MECHANISMS OF UREA EXCRETION IN SHEEP

A Thesis
presented to
The Faculty of Graduate Studies and Research
The University of Manitoba

In partial fulfillment
of the requirements for the Degree
Master of Science

by
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October, 1971

ABSTRACT

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The objectives of the research reported herein were to investigate the effects of different levels of water and dietary protein intake on the GFR on a long term basis and to study the renal mechanics of urea excretion on low and high protein intakes in sheep.

Water diuresis in both low and high protein diets resulted in marked increase in urine flow rate, free water clearance but only a slight increase in osmolar clearance. There were no consistent changes in inulin and para-aminohippuric acid clearances.

The effects of dietary nitrogen intake on urea nitrogen excretion in sheep could not be attributed to changes in GFR since there were no statistically significant changes in GFR between low and high protein diets. It was concluded that regulation of urea excretion in sheep is unlikely to be at the glomerulus.

On the high protein diet, during normal water intake, urine flow rate had no significant effect upon the fraction of filtered urea nitrogen excreted in the urine but the urine urea nitrogen to plasma urea nitrogen concentration ratio increased with increase in water reabsorption. Urea reabsorption under such circumstances could be explained by passive tubular

reabsorption aided by a counter-current mechanism in the Loop of Henle.

On the low protein diet, during normal water intake, the sheep had significantly lower levels of plasma urea nitrogen, urea nitrogen clearance, fraction of filtered urea nitrogen excreted in the urine and urea nitrogen excretion rate than those on high protein diet. The fraction of filtered urea nitrogen excreted in the urine decreased with decrease in urine flow rate but the urine urea nitrogen to plasma urea nitrogen ratio did not increase proportionally with decrease in urine flow rate. These results suggest that the decrease in fraction of filtered urea nitrogen excreted in urine on low protein diet cannot merely be explained by changes in tubular permeability. A urea absorptive mechanism is suggested in the collecting duct region of the nephron to help increase the tubular urea reabsorption. The biochemical characteristics and factors controlling this mechanism are unknown.

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ACKNOWLEDGEMENT

It is a great pleasure for the author to express his indebtedness to his advisor, Dr. G.D.Phillips, Professor of the Department of Animal Science, for his invaluable encouragement, advice and guidance which were extremely vital to the execution of this study. Constructive criticism given by Dr T.J. Devlin in the preparation of this report was exceedingly appreciated.

A note of gratitude also goes to my fellow student, Mr T. Cowan, for his advice and assistance in the operation of the autoanalyser and also to Miss J. Roper who cheerfully typed the draft of this report.

This research was financed by an assistantship from the Department of Animal Science, the University of Manitoba, and N.R.C. grant awarded to Dr. G. D. Phillips.

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LITERATURE REVIEW

Terminology

In order to avoid semantic confusion in the literature review and discussion in this report, the reviewer finds it desirable to clarify the terms and definitions used hereafter.

Plasma clearance

The term plasma clearance is used to express the ability of the kidneys to remove various substances from the plasma. The clearance (C) value of a plasma constituent is the volume (in ml) of plasma which contains the amount of the constituent which is excreted in the urine in one minute. Plasma clearance for a substance can be calculated by the following formula:-

$$C = \frac{\text{Urine concentration(U)xUrine flow rate(V)}}{\text{Plasma concentration (P)}} \text{ ml /min}$$

Thus, plasma clearance can be calculated for urea:

$$Cu = \frac{Uu.V}{Pu}$$

using, Uu = urea concentration in urine (mg/ml)

Pu = urea concentration in plasma (mg/ml)

Cu = urea clearance (ml/min)

V = urine volume (ml/min)

The term clearance can be somewhat misleading because no part of the plasma is completely cleared of a particular substance (Keele and Niel, 1965). Furthermore, this value tells nothing directly about how the particular substance is transferred from plasma to urine: whether by filtration plus tubular reabsorption, filtration alone or filtration plus tubular excretion (Smith, 1956). Consequently, the clearance value is a so called "virtual value"; it is the result of an arithmetical calculation.

Glomerular filtration rate (GFR)

Commonly, GFR is expressed in terms of millilitres of plasma filtered through glomerular capillaries per minute and consequently introduces an ambiguity in terminology (Pitts, 1965). Plasma is 93-94% water and some 6-7% protein but only the aqueous phase which contains soluble crystalloids is filtered. However, the crystalloids are commonly expressed in terms of milligrams per 100 millilitres or milliequivalents per litre of plasma. So if the GFR is 125 ml/min, it actually means that 125 x 93% = 116 ml of plasma water are filtered. One, therefore, has to bear in mind that even though GFR is defined as volume of plasma filtered per minute only the water and its dissolved crystalloids are actually filtered.

Active secretion

Active secretion is a mechanism by which substances are actively transported from interstitial fluid into the tubules of the nephron. In this case, the quantity of the particular substance which appears in the urine may exceed the amount filtered.

Active reabsorption

Active reabsorption is a mechanism by which a substance is transported against an electrochemical gradient i.e. against a gradient of electrical potential or chemical concentration or both. Work is performed directly on the substance reabsorbed by the cells effecting transport and energy is expended in the process.

Passive reabsorption

Passive reabsorption is a mechanism in which substances are reabsorbed due to electrochemical or potential gradient. No energy is expended directly in moving the substance in question although energy may be required indirectly, in establishing the gradients down which the substance diffuses.

Fraction of filtered urea excreted by the kidneys $\frac{Cu}{C_I}$

This fraction is also known as "tubular marker" (Goldstein and Levitt, 1970). It is calculated from the amount of urea excreted per minute divided by the urea filtered per minute. This can be shown algebraically.

Urea excreted per minute = $Uu.V$

Urea filtered load per minute = $C_I.Pu = \frac{U_I.V}{P_I}.Pu$

where, Uu = urea concentration in urine

V = urine volume (ml/min)

Pu = urea concentration in plasma (mg/100 ml)

U_I = inulin concentration in urine (mg/100 ml)

P_I = inulin concentration in plasma (mg/100 ml)

C_I = inulin clearance i.e. GFR (ml/min)

Therefore the fraction of filtered urea excreted per minute:

$$= \frac{\frac{Uu.V}{U_I.V}}{P_I}.Pu \qquad \left(\text{or} \quad Uu.V \div \frac{U_I.V}{P_I}.Pu \right)$$

$$= \frac{Uu.P_I}{U_I\ Pu}$$

$$= \frac{Uu}{Pu} \cdot \frac{P_I}{U_I} = \frac{\frac{Uu}{Pu}}{\frac{U_I}{P_I}} \qquad \ldots\ 1$$

But $\frac{Cu}{C_I} = \frac{\frac{Uu}{Pu} \cdot V}{\frac{U_I}{P_I} \cdot V} = \frac{\frac{Uu}{Pu}}{\frac{U_I}{P_I}} \qquad \ldots\ 2$

Therefore, the fraction of filtered urea excreted in urine per minute is equal to the urea:inulin clearance ratio $\frac{Cu}{C_I}$.

Conversely, the fraction of filtered urea reabsorbed per minute is equal to $(1 - \frac{Cu}{C_I})$.

$\frac{U_I}{P_I}$ ratio

The inulin in urine : inulin in plasma ratio is used as a measure of water reabsorption by the kidneys. Thus a $\frac{U_I}{P_I}$ ratio of 100 means that $\frac{99}{100}$ of the water filtered in the glomerulus has been absorbed. Numerically, this ratio increases as urine flow rate decreases and hence this is an indication of urine flow rate.

$\frac{Tu}{Pu}$.ratio

This is a ratio of concentration of urea in the renal interstitial fluid and urea concentration in the plasma.

Urea excretion rate (UER)

It is the amount of urea excreted in the urine per minute (Uu.V)mg/min.

Urine Flow Rate (UFR)

It is the volume of urine excreted per minute (ml/min).

Renal transport maximum (Tm) and threshold

Since any substance that is actively reabsorbed requires a specific transport system, the maximum amount that can be reabsorbed depends in part on the maximum rate at which the transport system itself can be operated, and this in turn depends on the total amount of carriers and specific enzymes, site, or energy available.

Consequently, for almost every actively reabsorbed substance, there is a maximum rate at which it can be reabsorbed; this is called the transport maximum for the substance, and the abbreviation is Tm (Ruch and Patton, 1966). Tm of a substance is often expressed in milligrams per minute (Davison and Eggleton, 1962; Guyton, 1965).

Every substance that has a transport maximum also has a "threshold" concentration in the plasma below which none of it appears in the urine and above which progressively larger quantities appear in the urine. Threshold concentration of a substance in plasma is expressed in mg% (Guyton, 1965).

Filtered load

Filtered load is also known as tubular load (Guyton, 1965). It is the amount of a certain substance filtered by the glomeruli per minute and is calculated from GFR x Plasma Concentration of that particular substance.

Osmolar clearance (Cosm)

The osmolar clearance is defined as the millilitres per minute of plasma completely cleared of osmotically active substances (Pitts, 1965). Cosm is calculated, as in the case of any clearance, as the quantity (mOsm) excreted per minute divided by the

quantity in each millilitre of plasma:-

$$Cosm = \frac{Uosm.V}{Posm}$$

where, Cosm = osmolar clearance (ml/min)

Uosm = osmolality (mOsm) in each ml of urine

Posm = osmolality (mOsm) in each ml of plasma

V = urine volume (ml/min).

Free water clearance (C_{H_2O})

The free water clearance is that moiety of water in the urine in excess of or less than the simultaneous osmolar clearance (Smith, 1956).

$$C_{H_2O} = V-Cosm$$

The induction of diuresis involves the inhibition of the secretion of the antidiuretic hormone (discussed later) which in turn decreases the water reabsorption in the distal part of the nephrons. However, this does not stop the reabsorption of electrolytes and non-electrolytes from the tubular fluid. So during diuresis, the urine consists of a mixture of osmotically obligated water, equal to the osmolar clearance admixed with solute-free, literally pure water. With reference to the latter, one may speak of the free-water clearance as representing the rate of excretion of osmotically free water in millilitres per minute, the total urine flow representing the sum of these two terms.

In normal water intake, the kidneys can elaborate urine some four to five times as concentrated as blood plasma (Pitts, 1965). Under such circumstances, the free water clearance is negative. This means that the kidneys are excreting hypertonic urine, and free water is said to have been reabsorbed to restore the body fluid values.

