow tight junctions and wide intercellular spaces. There are a few scattered microvilli and as in the descending thin limb there are few mitochondria. Evidence for active transport in the ascending thin limb is lacking.

One of the most important questions regarding the role of the ascending thin limb is whether it actively transports Na^+ and/or Cl^-. All attempts to show active transport from this segment have failed (Kokko 1982).

This segment is impermeable to water (Imai and Kokko 1974, Imai 1977), highly permeable to Na^+ and Cl^- and moderately permeable to urea (Imai and Kokko 1974). Na and urea are transported across this segment by simple diffusion. However Cl^- appears to be transported by two passive mechanisms : simple diffusion and carrier mediated facilitated diffusion (Imai and Kokko 1976).

Thick Ascending Limb of Henlé's Loop

The thick ascending limb of Henlé is divided into two parts, cortical and medullary. The thin ascending limb transforms abruptly into the thick ascending limb at or near the inner and outer medullary border. The cells are relatively tall (10μm) and interdigitating cell processes are filled with large mitochondria. The cortical thick ascending limb has lower epithelial cells with less interdigitation and fewer mitochondria (Kaissling and Kriz 1979). There are some microvilli (Allen and Tisher 1976).

Fluid that enters the distal tubule is markedly hypoosmotic (Gottschalk and Mylle 1959). The thick ascending limb's transport characteristics are fundamental to both the concentration and dilution of urine.

The thick ascending limb of Henlé is virtually impermeable to water. The permeability to Na^+ is relatively high, but decreases cortically. The permeability to Cl^- is very low. There is a strong lumen positive potential difference which is due to the active transport of Cl^- from this segment (Burg and Green 1973). More recent studies have shown that the transport of Cl^- is electroneutral (see discussion on sodium excretion, page 37).

There is also much ADH responsive adenyl cyclase in this segment (Imbert *et al* 1975). Parathyroid sensitive adenyl cyclase is also present.

Distal Convoluted Tubule

Regardless of the osmolality of the final urine, the fluid entering the distal tubule is always hypo-osmotic to plasma (Gottschalk and Mylle 1959).

The epithelium consists of heavily interdigitating cells. The apical surface possesses a dense border of microvilli (Kaissling and Kriz 1979).

The osmotic water permeability of this segment is virtually zero, with or without ADH (Gross *et al* 1975), although Ullrich *et al* (1964) found a four-fold increase in hydraulic conductivity (Lp) with the addition of ADH in vivo. Because of this relative impermeability, isotonicity is not regained in some species as the fluid traverses the tubule (Bennett *et al* 1967, 1968).

Only about 10% of the filtered load of Na^+ is reabsorbed in this segment (Giebisch and Windhager 1973). The transepithelial voltage becomes progressively lumen negative with distance along the distal convoluted tubule (Wright 1971). This negative voltage drives the passive reabsorption of Cl^- in this segment, although some active Cl^- reabsorption probably occurs (Giebisch and Windhager 1973).

Connecting Tubule

The transition from the distal convoluted tubule to the connecting tubule is

relatively distinct (Kaissling and Kriz 1979). The tubular cells are simple polygonous structures with no interdigitation. The basal cell membrane has numerous infoldings through which mitochondria are randomly scattered. There are some microvilli. There are two cell types; connecting tubule cells and intercalated cells.

In this segment adenyl cyclase is sensitive to parathyroid hormone and isoproterenol, whereas the distal convoluted tubule adenyl cyclase is sensitive only to calcitonin. In both segments the enzyme is insensitive to ADH (Charbardes *et al* 1975).

Collecting Tubule

The epithelium shows a gradual change along its course from the cortex to the papilla. It is composed of two types of cells. The principal cell, with few microvilli and the intercalated cell with prominent microvilli (Andrews and Porter 1974, Bulger *et al* 1975). In the cortex there are about twice as many principal cells as intercalated cells. In the outer medulla the proportion of these two cells is about the same, whilst in the inner medulla only principal cells exist (Kaissling and Kriz 1979). Both of these cells are simple polygonous structures which show no interdigitation. The tight junctions are deep and impermeable to lanthanum, except in the inner medulla (Tisher and Yarger 1973).

The principal cells are responsive to ADH (Jamison and Kriz 1982). When ADH is present, with considerable osmotic water flow, the epithelial cells swell and bulge into the lumen and the intercellular spaces become markedly widened (Ganote *et al* 1968, Grantham *et al* 1969).

The Renal Pelvis

For many years the pelvis has been regarded merely as an expanded upper end of the ureter. However Gertz *et al* (1966) showed that the pelvis certainly has a physiological role atleast in urea excretion.

The renal pelvis of the sheep is characterised by extensions or fornices that penetrate the outer zone of the outer medulla (Pfeiffer 1968). The pelvic epithelium therefore covers sections of the kidney from the cortex to the medulla.

This epithelium is characterised by a transition from that of the papillary collecting duct to that of the stratified squamous epithelium of the ureter. The epithelium of the pelvis covering the renal parenchyma is 3-4 cell layers thick and becomes thinner cortically until it becomes a single cell thick. The number of microvillous lining cells is greater in the inner and outer stripe regions of the outer medulla. The inner medulla is entirely covered with this cell type (Verani and Bulger 1982). The presence of microvilli on an epithelium generally suggests some absorptive function.

Ureter and Bladder

Although generally considered just as urinary conduits, with no physiological function other than an aqueduct and storage role, the ureters and bladder have been shown to have some absorptive function.

Levinsky and Berliner (1959) showed that the ureter has some water permeability that is not stimulated by ADH. Facilitated diffusion of urea has also been demonstrated by the ureteral epithelium of sheep (Carruthers *et al* 1980). The ureters probably play their major role in absorptive function by causing back flushing of urine (Reinking and Schmidt-Nielsen 1981) resulting from their inherent peristaltic activity. This peristaltic activity appears to be particularly active in sheep (Tabatabai *et al* 1980). The absorptive function of the bladder of sheep has been studied by McIntyre and Williams (1969). They found that the bladder is unlikely to play a physiological role in urea, water or Na homeostasis. However Shukla and Tripathi (1978) have shown that the permeability of the bladder of goats to water, urea and glucose is relatively great.