C_{PAH}

This stands for para-aminohippuric acid clearance. (See Page 14 for details).

Units

Some researchers, using microdiffusion cells to analyse urea concentration in plasma and urine, preferred to express urea concentration in terms of mg% of urea nitrogen as this would save the time and trouble to convert the values to mg% or millimoles of urea (Maloiy *et al.*, 1970; McIntyre and Williams, 1970). Since analysis of urea in the present experiment was done on an autoanalyser which also gives readings in mg% of urea nitrogen, the author has chosen the same convention used by the above-mentioned researchers. Hereafter, the subscript "u" used in the aforementioned symbols (namely Tu, Pu, Uu, Cu, $\frac{Cu}{C_I}$) refers to urea-nitrogen concentration, not urea concentration *per se*.

Glomerular filtration rate (GFR)

Measurement

The clearance of inulin, a fructose polysaccharide derived from Dahlia roots and Jerusalem artichokes, was proposed as a measure of GFR more or less simultaneously by Richards *et al.* (1934) and Shannon and Smith (1935). Up to the present time, inulin is still the substance most commonly used for measuring GFR. Inulin is physiologically inert. It has an Einstein-Stokes radius of 15 angstroms and molecular weight of 5,200 so that it passes through the glomerular membrane as freely as the electrolytes and water of the plasma and yet it is not reabsorbed or secreted to any significant extent by the tubules of the nephron. Mannitol is another polysaccharide which has similar properties and is also frequently used for measuring GFR. Since inulin is hydrolysed to fructose in the gastrointestinal tract and poorly absorbed from subcutaneous tissues or muscles, it must be administered parenterally. In the usual technique pyrogen free inulin is injected intravenously first giving a concentrated priming dose adequate to raise the plasma concentration to 10-20 mg/100 ml and then infusing at such a rate as to maintain the plasma level constant, i.e. the same rate at which inulin is excreted.

Creatinine clearance has been shown to be a valid measure of GFR in Necturus and in the rabbit, sheep, seal and dog (Smith, 1956). The major evidence in each instance is agreement of simultaneously measured inulin and creatinine clearances over wide ranges of plasma concentrations of both. It has been suggested that, for precise work, exogenous creatinine should be infused in amounts sufficient to raise the plasma concentration to about 15 mg/100 ml or more. The detailed mechanism for tubular secretion, excretion and chemical analysis of creatinine in urine and plasma have been discussed by Pitts (1965) and will not be mentioned here.

Factors influencing the changes in GFR

Glomerular capillary hydrostatic and osmotic pressures are the factors which operate to produce ultrafiltration. The capillary blood pressure is under neurohormonal control whereas osmotic pressure is mainly affected by diet, which in turn influences the volumes and osmolalities of the body fluids. Discussion herein will be made with respect to dietary changes.

Nielsen and Bang (1948) and Pullman et al. (1949) found that the protein content of the diet did not itself seem to affect the GFR to any marked degree. Chasis et al. (1950) observed that the Kempner rice diet (low protein, low salt) administered to hypertensive subjects caused an average decrease in GFR of 25% while renal

plasma flow decreased 20%. However, when the rice diet was supplemented by salt, the GFR returned to normal. A similar observation was made by Weston et al. (1950) who also found that a diet low in salt but with normal protein content caused a significant decrease in the GFR.

Similar effects have been reported in ruminants. Schmidt-Nielsen et al. (1957, 1958) found that in camels (pseudo-ruminants) as well as in sheep, the GFR was not significantly affected by different levels of protein intake (1.5% and 7.5% DCP) for normal urine flow rates with controlled salt intake. Similar observations were made by McIntyre and Williams (1970) in sheep.

Speculation concerning the nature and locations of plasma volume receptors have been controversial. However, the role played by these receptors in regulation of GFR and volumes of body fluids can be summarised briefly. Changes in extracellular fluid (ECF) volume can be sensed as changes in pressure or distension of the interstitial and venous reservoirs (Strauss, 1957), or distention of arterial reservoirs (Epstein, 1956), or distention of left atrium (Gauer et al., 1961), or as changes in blood flow (Davis, 1961) by volume receptors. During dehydration, the volume of ECF is decreased. Under such circumstances, the body will react in such a way as to conserve water and salt. (If dehydration is due to lack of water, the kidneys will excrete salt to maintain

osmolality of tissue fluid.) Change in ECF volume is detected by any of the aforementioned mechanisms and the response mediated through sympathetic impulses which control afferent renal arteriolar resistance. Increase in resistance will cause decrease in filtration force and hence result in reduction in GFR. Sympathetic constrictor fibres are very powerful in the kidneys and the gut (Guyton, 1965). At the same time, impulses generated by the volume receptors are delivered via the hypothalmic-hypophysial (also known as suproptic hypophysial) tracts causing the liberation of antidiuretic hormone (ADH) from the posterior lobe of the pituitary gland. ADH, an argine- vasopressin, acting directly on the adrenal glands, stimulates the secretion of hydrocortisone and aldosterone (Hilton, 1960). ADH also increases the permeability of distal convoluted tubules as well as collecting ducts to water and at the same time aldosterone stimulates the sodium pump. Hence water and salt are conserved.

Osmoreceptors also play an important part in restoration of ECF volume. The volume of the ECF compartments is largely determined by the amount of sodium and chloride. It is interesting to note that the osmoreceptors respond with maximal intensity to changes in concentration of these two principal ions of ECF, but do not respond readily to changes in concentration of urea

and glucose for they can pass across cell membranes with ease (Keele and Neil, 1965; Pitts, 1965). Verney (1954) has suggested that osmoreceptors are represented by vesicles in the supraoptic nuclei which act as tiny osmometers, swelling when the surroundings become hypotonic, shrinking when the surroundings become hypertonic. Shrinkage of the vesicles is presumed to stimulate processes of neurones of the supraoptic nuclei which are applied to the nuclei surfaces. Impulses, generated in these neurones and conducted over the supraoptic-hypophysial tracts to terminations in the median eminence and neural lobe of the posterior pituitary, cause the liberation of ADH. Antidiuresis results.

In water or osmotic diuresis, one can expect the reverse to take place, i.e. increase in GFR and inhibition of secretion of ADH and also aldosterone in the case of saline diuresis. Water and salt (in saline diuresis) excretion will increase until the output of these substances balances the intake (Pitts, 1965). It was found that on a short term basis, sodium chloride loading caused an increase in GFR in camels (Schmidt-Neilsen et al., 1957), in cattle (Weeth and Lesperance, 1965), and in sheep (Potter, 1961, 1963). However, Thornton (1970a) observed that a 24 hour estimate of GFR was not influenced by sodium chloride loading in cattle.

There remains some mystery regarding the relative importance of the volume receptors and osmoreceptors in the regulation of body fluid volumes. At this stage, one can only say that these two kinds of receptors, although basically independent, are nevertheless interrelated (Pitts, 1965; Johnson et al., 1970).

Para-aminohippuric acid (C_{PAH}) clearance

PAH, like inulin, passes through the glomerular membrane along with the remainder of the glomerular filtrate. However, PAH is different from inulin in that almost all the PAH remaining in the plasma after the glomerular filtrate is formed is secreted into the urine by the tubular walls (if the plasma load of the PAH is less than the Tm for PAH). One can use the clearance of PAH to estimate the flow of plasma through the kidneys. If haematocrit is known, one can also calculate the total blood flow through the kidneys each minute, (Guyton, 1965). In the human, to measure total blood flow precisely the concentration of PAH in plasma should be between 1 and 8 mg/100 ml because at plasma concentrations in excess of 10 mg/100 ml, the secretory mechanism becomes saturated (Pitts, 1965).

Kinetics of tubular secretion of PAH

$$(PAH) + B \rightleftharpoons (PAH)\cdot B \rightarrow B + (PAH)$$

A carrier, B, present in the cell membrane or perhaps within the cell in a fixed and limited amount,

combines with PAH to form a complex (PAH).B. The complex (PAH).B migrates to the cytoplasmic surface of the luminal membrane, where it is split. The carrier is presumed to shuttle back and forth between the two surfaces of the membrane, combining solute at one surface, freeing it at the opposite. Energy must be supplied either to effect combination of the solute with the carrier or to split the carrier-solute complex. The energy is derived from adenosine triphosphate (ATP). One must assume that the affinity of PAH for carrier B is high and that the rate of attainment of equilibrium in the reaction (PAH) + B (PAH).B is rapid in comparison with the rate of break down of complex (PAH).B. When all the carrier is used to combine with PAH, the secretory system is said to be saturated and the rate of secretion is determined by the rate of break down of the complex (PAH).B (Pitts, 1965).

Factors affecting renal excretion of urea in sheep

(A) General Discussion of Urea Excretion in Mammals

(a) Fate of ammonia in mammalian body

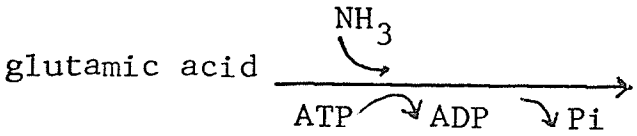
The fate of ammonia produced by various deamination reactions varies with the type of animals and their habitat. Since ammonia is very toxic, mammalian tissues are equipped with various mechanisms of converting ammonia into nontoxic materials, either for use by the animal or for excretion. The most significant methods for disposal of ammonia suggested by Allen (1970) are briefly listed below.

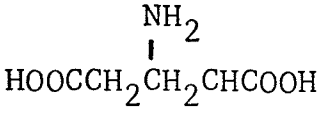
(i) Urea formation

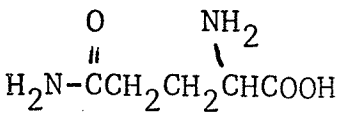
Ammonia is converted to urea in the liver through the Krebs Ornithine Cycle. The details of the biochemical reactions have been well summarized by Harrow and Mazur (1966).

(ii) Biosynthesis Reaction

Part of the ammonia resulting from deamination of amino acids is utilized in the formation of biologically useful nitrogenous compounds e.g. synthesis of glutamic acid, purines, pyrimidines and porphyrins. One of the more useful ways of removing toxic ammonia from a biological system is through the synthesis of glutamine. Glutamine is transported from various tissues by the blood to the kidneys where it may be stored to a limited extent.