The Renal Concentrating Mechanism

The mechanism whereby urine which is greatly hypertonic to plasma is produced has been the subject of much research and a comprehensive monograph on this subject has recently appeared (Jamison and Kriz 1982).

It has long been known that the proportion of the renal medulla compared to other sections of the kidney is directly related to the concentrating ability of the

kidney. Thus there is a good correlation between relative medullary thickness and the maximum urinary osmolality obtainable in a variety of mammals (Schmidt-Nielsen and Odell 1961). A more recent index, relative medullary area, which takes into consideration the relative area occupied by the medulla in a mid sagittal section of the kidney, appears to give a better correlation with maximum urinary osmolality (Brownfield and Wunder 1976).

Both of these indices are to some extent an index of the relative length and number of loops of Henlé. Early observations by Kuhn and his associates (Kuhn and Ryffel 1942, Wirz *et al* 1951) led to the association of the countercurrent arrangement of the renal tubules in the medulla and the renal concentrating process.

The active reabsorption of NaCl from the water impermeable ascending limb of Henlé was thought to create an osmotic gradient which is multiplied by countercurrent flow. However the fact that the ascending thin limbs of Henlé possess virtually no mitochondria (Schiller *et al* 1980) would preclude this segment from active reabsorption.

Because of this problem, Kokko and Rector (1972) proposed a concentrating mechanism which allows countercurrent multiplication without active transport in the inner medullary loop of Henlé. This study has remained as the basis for most subsequent modelling attempts and to date has not been substantially altered.

The Kokko and Rector Model

The key to this model is that the active reabsorption of Cl^- by the thick ascending limb of Henlé provides all the energy to drive the countercurrent multiplier. This active reabsorption of Cl^- and passive reabsorption of Na^+ (now thought to be Na^+ K^+ $2Cl^-$ cotransport, see section on sodium excretion, page 37) raises the interstitial salt concentration of the outer medulla.

This hypertonic interstitium abstracts water from the connecting tubule and the cortical and outer medullary collecting tubules in the presence of ADH. This

descending limb of Henle	- water permeable - solute impermeable
ascending thin limb of Henle	- water impermeable - NaCl permeable - permeable to urea (not as great as permeability to NaCl)
ascending thick limb of Henle	- impermeable to Cl^- - permeable to Na^+ - water impermeable - urea impermeable
distal tubule	- permeable to water - impermeable to urea
cortical collecting tubule outer medullary collecting tubule	- permeable to water - impermeable to NaCl - impermeable to urea
inner medullary collecting tubule	- permeable to urea - permeable to water

Table 2. The requisite permeability properties of the nephron segments (in the presence of ADH) for the Kokko and Rector model.

water removal raises the concentration of urea in the collecting tubules, which is then reabsorbed down a concentration gradient into the papillary interstitium. This urea then abstracts water from the descending limb of Henlé.

NaCl is the major solute in the fluid entering the descending limb of Henlé whereas interstitial fluid contains both urea and NaCl in appreciable quantities. The removal of water from the descending limb thus raises the concentration of NaCl in the tubular lumen and consequently upon negotiating the hairpin bend the NaCl will diffuse out passively, faster than urea will diffuse into the lumen. NaCl has thus been added to the interstitium in excess of water.

This hypertonic papillary interstitium osmotically draws water from the papillary collecting ducts, concentrating the non-urea solutes in the urine.

Most in vitro studies, using rabbits and rats, of the permeability characteristics of the loop of Henlé are consistent with the model, although the results of Morgan and Berliner (1968), Stoner and Roch-Ramel (1979) and data from Psammomys and hamster generally don't support the model (Jamison and Kriz

1982).

Foster and Jacquez (1978) computerised Kokko and Rector's model using transport coefficients, flow rates and solute concentrations from the literature and found that the model would not concentrate solutes in the inner medulla without active transport from the thin ascending limb of Henlé.

Stephenson (1972, 1978), using nonequilibrium thermodynamics, has shown that it is theoretically possible to concentrate fluid in the collecting tubule, given the right combination of permeabilities and solute concentrations, without active transport from the thin ascending limb of Henlé.

It is for this reason that the model is still regarded as the basis for further research.

Some recent findings have suggested that segments distal to the collecting ducts may be important in the urinary concentrating mechanism.

During antidiuresis the renal papilla is hypertonic due to the accumulation of NaCl and urea. The NaCl comes from the loop of Henlé. The collecting duct permeability to urea is not sufficiently high to account for all the urea found in the papilla.

Early work suggested that urea may be actively reabsorbed by the collecting ducts (Schmidt-Nielsen 1958, Truniger and Schmidt-Nielsen 1964, Ullrich *et al* 1970) because of lower levels of urea in urine than in papillary interstitial tissue.

Gertz *et al* (1966) showed that the urea concentration and the osmolality of the renal papilla decreases when the pelvis is opened and the papilla is no longer bathed in urine. Removal of the ureter also reduces concentrating ability (Schutz and Schnermann 1972). Schmidt-Nielsen *et al* (1980) have shown that during rising urine flow pelvic refluxes of urine occur which would facilitate washout of solute from the papilla during diuresis. The ureter and pelvis show peristaltic activity which is unaffected by urine flow rate (Reinking and Schmidt-Nielsen 1981).

As the pelvis contracts blood flow in the vasa recta temporarily stops, decreasing the solute removal from the papilla. Following the wave of peristalsis,

blood flow resumes, but the collecting tubule remains empty for a period of time, although flow continues in the ascending limbs of Henlé (Reinking and Schmidt-Nielsen 1981).

These effects on the vasa recta and collecting tubule according to Stephenson's (1972) theory of mass balance would enhance urinary concentrating ability because there is effectively less removal of solute from the medulla, and the urine flow at the papillary tip is effectively zero.

In the sheep ureteral peristalsis is characterised by a high rate and range of contractions with a higher intraluminal pressure than other species (Tabatabai *et al* 1980). Paralysis of the ureter without excision gives a reduction in concentrating ability, but not as great as that caused by excision alone (Jamison and Kriz 1982).

Other sources of papillary urea may be from red blood cells in the vasa recta. Urea may be released during osmotic shrinkage of the cells as they descend the vasa recta, faster than they gain it during cell expansion in the ascending vasa recta, thus resulting in a net gain of urea in the medulla (Jamison and Kriz 1982). Sheep that have been fasted and dehydrated have red blood cells that are higher in urea concentration than plasma, whilst the reverse is true for sheep fasted but given water ad libitum (Havassy *et al* 1971).

Urea Excretion

Early studies in man suggested that urea clearance is determined primarily by the urine flow rate (Austin *et al* 1921). In this study urine flow rate was decreased by witholding drinking water and increased by drinking large amounts of water or hypotonic saline. The clearance of urea increased linearly with the UFR until the UFR reached about 4 mL/min and then the P_{urea} plateaued. However Shannon (1936) using dogs that were dehydrated or water loaded showed that the U_{urea} increased continuously with the UFR. The FE_{urea} also increased with increasing fractional water excretion.

Many subsequent studies have confirmed these data and it was suggested

that urea was thus reabsorbed by passive means only, even when P_{urea} concentration was very high (Mudge *et al* 1949). Shannon's data were for animals in a steady state of hydration. He found that when UFR was rising after oral water loading there was a large increase in FE_{urea} which he termed 'exhaltation'. This phenomenon is caused by the rapid washout of medullary interstitial urea (Ullrich and Jarausch 1956, Atherton *et al* 1968). Conversely it has been shown that during falling urine flow rates a sudden decrease in FE_{urea} occurs called 'abatement' (Schmidt-Nielsen *et al* 1958).

These early experiments led investigators to believe that urea excretion was simply the net result of glomerular filtration and passive back diffusion of a portion of this filtered urea.

However Read (1925) found extremely low levels of urea in pregnant camel urine and many years later Schmidt-Nielsen and her associates demonstrated that several mammalian species, including ruminants, were able to alter urea excretion independent of changes in GFR or UFR in response to changes in dietary protein intake (Schmidt-Nielsen 1958). This finding indicated that some control of urea excretion must reside within the tubules. Later studies demonstrated that several mammalian species are capable of achieving a concentration of urea in medullary tissue that is higher than the final urine (Schmidt-Nielsen and Odell 1959, Bray and Preston 1961, Truniger and Schmidt-Nielsen 1964, Goldberg *et al* 1967). This led to the speculation that urea is actively reabsorbed at some site in the kidney tubules (Schmidt-Nielsen 1958).

Many studies published since the work of Schmidt-Nielsen (1958) have lent support to the theory of active urea reabsorption.

Firstly it was established that in amphibia active urea secretion occurs (Marshall and Crane 1925, Marshall 1932, Forster 1954, Schmidt-Nielsen and Forster 1954, Lore and Lifson 1958, Schmidt-Nielsen and Shranger 1963) primarily in the proximal and distal tubules (Long 1970). In elasmobranchs, active reabsorption of urea occurs (Smith 1936, Schmidt-Nielsen and Rabinowitz 1964, Forster 1970) however the countercurrent arrangement of the proximal and distal tubules

in elasmobranchs may possibly explain the apparent uphill transport of urea by passive diffusion (Mudge *et al* 1973).

Using micropuncture it was demonstrated that in protein deprived rats, the urea concentration at a given point in the collecting ducts is lower than at the same level in the vasa recta (Lassiter *et al* 1966, Ullrich *et al* 1967). The vasa recta concentration was assumed to reflect the concentration in the adjacent interstitium.

Renal cortical tissue has also been found to contain a higher urea concentration than the cortical tubular fluid (Roch-Ramel *et al* 1967, 1968). However this high urea concentration has been attributed to sequestration of urea in the cortical tissue, hence rendering it osmotically inactive, rather than accumulation due to active transport (Roch-Ramel *et al* 1970).

At similar urine flow rates, the proportion of injected urea that is excreted is much lower for rats fed low protein diets than those fed normal or high protein diets (Danielson *et al* 1970). A similar effect has been shown in sheep (Havassy *et al* 1974).

The uphill transport of urea in the dog kidney is inhibited by the inhibition of glycolysis with iodoacetate, and shows competitive inhibition with acetamide (Goldberg *et al* 1967), a urea analogue which can also be concentrated in the papillary tissue above the urine concentration (Truniger and Schmidt-Nielsen 1964).

A unique pattern of medullary urea accumulation was observed by both Schmidt-Nielsen and Odell (1959) and Rabinowitz *et al* (1973) in hydropenic sheep fed low protein diets. The pattern consisted of an increasing intrarenal tissue concentration from the cortex to the outer portion of the inner medulla, where the highest urea concentration was observed. The concentration was substantially higher than that of the final urine. In animals fed high protein diets the renal tissue concentration increased linearly from cortex to inner medulla.

Ethacrynic acid diuresis completely abolished these tissue gradients, suggesting that active urea reabsorption does not exist when water is not being

reabsorbed.

No tubular maximum for urea (a necessary condition for proof of active transport), has been achieved, despite tubular fluid concentrations of urea of up to 800 mmol/L (Mudge *et al* 1949, Schmidt-Nielsen 1970).

Active tubular secretion of urea has been demonstrated in the pars recta of the rabbit nephron (Kawamura and Kokko 1976) and interestingly it has been suggested that a genetic defect in active tubular urea secretion would explain the high plasma urea values observed in patients with otherwise normal kidney function and plasma creatinine (Hsu *et al* 1978, Hays 1978). A recent study in sheep has shown fractional urea clearances of greater than 100 % in a large number of animals, which means that urea excretion cannot be explained by glomerular filtration alone and that secretion of urea must have occurred (Nawaz and Shah 1984).

The transport of urea across the erythrocyte membrane by facilitated diffusion appears in evolutionary terms to have developed in relation to the adoption of a terrestrial habitat (Kaplan *et al* 1974).

The Effect of Antidiuretic Hormone (ADH)

Antidiuretic hormone apart from its well known effects on the permeability of membranes to water, also increases the permeability of some epithelia to urea (Andersen and Ussing 1957, Maffly *et al* 1960, Leaf and Hays 1962).

In 1972 Franki *et al* reported that ADH opens independent channels for water and urea in the toad bladder. The ADH sensitive urea transport channels have since been shown to have a cylindrical radius of 3.5-3.8 Åand are capable of hydrogen bonding with the molecular species traversing them (Petrucelli and Eggena 1982).