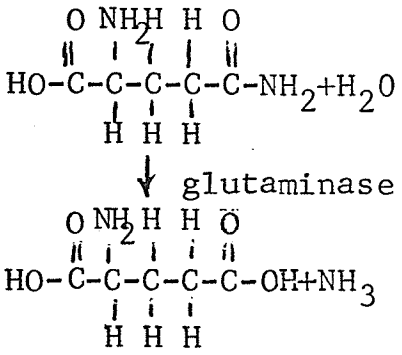






(iii) Direct excretion of ammonia in urine

Urinary ammonia is produced in kidney epithelial tissues from the hydrolysis of glutamine or the deamination of alpha amino acids. Glutaminase catalyses hydrolysis of the amide linkage of glutamine to produce ammonia and glutamic acid. Amino acid oxidases also appear to play a significant role in deamination of amino acids in the kidney (Allen, 1970).



The rate of ammonia secretion in the kidney depends on the acidity of the urine, the rate of ammonia production in the renal tubules and the urine flow rate if the urine is only slightly acidic (Pitts, 1965).

(b) Distribution and absorption of urea from alimentary tract in the human

Urea is an osmotically active substance in the body fluids. In the human, the osmolality contributed by urea in plasma, interstitial and intracellular fluids is approximately 4 mOsm/litre. Concentration of urea in the plasma depends on the level of protein in the diet. In the human, plasma urea concentrations vary between 15 and 40 mg% for normal range of protein diets. For high protein diets, the urine urea concentration can be as high as 5-6 times that in plasma and the kidneys are the main organs which are responsible for urea excretion (Guyton, 1965). A small amount of urea is also found secreted by salivary, sweat, and lachrymal glands (Oser, 1954). Urea is passively absorbed from the human stomach and absorption of urea from the alimentary tract is of no significance in carnivores as far as nutrition of carnivores is concerned (Davenport, 1966).

(c) Special nutritional significance and mechanism of reabsorption of urea in ruminants

(i) Evolution of ruminants

Herbivores can be divided into two groups: namely, those presenting their ingested food for fermentation prior to gastric digestion, such as true ruminants; and those in which fermentation occurs only in the hindgut, such as rabbits and horses. The great evolution of herbivores and the changes in dominance

within these groups, particularly the upsurge of the ruminants, coincided with the changing pattern of climate, and with the development and radiation of the grasses in the tertiary period (Dougherty, 1965). Paleontologists suggest that much of the evolution of the ruminant as a separate branch of mammals occurred during this prolonged dry period of the earth's history. It is likely that the special digestive tract modifications present in modern ruminants were developed in response to some such environmental pressure, and that the ability to survive a long period of semi-starvation is a result of these adaptations. Other evidence which supports this argument is that, although the nitrogen content of natural vegetation falls dramatically in the dry season, the cellulose content of plants from which herbivores can derive their energy is still high and the recycled urea in the rumen is important for microbial activities. Consequently, any nitrogen conservation mechanisms in these animals is a great advantage in survival (Houpt, 1970).

(ii) Present knowledge of protein regeneration cycle in ruminants

Lewis (1957) has shown that blood urea in ruminants varies according to the level of non-protein nitrogen in the diet. The higher the level of non-protein nitrogen intake, the higher is the plasma urea concentration and urea is most abundant in the blood when it is least needed

as a nitrogen supplement. It has also been demonstrated that urea enters the rumen from blood by diffusion across the rumen wall (Houpt, 1970) and in saliva (Somers, 1961a). The total amount of urea in saliva parallels the plasma urea nitrogen concentration and the amount of urea transferred is determined by the rate of salivary secretion and by the plasma urea concentration (Hungate, 1965).

Gillette (1967) found that, in goats, when the transport of endogenous urea to the rumen was increased by transplantation of one ureter to the rumen wall, the animals' apparent dietary requirement for nitrogen decreased from 1.69 to 1.33 gm/day. This result indicates that in these animals the transfer rate of urea nitrogen into the rumen, not the capacity of the rumen microbes to synthesize protein, was the limiting step for the protein regeneration cycle.

The full details of the mechanism of transport of urea across the rumen epithelium from blood to rumen are not yet known. The information available is contradictory. The latest hypothesis postulated by Houpt (1970) is summarized below.

The first event is the diffusion of urea from the blood vessels towards and into the basal layers of the epithelium. A small fraction of this urea will continue unchanged through the epithelium into the rumen interior

where it will be rapidly hydrolysed by microbial urease to ammonia. The remainder of the urea will be hydrolysed at some site probably within the cornified layers, by bacterial urease which has penetrated from the rumen interior. The tissues in the cornified layers therefore contain a relatively high concentration of NH_3 which tends to diffuse both towards the rumen and back towards the blood vessels. However, diffusion of NH_3 towards the rumen is more favoured because the rumen content is slightly acidic (a pH of approximately 6.5) and will keep the NH_3 concentration low, trapping it in the form of NH_4^+.

If one is convinced that recycling urea in ruminants is a means of conserving urea, one will face an unanswered question: what role do the kidneys play in urea conservation since these are the main organs by which urea is excreted as waste product of protein metabolism? Teleologically, the ruminant should decrease the amount of renal urea excretion on low protein diet so that more urea could be recycled to the rumen for protein synthesis.

Several researchers have reported that more urea is excreted in urine with high than with low protein diets in cattle (Thornton, 1970b), and in sheep (Schmidt-Neilson et al., 1958; McIntyre and Williams, 1970; Scott and Mason, 1970; Maloiy et al., 1970). The decrease in renal urea excretion with low protein diets does not, however,

necessarily suggest that urea is being conserved. It may merely mean that there is less urea in the plasma and hence less urea is presented to the kidneys for excretion. On the other hand, there may be a specific mechanism in ruminant kidneys to increase renal urea reabsorption when protein intake is low. So far there is no concrete evidence to conclude whether urea is actively or passively reabsorbed in the kidneys. Before reviewing the hypotheses that have been postulated and experiments that have been reported on this subject, reference is made to the kinds of theoretical evidence one would expect to find if one assumes active or passive urea reabsorption in the kidneys.

(d) Theoretical evidence for passive and active reabsorption of urea in the kidneys

The evidence for passive renal reabsorption of urea can be derived from two main types of observations: (i) the urea clearance is independent of plasma urea over a wide range of concentration and (ii) the urea clearance varies with urine flow rate, especially at flow rates less than two millilitres per minute (Pitts, 1965).

Independence of urea clearance on plasma urea concentration suggests, although it does not prove, that the reabsorption of urea is passive. The plasma urea concentration varies with the levels of protein intake

and if the urea is passively reabsorbed, the rate of excretion of urea will be directly proportional to plasma urea concentrations; clearance remains unchanged. The fraction of the filtered urea reabsorbed $(1 - \frac{Cu}{C_I})$ is likewise unchanged. Therefore, the absolute amount of urea reabsorbed in milligrams per minute varies in direct proportion to the plasma urea concentration. One hundred times more urea may be reabsorbed at high than low plasma urea concentration. This also indirectly suggests that no limit to reabsorption exists and therefore, that reabsorption depends on passive diffusion, not on an active energy requiring transport mechanism for which Tm exists.

Variation of urea clearance with urine flow rate also suggests that passive diffusion determines reabsorption. If it were possible to induce such a massive diuresis that urine flow rate was equal to GFR, no difference in concentration of urea would exist across any portion of the renal tubules. The urea $\frac{u}{p}$ ratio would be 1. In the absence of a diffusion gradient, no reabsorption of urea should occur. Assuming GFR is 100 ml/min, at a urine flow of 50 or 10 ml/min the urea $\frac{u}{p}$ ratio would be 2 and 10 respectively. The lower the urine flow rate and the higher the urea $\frac{u}{p}$ ratio, the greater would be the tendency for urea to diffuse from lumen to peritubular fluid (Pitts, 1965).

On the other hand, the observations that Cu and $(1 - \frac{Cu}{C})$ ratio vary with different levels of Pu and that urea $\frac{u}{p}$ ratio does not increase with decrease in urine flow rate may suggest that a mechanism other than passive reabsorption of urea also takes place. If active transport of urea takes place in a normal protein diet, one can easily observe renal Tm of urea by intravenous infusion of different levels of urea. However, so far there has not been any known suggestion to detect the Tm of urea on a low protein diet.

(B) Historical Concept of Urea Excretion in Mammals

Up to the early 1950's, the mechanism for renal excretion of urea in mammals had been regarded as consisting of glomerular filtration and a passive back diffusion in the tubules of the nephron. It had been assumed that no tubular regulatory or secretory mechanisms were involved in the process.

Rehberg (1926) investigated urea excretion in man and found that the amount of urea which appeared in the urine was less than the amount filtered, using exogenous creatinine to measure GFR. He also found that the fraction of filtered urea which was excreted, decreased in a regular manner with decreasing urine flow. Variations in plasma urea concentration did not alter this relationship. He concluded that excretion of urea was a process of glomerular filtration and tubular reabsorption and urea was not a threshold substance.

The next intensive investigations of the renal mechanism for urea excretion were those of Shannon (1936, 1938), Jolliffe and Smith (1931) and Chasis et al. (1938). Shannon (1936), working with dogs, confirmed Rehberg's findings that the fraction of filtered urea which was excreted decreased regularly over a wide range with increasing tubular reabsorption of water. Smith (1937) did not agree with the conclusion of Shannon that urea reabsorption was merely a passive process. Smith argued that if one extrapolated Shannon's data to zero tubular reabsorption of water, the amount of urea excreted in the urine would still be 40% lower than the amount filtered, indicating that this fraction had been actively reabsorbed. Shannon (1938) extended his experiments to investigate urea excretion in normal dogs during forced diuresis. He found that, when the creatinine $\frac{u}{p}$ ratio increased to values lower than 10-15, the $\frac{\text{urea}}{\text{creatinine}}$ clearance ratio increased rapidly towards unity with increases in urine flow. Chasis and Smith (1938) found a similar relationship between urea clearance and filtration rate in normal man and consequently agreed that urea reabsorption is a passive process. This concept has been presented repeatedly by Smith (1951, 1956).

(c) Current Concept of Urea Excretion in Mammals

The first indication in the literature that ruminants under certain conditions might excrete very small amounts of urea was given by Read (1925), who analysed the urine of a pregnant camel on a rather low protein diet and found the urine to be practically free of urea. Smith and Silvette (1928) were doubtful about this finding because they analysed the urine of camels maintained on a normal diet and found the normal concentrations of urea. It was not until the late 1950's that the mechanisms of urea excretion and reabsorption in ruminants were investigated further.

Schmidt-Neilsen et al. (1957), experimenting with camels, found that with normal nitrogen intake, about 40% of the urea filtered in the glomeruli was excreted in the urine, but with low nitrogen intake only 1-2% was excreted. No evidence of active tubular reabsorption was observed since the urine urea concentration at all times remained higher than the simultaneous plasma urea concentration. They postulated that during low nitrogen intake in camels, the renal tubules must either vary their permeability to urea in a highly selective manner, or secrete urea actively.

Schmidt-Neilsen et al. (1958) fed diets containing either 7.5% or 1.9% digestible crude protein (D.C.P.) to sheep. They observed that when the low protein diet was fed, an increase in the inulin $\frac{u}{p}$ ratio of from 10 to 200 was accompanied by an increase in the percentage of filtered urea reabsorbed from 40% to 90%. In comparison, when the high protein diet was fed, percentage reabsorption of filtered urea was steady at between 35-55% over the same range of inulin $\frac{u}{p}$ ratios. Schmidt-Nielsen et al. (1958) concluded from these and other experiments that urea reabsorption by the renal tubules may be regulated through changes in tubular permeability or by "counter-current multiplier system" which involves active transport somewhere in the distal parts of the nephron.

A different conclusion was reached by McIntyre and Williams (1970), who studied urea reabsorption in experiments in which three diets varying in protein content were fed to sheep (1.14%, 3.49% and 11.18% D.C.P.). They observed that the amount of urea excreted in the urine was linearly related to the concentration of urea in the plasma ($P<0.01$) and also to urine flow rate ($P<0.01$). Urea reabsorption appeared to be largely restricted to the proximal tubule and the authors concluded that the concentration of urea in the plasma rather than any special renal mechanism determined the degree of tubular reabsorption.

In the last decade, micropuncture technique has been the most popular method used to study the trans-tubular movements of urea. Gottschalk et al. (1961) studied the trans-tubular movements of water and urea in the kidney of nondiuretic rats with normal protein intake and urine flow rate. It was found that 50% of the filtered urea had diffused out by the end of the proximal convoluted tubules due to concentration differences established by the reabsorption of ions and water. The tubular fluid that had traversed the Loops of Henle contained about 110% of the original filtered urea, 70% of the filtered moiety entered the collecting ducts and since inulin was concentrated from a $\frac{u}{p}$ ratio of 14.9 in the fluid entering the collecting ducts to a $\frac{u}{p}$ ratio of 690 in that leaving as urine, these authors believed that a gradient could be established to cause the diffusion of urea from the collecting duct urine into the medullary and papillary interstitial fluid. Furthermore, by the same technique it was found that during mannitol diuresis (Ullrich et al., 1963) as well as saline diuresis in rats (Lassiter et al., 1964) less urea was lost from the proximal convoluted tubules and collecting duct and less was consequently added to the descending limb of the Loop of Henle. The urea clearance was consequently higher during diuresis.

All the findings thus far from micropuncture technique indicate the recycling of urea from the medullary and papillary interstitial fluid into the descending limb of the Loop of Henle but lend no support to the hypothesis of active transport of urea in the renal tubule as proposed by Schmidt-Neilsen et al. (1958). Of course, these findings were not conclusive because concurrent measurements on the urea concentration in the papillary and medullary interstitial fluid were not made.

Clapp (1966) investigated the renal reabsorption of urea in protein-depleted rats (fed 6% D.C.P.) during mannitol diuresis by micropuncture technique. In the same experiment, the papillary tissues of another group of rats under the same treatment were removed and analyzed for urea in the tissue water. It was found that the urea concentration in the distal convoluted fluid was higher than that at the end of the collecting duct while the urea concentration in the papillary tissue water was consistently higher than that in the final urine. These data seem to suggest that urea is reabsorbed from the collecting duct by active transport. One criticism of this experiment is that all the tubular fluids were taken from the cortical nephrons and none from juxtamedullary nephrons, while the final urine is derived from the tubular fluids of both kinds of nephrons. The surface nephrons which are accessible to micropuncture do not extend deeply into the medullary and papillary tissues

whereas juxtamedullary nephrons which are not accessible to micropuncture extend deeply into the papillary tissues, thus passing through an area of higher urea concentration which may favour increased net urea addition to the Loop of Henle in these nephrons and hence passive reabsorption from the collecting duct fluid. Another possibility one has to consider is that portion of the medullary and papillary tissue urea exists in an inactive or bound form (to protein) so that a measurement of total urea concentration in these tissues would not accurately reflect the true concentration gradient against which net urea reabsorption occurs.

Lassiter et al. (1966) repeated their investigations of urea transport in rats (Lassiter et al., 1964) by micropuncture technique with the following modification. Young rats were used in this study because it was possible to expose the tips of the renal papillae by opening the upper portion of the ureter and hence samples of blood and tubular fluid could be obtained at the tip of the papilla from the vasa recta and collecting ducts of juxtamedullary nephrons respectively. Urea C^{14} and inulin-methoxy - H^3 were injected to anaesthetized young rats and radio-activities were determined in vena cava plasma and in fluid obtained by micropuncture from the Loops of Henle, vasa recta, and collecting ducts in the papilla, and from distal convoluted tubules of cortical nephrons on the surface of the kidney. Mean urea concentration was

significantly greater in vasa recta (VR) than in adjacent collecting ducts (CD) in protein depleted rats fed 6% D.C.P. (mean VR/CD = 1.14; $P < 0.02$) and also marginally significant in rats on a normal protein diet (23% D.C.P.) during osmotic diuresis (mean VR/CD = 1.08; $P < 0.10$). Urea concentration in fluid from the Loops of Henle did not differ significantly from that in collecting duct fluid in protein depleted diuretic rats. Urea and inulin fluid-to-plasma ratios were higher in distal convoluted tubules than in the collecting ducts. The authors felt that the most reasonable interpretation of these data was that the higher urea concentration in vasa recta than in collecting ducts was a reflection of active transport out of the collecting ducts. Although this phenomenon was most readily demonstrated in protein-depleted animals, the data also suggested that it might also be observed in normal rats if the urine urea concentration was sufficiently reduced by osmotic diuresis. One important effect of protein depletion might be to reduce the urine urea concentration sufficiently to unmask the active transport, which under ordinary circumstances was obscured by a much larger movement of urea in the direction of its activity gradient out of the collecting ducts.

Of course, all the micropuncture experiments reviewed so far have a few common disadvantages. These experiments have to be carried out under anaesthesia and on a short term basis. Anaesthesia may temporarily

change the renal haemodynamics. While it takes at least a few hours for the osmotic pressure and concentration of various ions in the renal tissues to establish or equilibrate their gradients after infusion, data obtained on a short term basis can be misleading. Last but not least, no technique has yet been developed to micropuncture the juxtamedullary nephrons at the level of proximal and distal convoluted tubules.

Goldstein and Levitt (1970) investigated the mechanism of urea excretion in man and dog by the conventional infusion method. It was observed that urea excretion in man and dog was influenced by urine flow rate. Inhibition of sodium and water reabsorption in the proximal convoluted tubules by infusion of 3% mannitol or by acetazolamide was associated with a parallel change in urea reabsorption while inhibition of sodium and water reabsorption in the distal tubules by infusion of 3% mercaptomerin or ethacrynic acid did not affect urea excretion significantly. However, no mention of amount of dietary protein was made in this report. The author concluded that urea in man and dog was passively reabsorbed and dependent on the reabsorption of sodium and water, and the major fraction of urea reabsorption took place in the proximal convoluted tubules.

OBJECTIVES OF THE EXPERIMENT REPORTED HEREIN

Experiments reported on renal tubular reabsorption of urea in ruminants and monogastrics have been contradictory. Opinions differ as to whether this reabsorption is passively or actively regulated. The purpose of this experiment, therefore, was to study the long term effects of different levels of dietary non-protein nitrogen and water intake on GFR and urea excretion in sheep.

MATERIALS AND METHODS

Three Suffolk x Rambouillet ewes about two years of age were used. Several months prior to the start of the experiments, an exteriorised loop of the right carotid was established in each animal using the operative techniques described by Phillips (1968). The ewes were also fitted with permanent cannulas into the rumen. The ewes weighed 60-70 Kg. and were selected for uniform body size at the time of the operation.

Management

The ewes were chained to a continuous feeder in which the feed pellets were spread on conveyors and the speed of the conveyors so set that a small quantity of pellets was dropped from the conveyors to the respective mangers every three minutes. The pellets were spread out on the conveyor so that it took 24 hours to convey 0.8 Kg. of pellets into each manger. A 60 watts ceiling light was kept on all the time. The experimental animals were subjected to training periods until they would eat the pellets immediately after they were dropped into the manger. During this training period, animals were allowed water ad lib. Three days before each collection, water was infused into the rumen by a Harvard peristaltic pump (Model 600-1200) at the rate of 3 litres per 24 hours. (S.D.$\pm$ 300 ml/day).

Reason for using the continuous feeder and for continuous infusion of water into the rumen

The pellets were fed to the experimental animals by continuous feeder in order to achieve constant fermentation and reabsorption of nutrients whereby the urea concentration in the plasma would not vary a great deal. Constant plasma urea concentration is important because it may be a factor in regulation of urea clearance. Ibrahim et al. (1969) found that cows fed by continuous feeder had virtually a constant rate of fermentation in the rumen. Continuous infusion of water into the rumen was used as a means to regulate water intake in order to produce constant low urine flow rate. This prevents sudden large urine volume output which may result in a washout effect of urea and period to period variability of urea clearance if the animals are allowed to drink ad lib.