The only tubular segment in mammals which appears to show a change in urea permeability with the addition of ADH is the medullary collecting duct (Morgan *et al* 1968, Morgan and Berliner 1968). However these results have not been confirmed during microperfusion studies (Grantham 1978). The effects of

ADH on urea transport are thus considered secondary to its effects on water transport. Cross *et al* (1966) found that ADH decreases the clearance of urea in diuretic sheep without altering the glomerular filtration rate. The largest decreases in urea clearance were seen in those experiments in which there was no decrease in urine flow rate, suggesting that ADH increases the permeability of the tubular cell membrane to urea in this species.

Antidiuretic hormone is often infused as a urine flow rate stabilizing agent to reduce the phenomena of exhaltation and abatement, mentioned earlier, in renal studies (Ergene and Pickering 1978a, 1978b). This infusion of ADH may complicate the determination of parameters of urea excretion.

Urea as a Diuretic

Urea has been used widely as a diuretic agent and is now the prescribed therapy for preventing overhydration in the syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Decaux *et al* 1980).

Urea is classified as an osmotic diuretic, because when its concentration in glomerular filtrate is sufficiently high it will retard tubular fluid reabsorption, by virtue of the osmotic pressure it creates in the tubular lumen.

Earlier studies assumed that increased filtered loads of urea resulted in a decreased fractional reabsorption of urea from the proximal tubules, hence leaving a large amount of urea in the tubules to prevent water reabsorption. In 1970 Kauker *et al* using micropuncture techniques, showed that proximal tubular fractional reabsorption of urea increases with increasing filtered load of urea, even though the UFR was substantially increased. It was therefore concluded that the osmotic diuretic effect of urea was located in the distal tubular region as this segment is relatively impermeable to urea (Ullrich *et al* 1970).

It is of interest that during urea diuresis the clearance of free water increases (Wesson and Anslow 1948, Page and Reem 1952, Zak *et al* 1954, Maude and Wesson 1963). This suggests that urea may play a diuretic role, other than that of an osmotically active non-reabsorbable solute.

Sodium Excretion

Sodium is the most abundant cation in the extracellular fluids. In sheep under natural feeding conditions the intake, and hence the load of Na presented to the kidneys can show wide variation.

Na^+ is freely filtered at the glomerulus and the bulk of this filtered Na^+ is reabsorbed in the proximal tubule and ascending limb of Henlé's loop. The proximal tubule reabsorbs 50-60% of the filtered sodium.

The tubuloglomerular feedback mechanism discussed earlier controls the GFR according to the amount of NaCl reaching the macula densa. However when there is a spontaneous change in GFR in spite of the feedback mechanism there will be a difference in the amount of Na^+ delivered to the proximal tubule. When this occurs absolute reabsorption from the proximal tubule changes to maintain a constant proportional reabsorption (Lewy and Windhager 1968, Schnerrman *et al* 1968). This phenomenon is known as glomerular-tubular balance and its mechanism of control has been the subject of much research.

A rise in GFR without any change in renal plasma flow (increased FF) results in an increase in peritubular protein concentration which, in turn increases the reabsorption of Na^+ (see Fig.3.). This change in peritubular protein concentration has been suggested as the mechanism responsible for glomerular-tubular balance (Brenner and Troy 1971, Brenner *et al* 1971). However several studies give conflicting evidence as to its role in the phenomena (Bartoli *et al* 1973, Conger *et al* 1976, Leyssac 1976).

The proximal tubule microvilli appear to be motile and it has been suggested that increased motility, resulting from an increased GFR stirs the tubular fluid facilitating permeation by subjecting a greater amount of the fluid to the surface of the microvilli and by reducing unstirred layer effects (Trueberg and Rostguard 1969 cited in De Wardener 1978).

The loop of Henlé reabsorbs 15-20% of the filtered NaCl. Early work by Burg and Green (1973) showed that Cl^- was actively reabsorbed in the thick ascending limb and that this reabsorption was inhibited by ouabain, an inhibitor of Na-K-

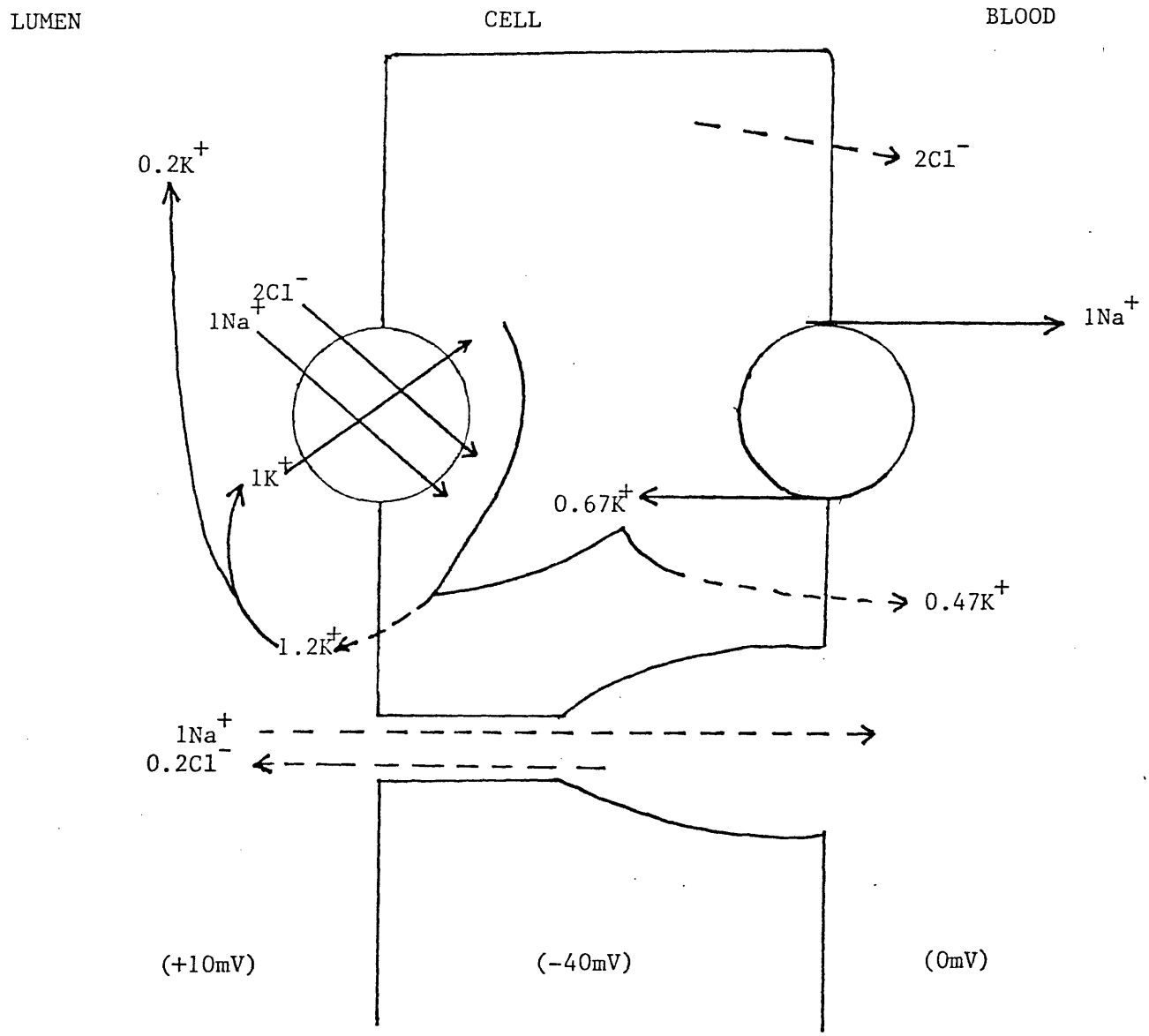


Fig.4. Model of NaCl reabsorption by the thick ascending limb of Henlé, proposed by Hebert and Andreoli (1984). Dashed arrows indicate movement down an electrochemical gradient. Stoichiometry of Na-K-ATPase is assumed to be $3Na^+ : 2K^+$.

ATPase. Recent evidence suggests that the reabsorption of Cl^- by the thick ascending limb involves a secondary active transport process where luminal Cl^- entry is mediated by an electroneutral ($1Na^+$,$1K^+$,$2Cl^-$) co-transport mechanism (Greger 1981, Hebert *et al* 1981, Hebert and Andreoli 1984). Na^+ is then actively transported out of the tubular cells across the basolateral cell membane by Na-K-ATPase (see Fig.4.). This produces an electrochemical gradient favouring the passive removal of Cl^- from the tubular cell and explains why ouabain inhibits Cl^- reabsorption from this limb (Hebert and Andreoli 1984).

Factors Influencing Na Excretion

Aldosterone

Aldosterone is the major factor regulating Na^+ excretion in normal animals. Twenty to sixty minutes after injection of aldosterone Na excretion is reduced and K excretion enhanced. The hormone exerts its major effects in the distal tubule and collecting ducts, although it does increase the epithelial leak permeability of the proximal tubule (Gutsche and Hegal 1980).

Aldosterone is believed to induce new ribonucleic acid formation followed by the synthesis of enzymes which mediate the action of the hormone (Edelman and Fanestil 1970). These enzymes have not yet been isolated (Suzuki 1981).

Aldosterone also restores the depressed Na-K-ATPase activity of adrenalectomised rabbits (Horster *et al* 1980) although the effect is not thought to be the mechanism of salt retention caused by the hormone (Schwartz *et al* 1975).

The rate of Na reabsorption by the cortical collecting duct is related to the potential difference across the segment. A more negative potential is observed in animals fed low Na, high K diets (Frindt and Burg 1972). Both the potential difference and the Na reabsorption were correlated with the concentration of aldosterone present (Gross *et al* 1975, Schwartz and Burg 1978).

The medullary collecting tubule appears to reabsorb Na by passive mechanisms only (Stokes 1981).

Prostaglandins

Chronic salt loading decreases and salt restriction increases PGE_2 excretion (Scherer *et al* 1977, Stahl *et al* 1979).

The effects of prostaglandins on sodium homeostasis are generally regarded as being due to modulation of other effectors such as GFR, ADH, and renin release, rather than a direct effect on Na transport in the tubules. Nevertheless inhibition of cyclooxygenase with drugs such as indomethacin leads to substantial Na retention (Weber and Siess 1982).

Antidiuretic Hormone

A large amount of work with toad bladders has shown that ADH is considered to have a natriferic effect. ADH stimulates Na transport by a primary cyclic AMP-dependent increase in the permeability of the apical epithelial membrane (deSousa and Grosso 1981), although a direct effect on the active Na pump has been shown in the toad bladder (Finn 1971). These effects are noticeable in the cortical collecting tubule and connecting tubule segments of mammals, although the effect is transient and only elicited at high doses of ADH. ADH increases NaCl efflux from the thick ascending limb of Henlé (Hall and Varney 1980, Sasaki and Imai 1980) which aids in the urinary concentrating mechanism. Pharmacological doses of ADH given to dehydrated sheep also increase the excretion of Na (Kinne *et al* 1961), however this effect is thought to be due to changes in renal haemodynamics (Yesberg *et al* 1979).

ADH appears not to play a major role in the control of Na excretion. In fact Grantham (1974) concluded by stating *'it would appear, with due apology to the scores of fallen toads, that vasopressin in physiological concentrations probably has little to do with the regulation of sodium excretion by the mammalian kidney'*.

Natriuretic Hormone

Increasing evidence for the existence of two circulating natriuretic factors has come after much work, mostly by De Wardener's group in London (De Wardener 1982a, De Wardener 1982b, De Wardener and Clarkson 1982).

One of the natriuretic hormones is probably derived from the hypothalamus (Alaghband-Zadeh *et al* 1981). This hormone inhibits Na-K-ATPase apparently in all tissues of the body (Grantham and Edwards 1984). Inhibition of this enzyme located on the basolateral membranes of the kidney tubules results in the natriuresis.

The hormone is found in the plasma and urine of salt loaded and ECV expanded animals including sheep (Sealey *et al* 1969).

A second natriuretic factor is derived from the granular structures in the atria of the heart. This factor does not affect Na-K-ATPase but inhibits the reabsorption of NaCl and water in the distal nephron (Briggs *et al* 1982, Sonnenberg *et al* 1982). The hormone probably acts on the luminal rather than the basolateral epithelium of tubular cells (Sonnenberg *et al* 1982).