Diets

Three dietary treatments (Table 1) were employed. When formulating the diets, it was expected that each experimental animal would consume approximately 1.3 Kg. of pellets per day so that animals fed diets 1, 2 and 3 would be on high, medium and low protein intake respectively. However, during all the experimental periods, each animal only consumed about 0.8 Kg. of pellets per day and calculation based on this amount of food consumption shows that animals on treatment 1 had high protein intake while

Table I Diet Constituents

Trt.	No. of Animals	Description		Calculated D.C.P.(%)	Calculated D.C.P.* intake per day (gm)
1 (High Protein Diet)	3	Oat straw	34%	12	96.0
		Starch	2%		
		Molasses	4%		
		Sugar	2%		
		Brewer's Grain	58%		
2 (Medium Protein Diet)	3	Oat straw	51%	5.7	45.6
		Starch	10%		
		Molasses	4%		
		Sugar	10%		
		Brewer's Grain	25%		
3 (Low Protein Diet)	3	Oat straw	60%	2.1	16.8
		Starch	18%		
		Molasses	4%		
		Sugar	10%		
		Brewer's Grain	8%		

*D.C.P. = Digestible Crude Protein

those on treatments 2 and 3 had low protein intake (Table 1) based on N.R.C. requirements for maintenance of non-pregnant ewes (N.R.C., 1964). The basal ingredients were the same in all three diets so that the nitrogen and energy sources were the same in all treatments. Even though the consumption levels of dietary protein were not as high as expected, for the sake of convenience, treatments 1, 2 and 3 are termed high, medium and low protein diets respectively.

The pH of the urine in sheep can vary between 5 and 8 (Hungate, 1966). More nitrogen is excreted in the form of ammonia in acid urine than in alkaline urine (Christensen, 1965). Preliminary trials showed that all the experimental animals secreted acid urine on all three diets. Therefore 70 gm of potassium bicarbonate were dissolved into the daily allowance of water infused into the rumen, to ensure alkaline urine.

Minerals and salts contents of each of the diets were calculated and extra minerals, salts and vitamins were added so that they all met the N.R.C. requirements (N.R.C., 1964). In this way, all the three diets contained approximately the same amount of salts and minerals per Kg.

Catheterization techniques

The techniques used were similar to those described by Phillips (1968). Jugular catheterization involved inserting a "Selflex" guide wire (O.D. 0.035) through a 16 SWG needle and into the blood vessel. The needle was then completely withdrawn and then the catheter (Clay-Adams Polythene PE160) was threaded over the guide wire and into the blood vessel. The guide wire was then removed slowly. About 6 inches of the catheter was inserted in the blood vessel. The exterior end of the catheter was connected to a nylon 3-way stopcock by means of a "Tuohy-Borst" adaptor. The catheter was threaded through some of the wool on the back and the connector was tied on to a small tuft of wool. The catheter was taped around the neck region. After flushing with heparinized saline, the catheter was ready to use.

Exactly the same technique was used to catheterize the carotid artery except that 18SWG needle, Polythene catheter (PE 90) and "Selflex" guide wire (O.D. 0.025) were used.

The urethra and vulva were anaesthetized with "Xylocaine Gel" and the bladder was catheterized with a 12 FG Foley balloon catheter. A wire stiffener was first put into the catheter and this facilitated guiding the catheter into the urethra with the tip of the index

finger inserted into the vulva. After withdrawing the guide wire, about 7-8 ml of water were injected to inflate the balloon so as to retain the catheter in place. A 3-way nylon stopcock was attached to the end of the catheter to enable anaerobic sampling of the urine. Attached to the 3-way stopcock was a small rubber drainage tube leading to a measuring cylinder.

Blood and urine sampling technique

The dead space in a 10 ml plastic syringe and that of an attached 3-way stopcock was filled with heparin solution (1000 units/ml). On the side arm of this stopcock there was an empty 5 ml syringe. The stopcock of the 10 ml syringe was connected to the stopcock on the carotid catheter and about $1\frac{1}{2}$ ml of blood was drawn into the side arm syringe so as to flush the catheter. Then 10 ml of blood was drawn slowly into the sample syringe after which the stopcock was closed. The blood in the side arm syringe was returned to the animal. The catheter was then flushed with heparinized saline from a third syringe attached to the side arm of the catheter stopcock. About 8 ml of blood was transferred to a centrifuge tube and the plasma was separated into a sample bottle and stored in a freezer. The syringe containing the remaining blood sample was chilled in iced water to reduce glycolysis.

Anaerobic urine samples were taken into a 2 ml plastic syringe. All the small air bubbles trapped in the dead space of the syringe were ejected and the 3-way stopcock of the sample syringe was closed. The urine samples were also chilled in iced water. Whenever time allowed during the experiment, the pH's of the blood and urine samples were measured. Urine samples contained in small bottles were also stored in the freezer.

Measurement of pH

Blood and urine pH's were measured in the microelectrode attached to a "Radiometer pH meter" (Model PHM 25). Duplicate readings of each sample were made.

Chemical analysis

Urea in plasma and urine samples were analysed with a Technicon autoanalyser II (Model 7-70-140A). The methodology employed was that according to the manual supplied by the manufacturer. Inulin and PAH analysis on blood and urine samples were done by the Medical College Laboratory, the University of Manitoba, using a Technicon autoanalyser. A Fisher Diluter (Model 240) was used to dilute urine samples for autoanalysis.

Measurement of Osmolality

Osmolalities of plasma and urine samples were measured by osmometer (Model 31LAS, Advanced Instruments Incl.), using the 2 ml sample technique for both plasma and urine samples.

Statistical analysis

Regression and correlation, standard error, mean and one-way analysis of variance were calculated on an electronic desk computer (Olivetti Programma 101), while tests of significance of the differences between means were made using Duncan's Multiple range test (Snedecor, 1956). In the evaluation of the statistics the level of probability accepted as being significant was $P < 0.05$.

Experimental procedure and sampling schedule

The experimental animals were chained to and fed with the continuous feeder throughout all the experiments. Fourteen days of adjustment period were allowed for each diet. The diets were allotted to each animal at random and each animal was to go through all three different diets. Thus a Latin Square sequence of dietary treatments was used.

At the beginning of each experiment, the carotid artery, jugular vein and urinary bladder were catheterized. After a blood and urine sample had been taken from the carotid catheter and bladder catheter respectively to provide blank values, two loading doses (50 ml of 10% inulin solution, and 50 ml of 0.6% PAH solution) were injected through the venous catheter. Immediately afterwards, a continuous infusion of inulin-para-amino-hippuric acid solution at the rate of 0.7 ml per minute (0.7-0.9 ml/min) was started, using a syringe pump.

The inulin-para-aminohippuric acid infusion solution contained 1.263 gm% of inulin and 0.368 gm% of PAH.

One hour after the start of the infusion, the first of the four 30 minute blood and urine sample collection periods for normal urine flow rate was started. After the fourth period, water was pumped into the rumen at an average rate of 456 ml/hour (400-555 ml/min) by a peristaltic pump to produce water diuresis. The first of the six 30 minute blood and urine sample collection periods for water diuresis was started when urine flow rate reached a steady rate of at least 7 ml/min. The time required to produce this urine flow rate varied between animals.

RESULTS AND DISCUSSION

The results and discussion of this experiment were reported in two sections.

Section I

Observation on C_I, C_{PAH}, C_{H_2O}, Posm and Uosm during normal and high water intake

Since the fraction of filtered urea nitrogen excreted in urine is calculated by the ratio $\frac{Cu}{C_I}$, any changes in GFR at different levels of water intake is an important parameter in the interpretation of factors influencing urea excretion in Section II of Results and Discussion. This section, therefore, is to investigate whether the GFR changes significantly in long term diuresis under the same dietary treatment.

Results

Tables II and III respectively show that C_I and C_{PAH} did not change significantly during diuresis. Diuresis resulted in increased UFR and C_{H_2O} and slightly increased Cosm (Fig.I). The Posm was not significantly changed while the Uosm changed from hypertonic in low UFR to hypotonic during diuresis (Appendix Tables 1-9).

Fig.II illustrates the changes in urine volumes during the period of inducing steady diuresis. In all

Table II Glomerular filtration rates during normal and high water intakes (means ± S.D.)

Period	Dietary Treatment and Protein Content	Animal No. and Date of Experiment	Glomerular Filtration* Rate (ml/min) Normal water intake	Glomerular Filtration* Rate (ml/min) High water intake
1	1 (High)	11/8 50	59 ± 8 (4)	67 ± 7 (6)
	2 (Medium)	12/8 103	104 ± 5 (4)	94 ± 11 (6)
	3 (Low)	4/11 Y*	95 ± 10 (4)	102 ± 8 (6)
2	2 (Medium)	10/9 103	96 ± 8 (4)	89 ± 10 (6)
	1 (High)	11/9 164	67 ± 7 (4)	74 ± 7 (6)
	3 (Low)	17/9 50	71 ± 8 (4)	63 ± 8 (6)
3	3 (Low)	24/9 103	94 ± 13 (4)	92 ± 3 (6)
	2 (Medium)	29/9 164	83 ± 6 (4)	75 ± 9 (6)
	1 (High)	2/10 50	93 ± 7 (4)	86 ± 8 (6)

(Numbers in parentheses are the number of collection periods for which the respective means and S.D. were calculated.)

In the first period, treatment 3, animal No.2 died during the experiment.

* Starting from the second period, animal No.164 was used. Unfortunately No.164 also died two weeks after the completion of the third period; so animal Y was used to complete the first period.

* Glomerular Filtration Rate is measured by C_I.

Table III Means and standard deviation of para-aminohippuric acid clearances during normal and high water intakes

Animal No. and Date of Experiment		Dietary Treatment	Para-aminohippuric acid clearance (ml/min)	
			Normal UFR	Diuresis
50	11/8	2 (Medium Prot.)	495 ± 38 (4)	500 ± 30 (6)
164	11/9	1 (High Prot.)	504 ± 47 (4)	526 ± 42 (6)
103	24/9	3 (Low Prot.)	547 ± 21 (4)	532 ± 27 (6)
Y	2/10	3 (Low Prot.)	573 ± 43 (4)	604 ± 45 (6)

(Numbers in parentheses are the number of collection periods for which the respective means and S.D. were calculated.)

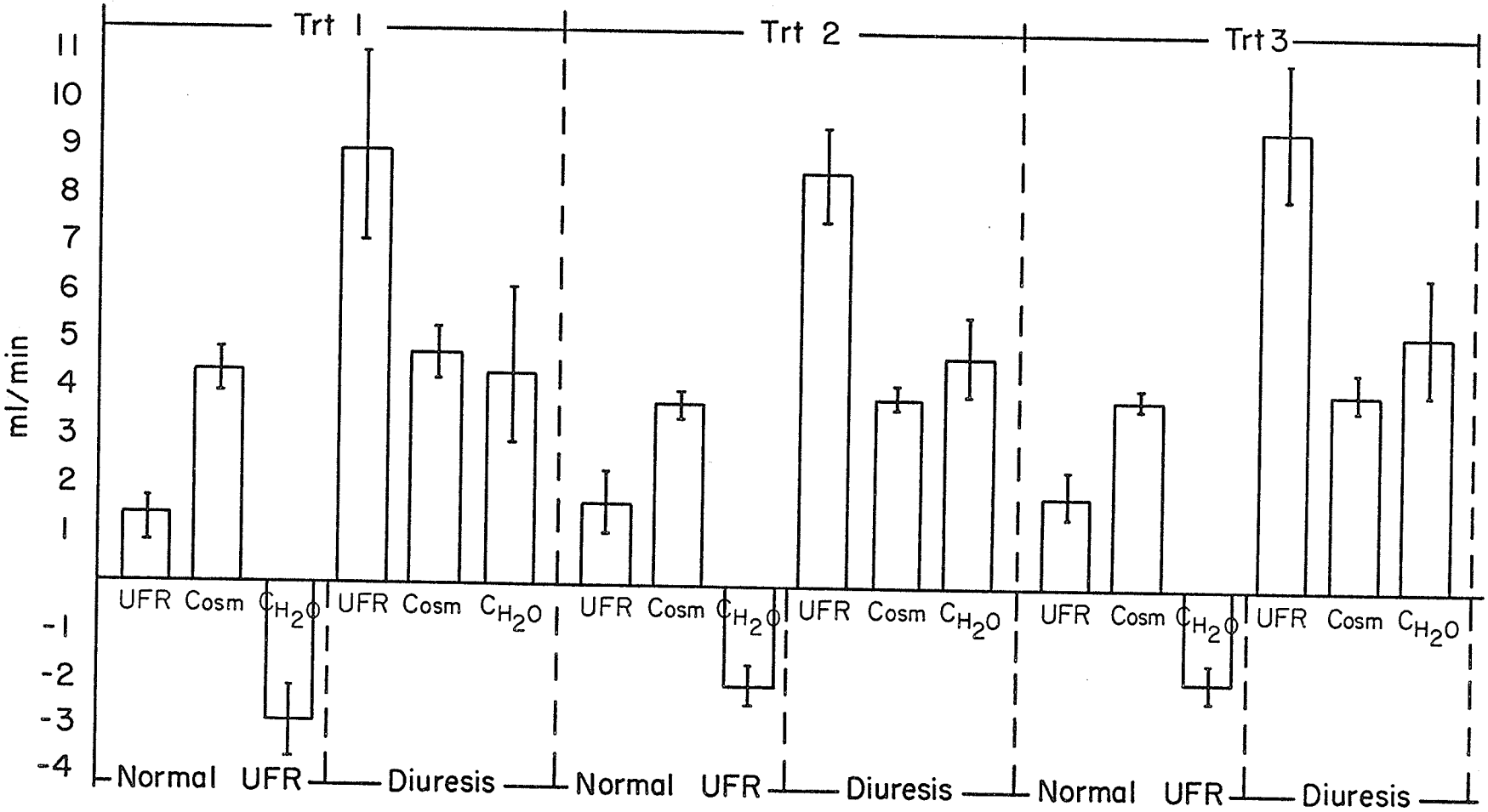


Fig. I Urine flow rate, osmotic clearance and free water clearance on high, medium and low protein diets during normal urine flow rate and water diuresis. (Bars represent standard deviations.)

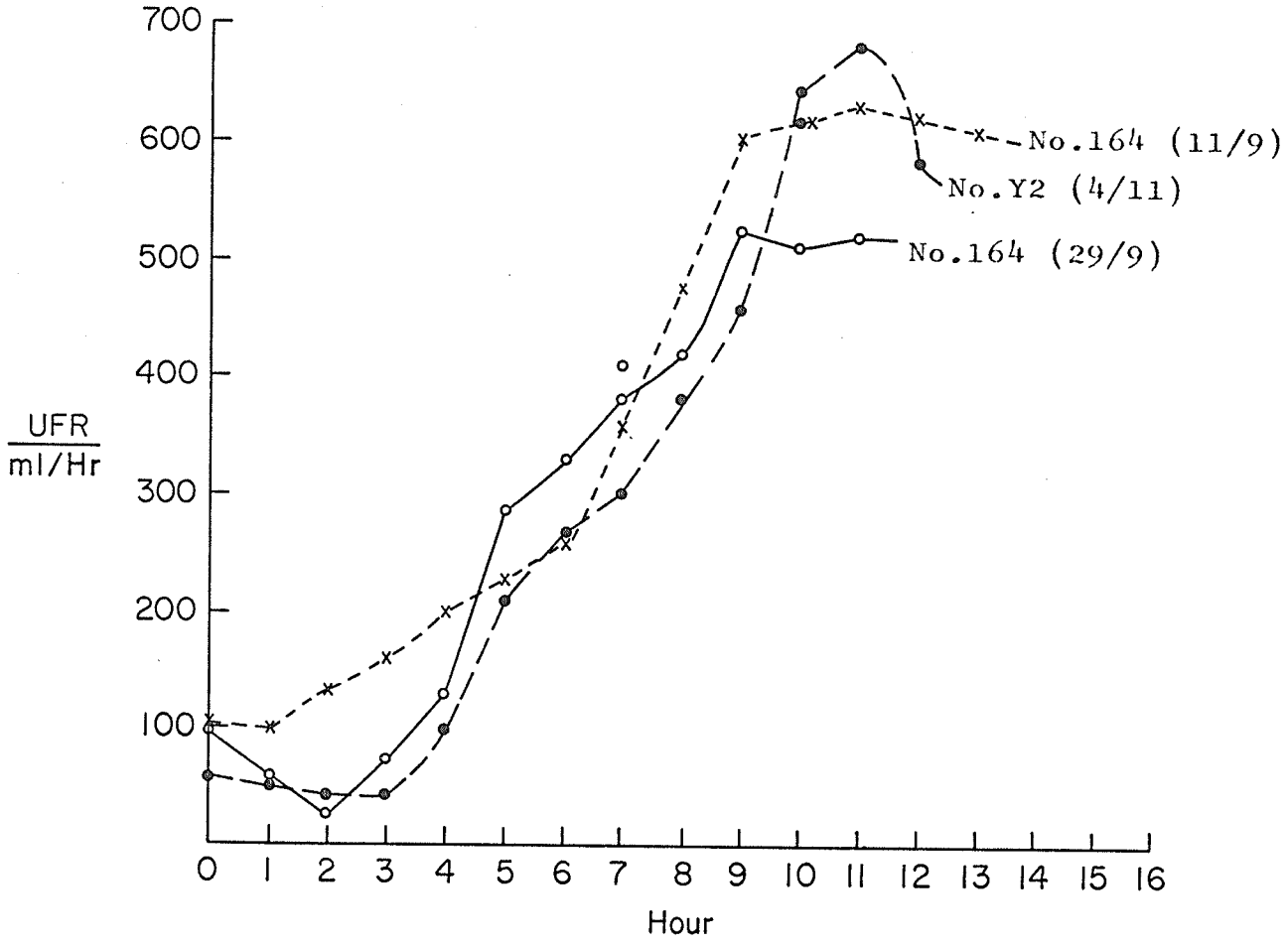


Fig. II Changes in urine flow rate in producing water diuresis

cases, diuresis started gradually after a latent period of 3-4 hours and maximum renal response occurred after 9-12 hours during which time the volumes of urine output were higher than the volumes of water infused per hour.

Discussion

The present observations that the kidneys, in water diuresis, responded selectively by an enormous increase in water output with little associated loss of osmotic particles, no change in renal plasma flow rate or GFR are all in keeping with the hypothesis of Keele and Neil (1965) and observations in dogs reported by Atkins and Pearce (1959). The slight increase in Cosm due to wash-out effect did not affect the Posm to any significant extent but the increase in C_{H_2O} was responsible for the hypotonicity of urine during diuresis (Figs.I and Appendix Tables 1-9).

The factors which effect changes in GFR are the mean arterial blood pressure, glomerular capillary pressure and colloid osmotic pressure and intracapsular tissue hydrostatic pressure (White, 1929). Despite the fact that no attempts had been made to measure the aforesaid parameters in the present experiments, the following indirect evidence may suffice to support the above observations on GFR in water diuresis.

The observations that C_{PAH} (which measures effective renal plasma flow) and Posm remained essentially unchanged in long term water diuresis would indicate that the renal haemodynamics did not change significantly between normal UFR and long term water diuresis. Furthermore, all the experimental animals were fed pellets using a continuous feeder and since the amounts of protein and

mineral salts were the same per kilogram of feed in the same diet, one would expect little or no change in plasma oncotic and osmotic pressures under the same dietary treatment if the volumes of body fluids were regulated by the kidneys. Marked increase in C_{H_2O} together with slight increase in C_{osm} further indicate the renal mechanism in defence of body fluid compartment in face of excessive water. During the early stage of diuresis, the total body fluid volumes might have been expanded but delay could reflect time of absorption from G.I. tract rather than in the body fluids. The osmoreceptors and volume receptors presumably acted to inhibit the secretion of ADH from the posterior pituitary gland which then rendered the distal convoluted tubules and collecting ducts impermeable to water (Pitts, 1965) and hence large amounts of water were excreted in urine in order to remove from the body the excess amount of water which had accumulated. Concomitant decreases in sodium concentration in plasma might have stimulated the excretion of aldosterone from the adrenal cortex (Renin-Angiotensin Theory) to activate the sodium pump in the distal convoluted tubules so that the rate of reabsorption of sodium was increased to compensate for the washout effect of high UFR. This can be deduced from the observation that even though C_{H_2O} increased, C_{osm} only slightly increased. All these observations seem to agree with the hypothesis that the

kidneys are very efficient in defending the body fluids against dilution (Pitts, 1965).