Renal Nerves

Stimulation of renal sympathetic nerve activity causes parallel changes in tubular Na and water reabsorption mediated by adrenergic nerve terminals in direct contact with tubular epithelial cells particularly in the proximal tubule (DiBona 1977). The renal nerves also control Na excretion indirectly by their effects on GFR, ERPF, renin release and various systemic effects.

Hormonal Effects

The kallikrein-kinin system antagonizes the renin-angiotensin system and thus causes increased Na excretion when renal kallikrein is released (Mills 1982).

Thyroidectomised animals have a lower excretion of Na primarily because of lowered Na-K-ATPase activity which can be corrected by thyroid hormone administration. This is both an adaptive response to a different Na load and to a direct effect on Na-K-ATPase activity (Edelmen 1975).

Calcitonin is strongly natriuretic in most animals including sheep (Barlet 1972). The effect is principally located in the proximal tubule and is mediated via the adenyl cyclase system. It may be related to the sodium linked amino acid reabsorption as amino acid excretion is also enhanced by calcitonin administration (Bijvoet *et al* 1971).

Insulin enhances Na reabsorption in the ascending limb of Henlé by an undetermined mechanism (Defronzo *et al* 1975) although Na-K-ATPase is probably involved (Katz and Lindheimer 1977).

Glucagon is natriuretic but the effect is mostly caused by a rise in GFR although glucagon sensitive adenyl cyclase has been found in the renal medulla of man (Mulvehill *et al* 1976).

Prolactin is antinatriuretic in most species including sheep (Burstyn *et al* 1972). The effect is located in the proximal tubule and is probably caused by a widening of the paracellular spaces in the epithelium (Lucci *et al* 1974).

Oestrogens generally cause Na retention although the mechanisms are unclear and inconsistencies across different species exist (Christy and Shaver 1974, Katz and Lindheimer 1977). During the reproductive cycle of sheep, sharp increases in Na excretion are noticed during oestrus but this effect is mostly accounted for by a reduction in food and water intake during this period (Michell 1981). Oestrogens are not elevated during pregnancy in sheep except just prior to parturition (Bedford *et al* 1972).

Progesterone increases Na excretion in man. This effect is caused mostly by competitive inhibition of aldosterone (Oparil *et al* 1975). During pregnancy in the sheep the blood levels of progesterone are only 2-5 times those found in the non-pregnant animal (Basset *et al* 1969, Fylling 1970). This level may be insufficient to act as an inhibitor of aldosterone (Wintour *et al* 1976).

Regulation of Acid-base Balance by the Kidney

The kidneys play a major role in the regulation of acid-base balance of mammals.

The HCO_3^- - H_2CO_3 buffer system plays a key role in the maintenance of acid-base equilibrium in the body fluids, not because of its buffering action, as the pKa_3 at 6.1 is too distant from the body fluid pH, but because of the effective excretion of bicarbonate by the kidney and CO_2 by the lungs.

Recent studies (Atkinson and Camien 1982, Halperin and Jungas 1983) have appeared which challenge the traditional view of the role of ammonia excretion in acid-base homeostasis. A summary of these views is included in the following discussion, however until further studies confirm these theories, the role of NH_3 as a buffer for secreted H^+ ions will remain.

CO_2 and H_2O are the only end products of the total oxidation of carbohydrates and fats. However, as noted by Atkinson and Camien (1982), oxidation of any carboxylate anion at physiological pH produces HCO_3^- not CO_2 (see equation 1.).

$$CH_3\text{-}(CH_2)_{n-2} - COO^- \longrightarrow (n\text{-}1)CO_2 + HCO_3^- \qquad (1)$$

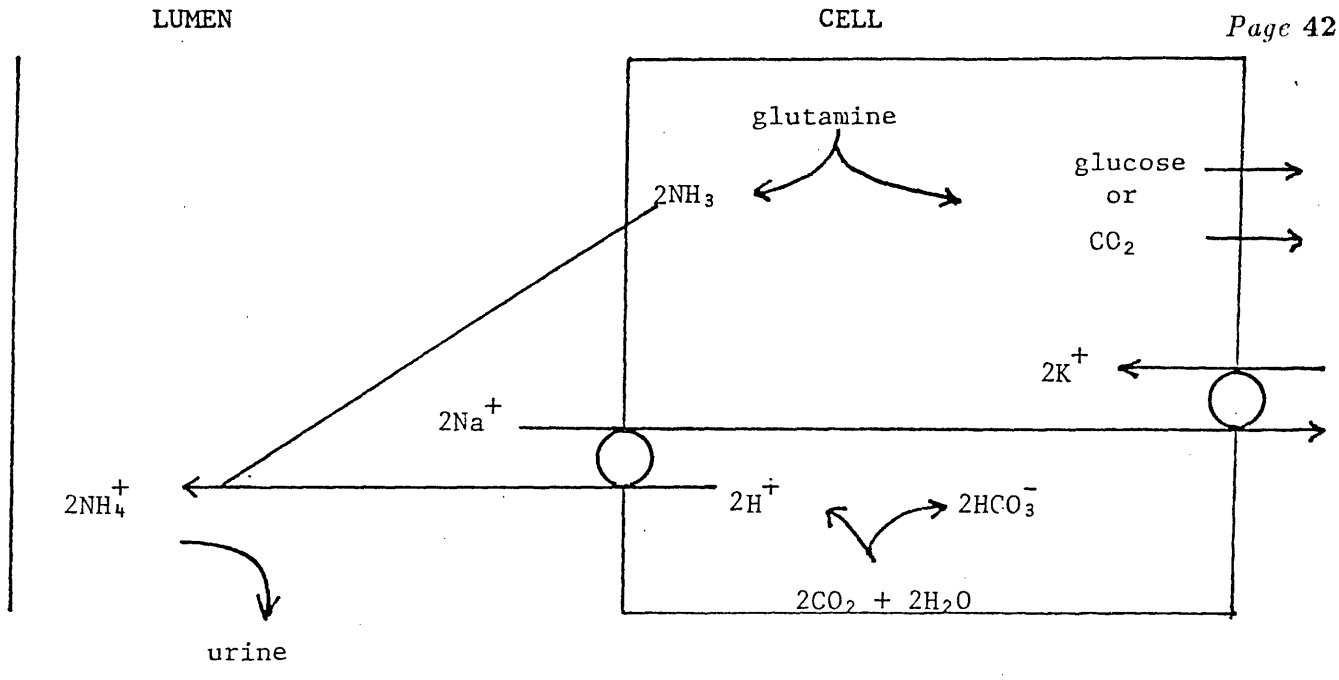
Similarly at pH 7.4 oxidation of simple amino acids yields HCO_3^- and NH_4^+ in equimolar quantities (see equation 2.).