Similar observations were reported by Johnson et al. (1970) who infused hypotonic saline intravenously (7 ml/Kg of B.W. in 30 min.) into sheep to produce diuresis. They also reported that the left atrial blood pressure and mean arterial blood pressure, which rose in the beginning of diuresis, gradually returned to normal after one and a half hours and the plasma ADH concentration dropped from about 2.5 uU/ml to 0.5 uU/ml during the first two hours of diuresis. It has been found that ADH which has already been produced can be destroyed by body tissues at a rate of approximately one-half every eight minutes (Guyton, 1965). These findings from Johnson et al. (1970) lend further support to the present observations that GFR and C_{PAH} did not change significantly in long term water diuresis but that water diuresis did result in alterations of renal tubular function to regulate body fluids and electrolytes.

Section II

It is realized that any interpretation of results from experiments with small number of animals and large variations in body weight and age must be guarded. Difference in body weight and metabolism may affect the GFR. Poulsen (1957) suggested that GFR in heifers could be related to the body weight while Sellers *et al.* (1958) suggested that GFR in heifers could be related to the surface area. In view of this controversy, no attempt had been made to correct for the GFR of the experimental animals. In consideration of the above limitation, the discussion concerning urea excretion in this report will stress the relationship of the fraction of filtered urea nitrogen excreted in urine $(\frac{Cu}{C_I})$ and the ratio of plasma urea nitrogen and urine urea nitrogen $(\frac{Uu}{Pu})$ with water reabsorption rate by the kidneys $(\frac{U_I}{P_I})$ at different levels of water and dietary protein intake. These ratios are not significantly affected by the variations mentioned above and hence are good estimates of renal tubular function.

Results

(a) Treatment differences

The measurements shown in table IV are representative of four collection periods for each sheep on each dietary treatment during normal UFR. There were three sheep in each treatment. The UFR and GFR were not significantly different among treatments apart from period

Table IV Means and standard deviations for plasma urea nitrogen, urine flow rate, urea nitrogen clearance, fraction of filtered urea nitrogen excreted in the urine and GFR during low urine flow rate. (Measurements are taken at 30 min. intervals for 2 hours. There are 12 observations per treatment.)

Treatment	1 (High Prot.)	2 (Medium Prot.)	3 (Low Prot.)	Stat.
Plasma urea - N (mg%)	13.40 ± 0.90	6.14 ± 1.52	3.50 ± 0.38	1 > 2 & 3 2 > 3
Urine Flow Rate (ml/min)	1.45 ± 0.80	1.74 ± 0.72	1.89 ± 0.50	N.S.
Urea-N clearance (ml/min)	45.83 ± 8.80	30.83 ± 9.22	27.96 ± 7.49	1 > 2 & 3
$\frac{Cu}{C_I}$	0.523± 0.04	0.398± 0.08	0.327± 0.06	1 > 2 & 3 2 > 3
Urea-N Excretion Rate (mg/min)	6.22 ± 1.19	1.94 ± 0.84	0.96 ± 0.20	1 > 2 & 3 2 > 3
GFR (ml/min)	88 ± 15	79 ±17	86 ± 15	N.S.

to period variability.

The sheep on treatments 2 and 3 (fed 45.6 and 16.8 gm. of calculated D.C.P. per day respectively) had significantly lower levels of Pu (mg%), Cu (ml/min), $\frac{Cu}{C_I}$ and UER (mg/min) than the sheep on treatment 1 (fed 96 gm. of calculated D.C.P. per day). The sheep on treatment 3 also had significantly lower levels of Pu, $\frac{Cu}{C_I}$ and UER than sheep on treatment 2.

In sheep on high protein diet (treatment 1), the UFR had very little effect upon the fraction of filtered urea nitrogen excreted in the urine. The average fraction falls between 0.45-0.57 throughout the range of $\frac{U_I}{P_I}$ ratios between 30-140 (fig.IIIa). Statistical analysis of the regression line shows that the slope is not significantly different from zero. However, the $\frac{Uu}{Pu}$ ratio increased in proportion to the increase in $\frac{U_I}{P_I}$ ratio (fig.IVa).

The data obtained when the sheep were on treatments 2 and 3 (medium and low protein diets respectively) show a different relationship within treatment. The $\frac{Cu}{C_I}$ ratio decreased steadily with decrease in UFR (figs.IIIb and IIIc). Both slopes are statistically significant. However, the $\frac{Uu}{Pu}$ ratios in both cases start to level off when $\frac{U_I}{P_I}$ is greater than 30 (figs.IVa and IVb). Over the range of $\frac{U_I}{P_I}$ ratio approximately between 30-100, the slopes are not significantly different from zero.

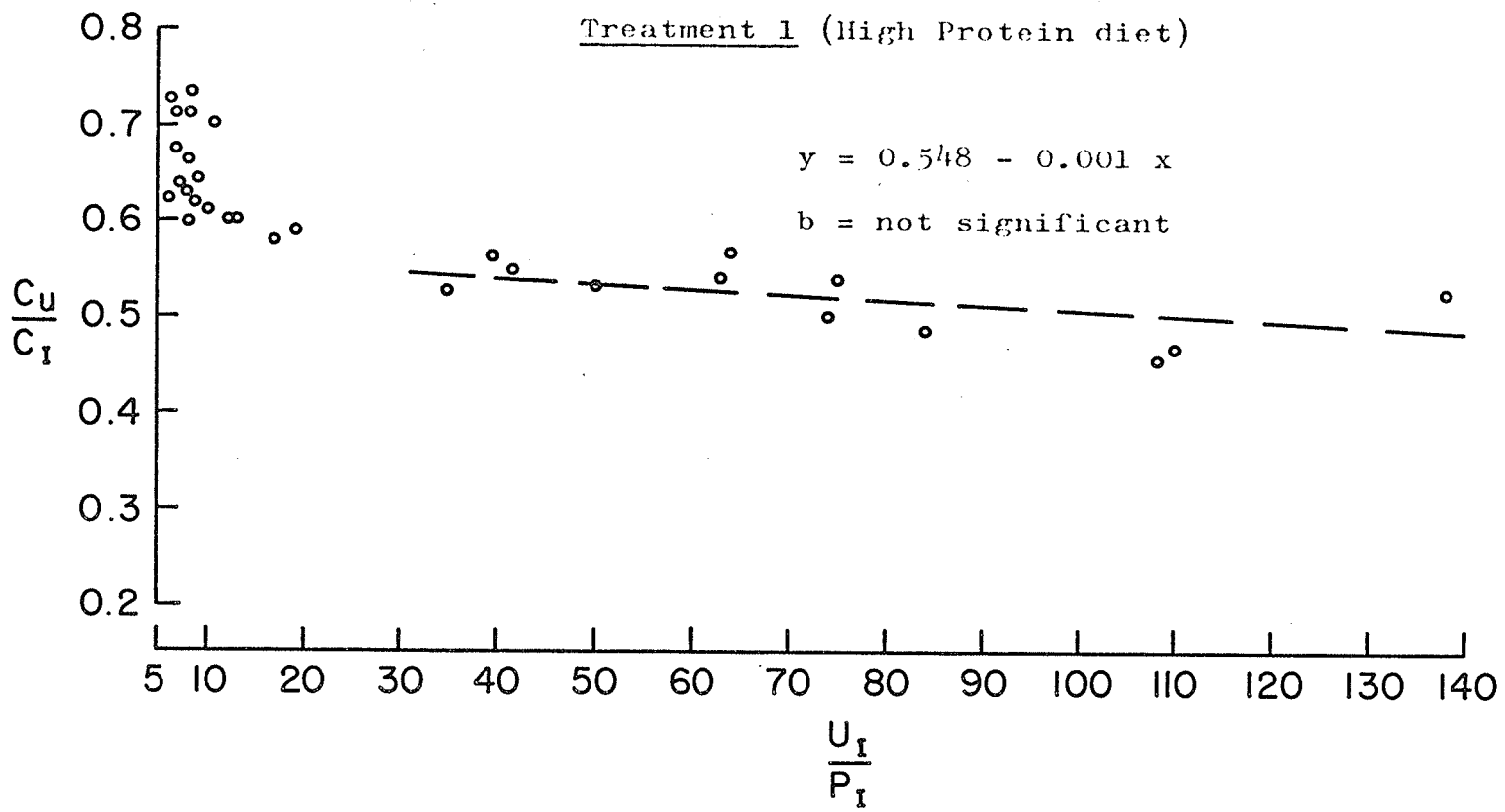


Fig.IIIa The relationship between inulin $\frac{u}{p}$ ratio and fraction of filtered urea nitrogen excreted in the urine during low urine flow rate