$$CH_3\text{-}(CH_2)_{n-3} - \underset{\displaystyle NH_3^+}{CH}\text{-}COO^- \longrightarrow (n\text{-}1)CO_2 + HCO_3^- + NH_4^+ \qquad (2)$$

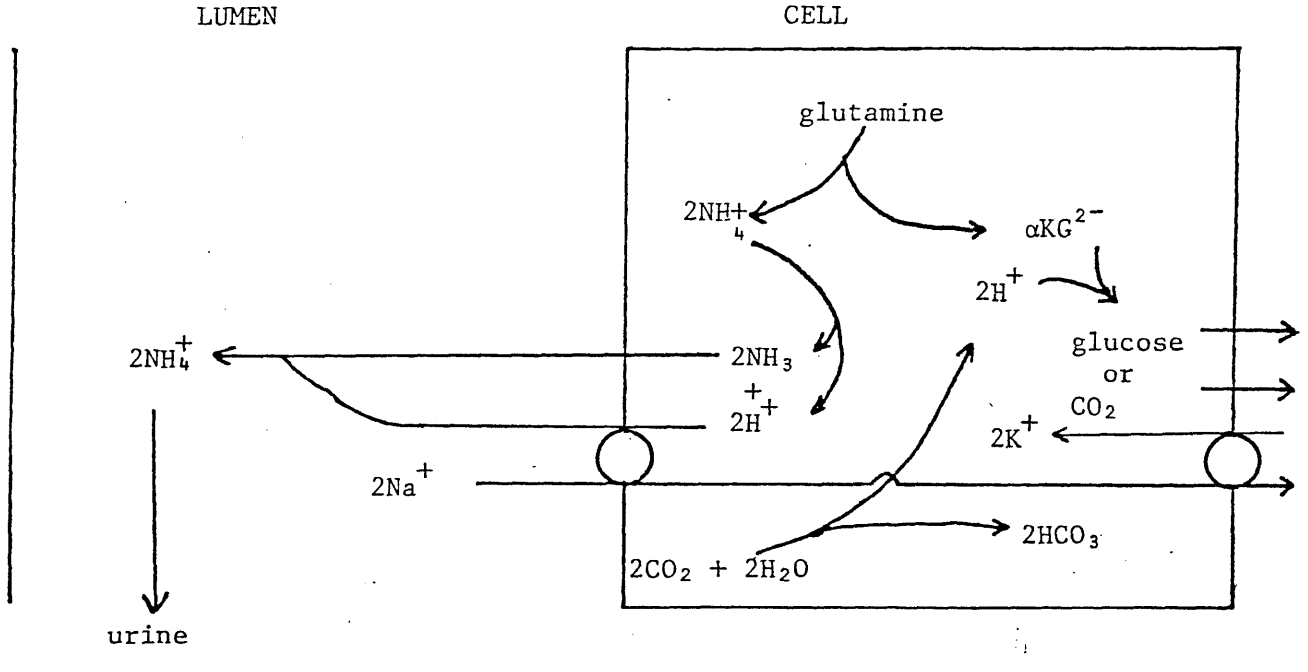
At pH 7.4 NH_3 is rapidly protonated to form NH_4^+. The traditional view that the excretion of NH_4^+ is accomplished by the diffusion of NH_3 through the tubular lumen membrane where it combines with a secreted proton, to form NH_4^+ is not, according to Halperin and Jungas (1983), a means of excreting excess H^+ ions.

The metabolism of glutamine in the kidney produces NH_4^+ and not NH_3 such that the excretion of ammonia can be summarised as in Fig.5.

The increased excretion of ammonia during acidosis is simply a means of removing the excess nitrogen produced from protein wasting (Haussinger *et al* 1984).



a) The traditional view of ammonia production and excretion.



b) The modified view of ammonia production and excretion according Halperin and Jungas (1983).

Fig.5. The production and excretion of ammonia by the kidney tubules.

The Renal Handling of Ammonia

Urinary ammonia is derived almost entirely from renal synthesis (Pitts 1973) in the proximal tubular cells (Good and Burg 1984, Guder and Ross 1984). The majority of ammonia synthesized is excreted rather than returned to the systemic blood circulation (Tizianello *et al* 1982).

The traditional view holds that the excretion of ammonia depends largely on 'diffusion trapping', ie. the diffusion of NH_3 through the renal membranes and its protonation in the acidic environment of the tubular lumen to form NH_4^+.

The addition of ammonia to the proximal tubular fluid cannot all be accounted for by diffusion trapping as the change in H^+ concentration that can occur in the proximal tubular fluid is atleast an order of magnitude lower than that which can be achieved for NH_4^+ ion concentration (Warnock and Rector 1981, Nagami and Kurokawa 1985).

The brush border of the proximal tubular epithelium contains ammonia 'cleaving' enzymes such as γ-glutamyltransferase (Welbourne and Pass 1982) and these may produce ammonia from glutamine within the tubules.

NH_4^+ has an identical ionic radius to K^+ (Weast and Selby 1966) and hence the permeability of nephron segments to these two ions are likely to be similar. For this reason substantial reabsorption of NH_4^+ is thought to occur in the thick ascending limb of Henlé, by diffusion down an electrochemical gradient (Good *et al* 1984), or alternatively by substitution of NH_4^+ for K^+ in the Na^+-K^+-$2Cl^-$ transport system (Hebert and Andreoli 1984).

In normal rats, the NH_4^+ concentration in the distal tubular fluid was similar to that of the proximal tubule, despite the absorption of more than half of the water in the loop of Henlé (Sajo *et al* 1981). There is no loss of ammonia from the distal tubular fluid in the rat (Hayes *et al* 1964).

There is a very large rise in the NH_4^+ concentration in the tubular fluid between the distal tubule and the final urine, which is due mostly to water abstraction from this segment (Hayes *et al* 1964, Sajo *et al* 1981).

In acidotic rats there is a large augmentation of NH_4^+ secretion in the cortical

collecting ducts (Sajo *et al* 1981), whilst in rats fed high protein diets the major site of ammonium addition is the medullary collecting ducts.

Bicarbonate Reabsorption

Most studies indicate that HCO_3^- reabsorption in the proximal tubule is mediated by Na^+–H^+ exchange rather than by direct reabsorption of HCO_3^- (Burg and Green 1977).

H^+ originates from the intracellular hydration of CO_2 to H_2CO_3 which in turn, dissociates to yield H^+ and HCO_3^-. The secreted H^+ reacts with lumenal HCO_3^- to yield carbonic acid which then dissociates to form H_2O and CO_2.

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

The latter reaction is catalysed by the enzyme carbonic anhydrase which is bound to the lumenal membrane of the proximal tubule (Wistrand and Kinne 1977). The CO_2 diffuses into the proximal tubular cells to start the cycle over again (see Fig.6.).

Na-K-ATPase located on the basolateral membrane provides the driving force for the Na^+–H^+ exchange.

ECV expansion results in an inhibition of HCO_3^- reabsorption (Purkerson *et al* 1969, Kurtzman 1970). The mechanism of this inhibition has not been resolved (Battle and Kurtzman 1983) but is probably related to natriuretic hormone inhibition of the basolateral membrane Na-K-ATPase which occurs during ECV expansion (Grantham and Edwards 1984).

Blood pCO_2 is an important factor in determining HCO_3^- reabsorption. However the pH of the blood is probably the controlling factor as respiratory and metabolic alkalosis produce a similar depression in HCO_3^- excretion despite large changes in the blood pCO_2 (Langberg *et al* 1981).

Acid Excretion

The kidney tubules cannot maintain a H^+ gradient between lumenal fluid and blood of more than about 3 pH units, consequently urine pH never falls much lower than about 4.4.

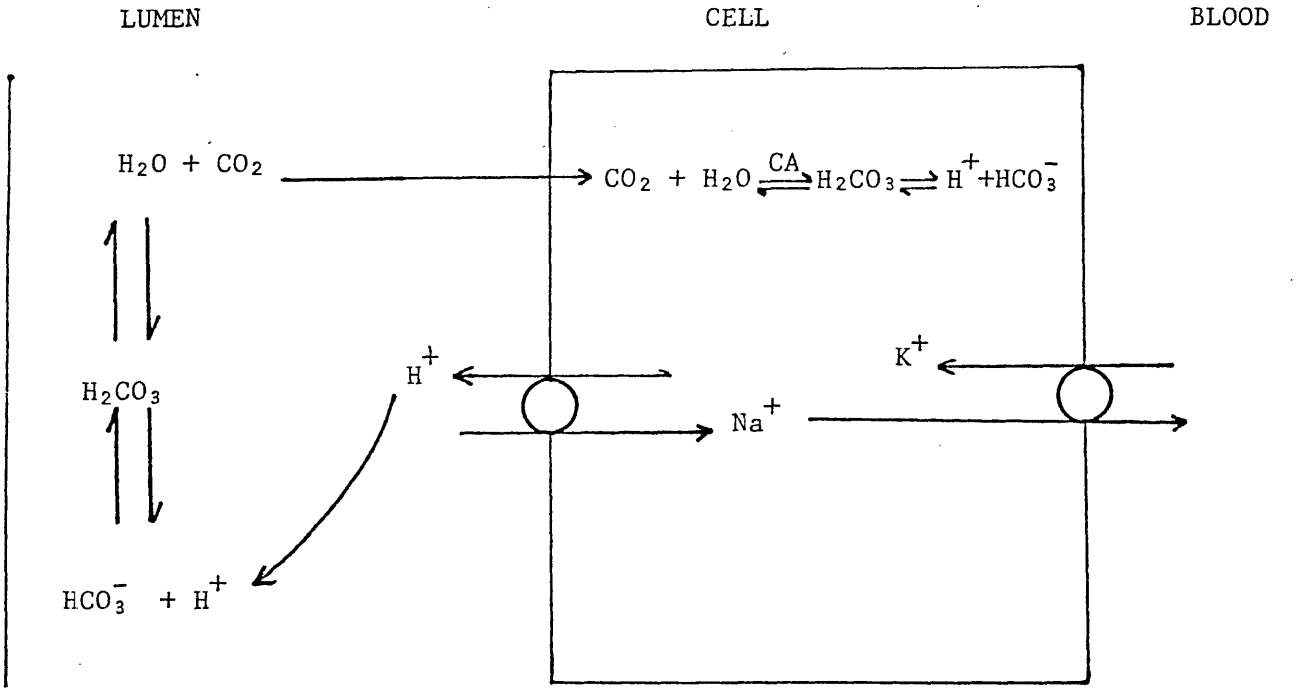


Fig.6. The mechanism of bicarbonate reabsorption in the proximal tubule.

Most of the acid excreted is in a buffered form, either as NH_4^+ as mentioned earlier, or as $H_2PO_4^-$. Normally the amount of acid which can be excreted in the phosphatic form is limited by phosphate excretion. The excretion of phosphate is enhanced during acidosis, which helps provide extra buffer for the excretion of acid. The sheep normally excretes very little phosphate in the urine, the preferred route of excretion being via saliva and hence in the faeces (Tomas 1974).

During acidosis in sheep there is a slight increase in renal phosphate excretion (Williams and Pickering 1980, Scott and Buchan 1981). Increased intake of concentrate diets in sheep also enhances the renal excretion of Pi, probably by reducing salivary Pi removal together with a phosphaturia of acidosis as these diets tend to produce some degree of acidosis (Godwin and Williams 1982). Sheep fed roughage diets normally excrete a very alkaline urine (pH 7.9 - 8.4).

Scott and associates have suggested that because ruminants excrete so little

phosphate in the urine, they rely on NH_3 as a vehicle for eliminating H^+ ions (Scott 1969, Scott 1971, Scott *et al* 1971). According to Halperin and Jungas (1983) (see earlier section), NH_4^+ excretion is not a means of removing H^+ ions. However, another chemical buffering mechanism(s) other than ammonia or phosphate that could be utilized by sheep kidneys during acidosis is unlikely although creatinine and hippuric acid may possibly play a small role. Hence the traditional view of NH_3 as a vehicle for H^+ ion elimination appears more likely, at least in sheep.