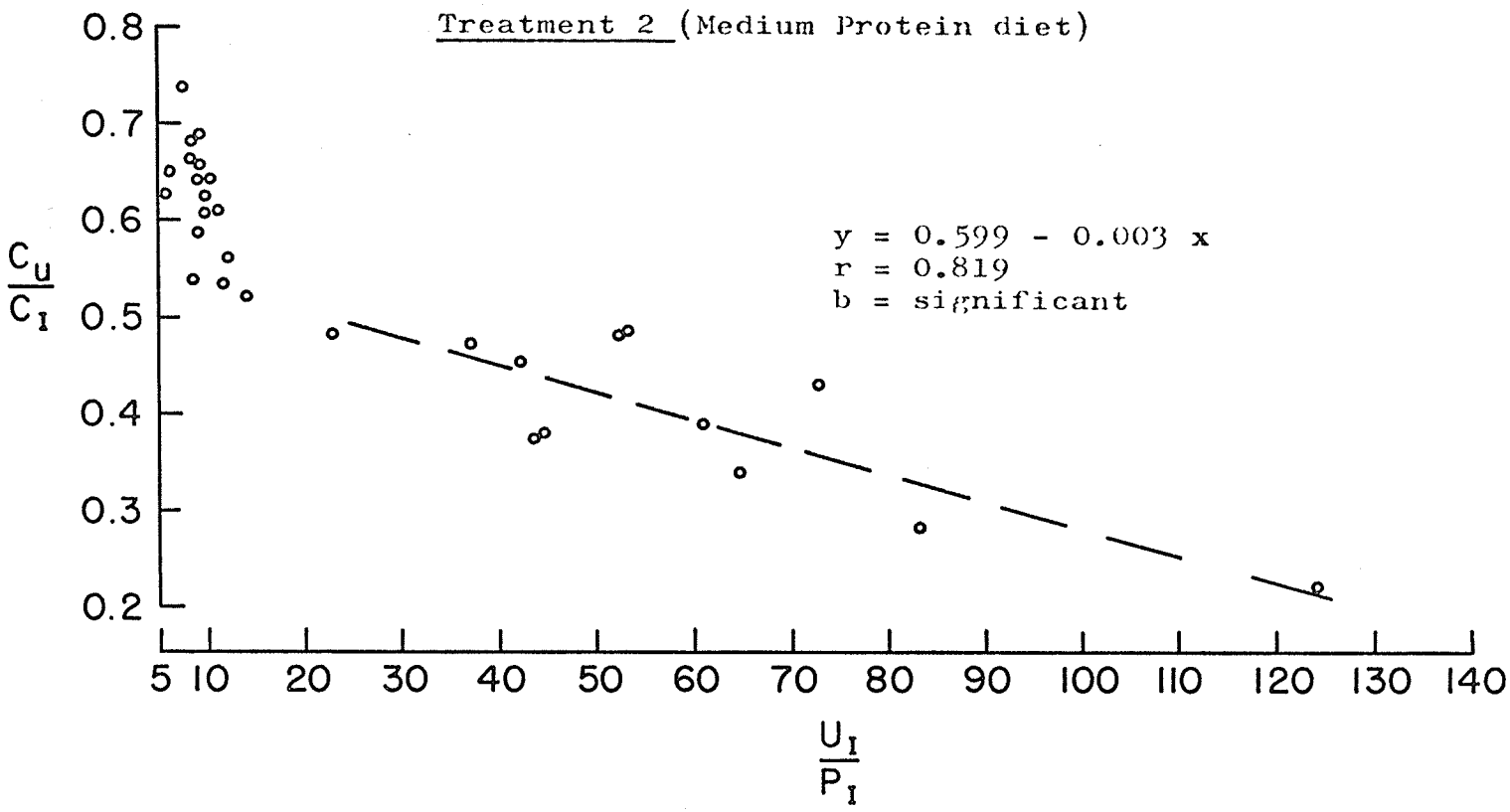


Fig.IIIb The relationship between inulin $\frac{u}{p}$ ratio and the fraction of filtered urea nitrogen excreted in the urine during low urine flow rate

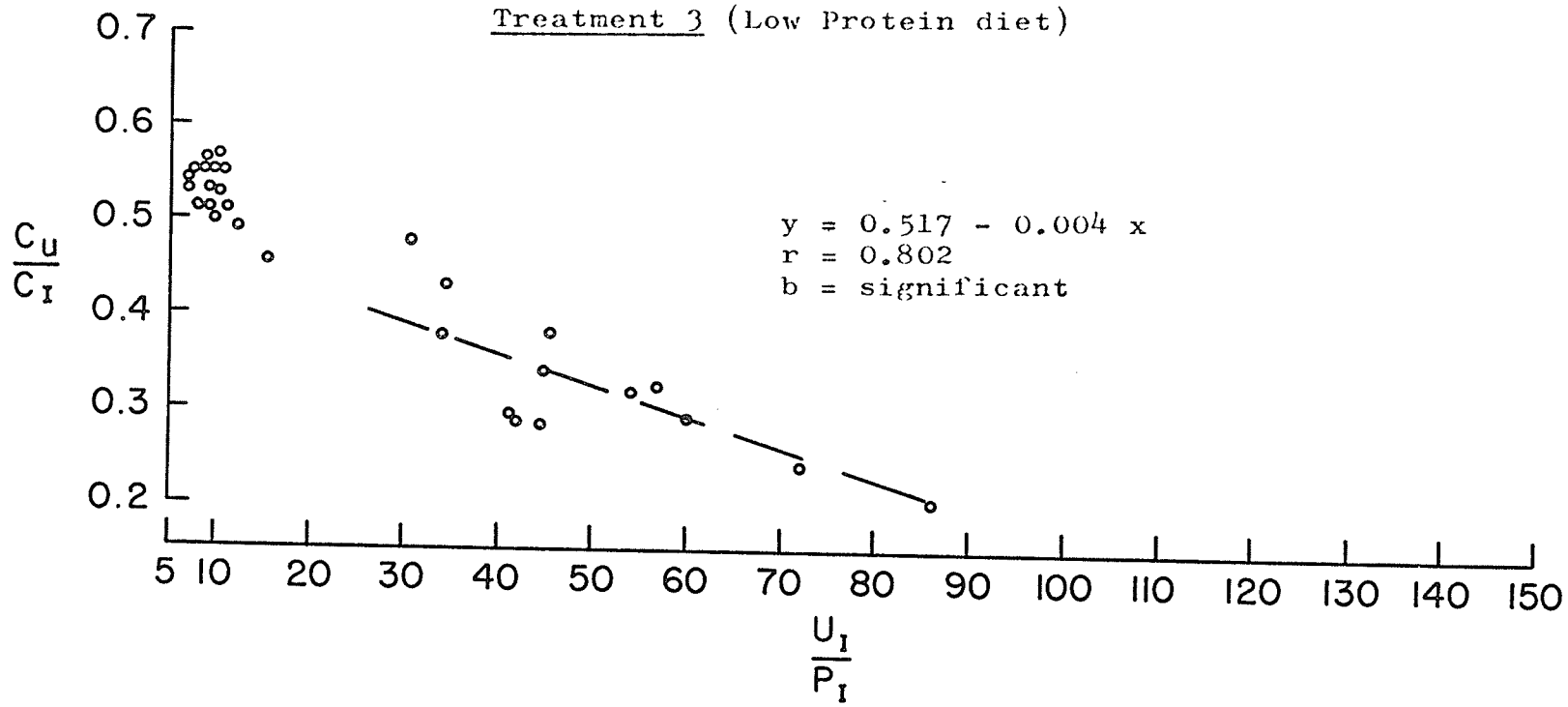


Fig.IIIc The relationship between inulin $\frac{u}{p}$ ratio and the fraction of filtered urea excreted in the urine during low urine flow rate

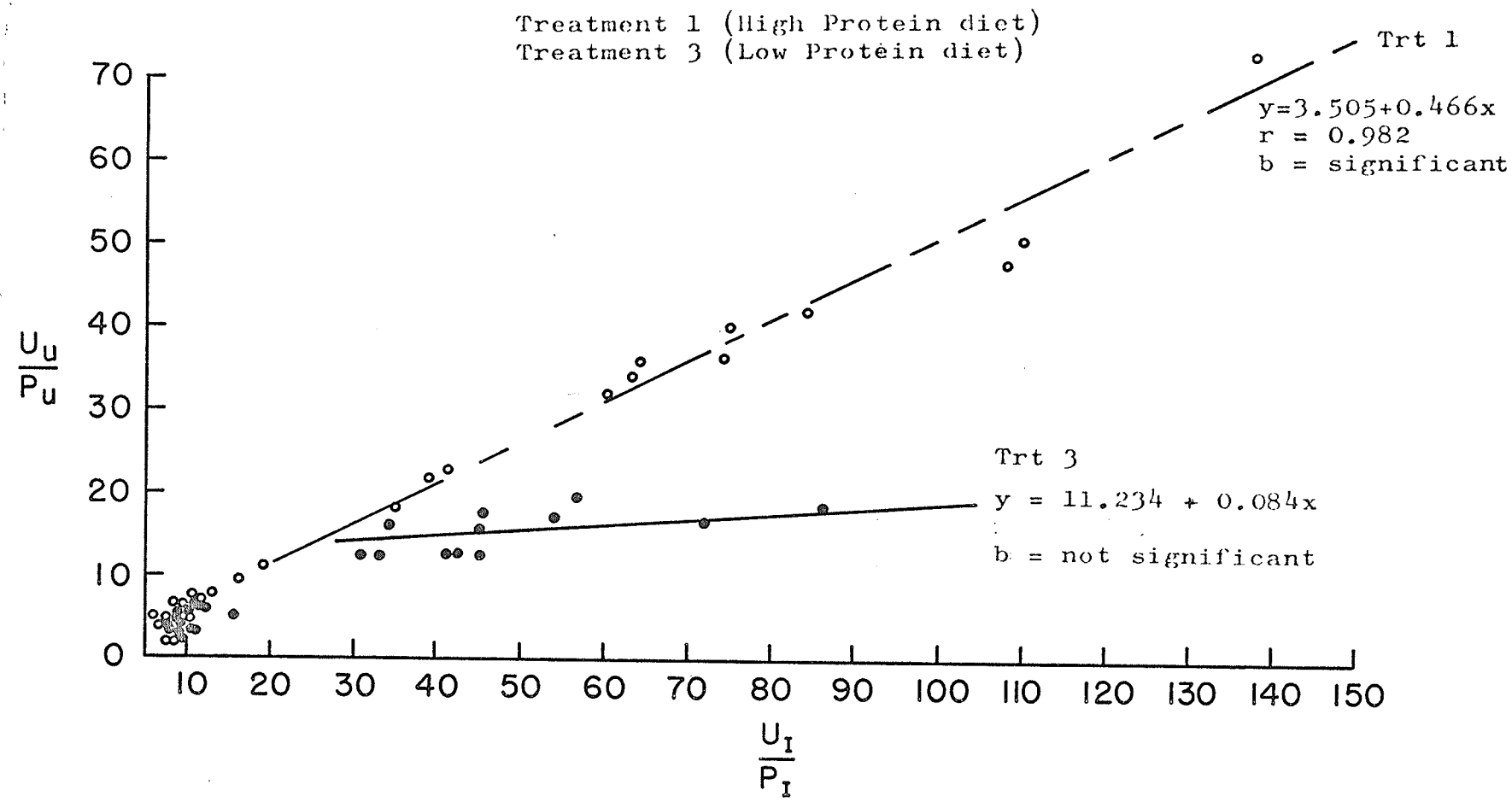


Fig.IVa The relationship between inulin $\frac{u}{p}$ ratio and urea nitrogen $\frac{u}{p}$ ratio during low urine flow rate

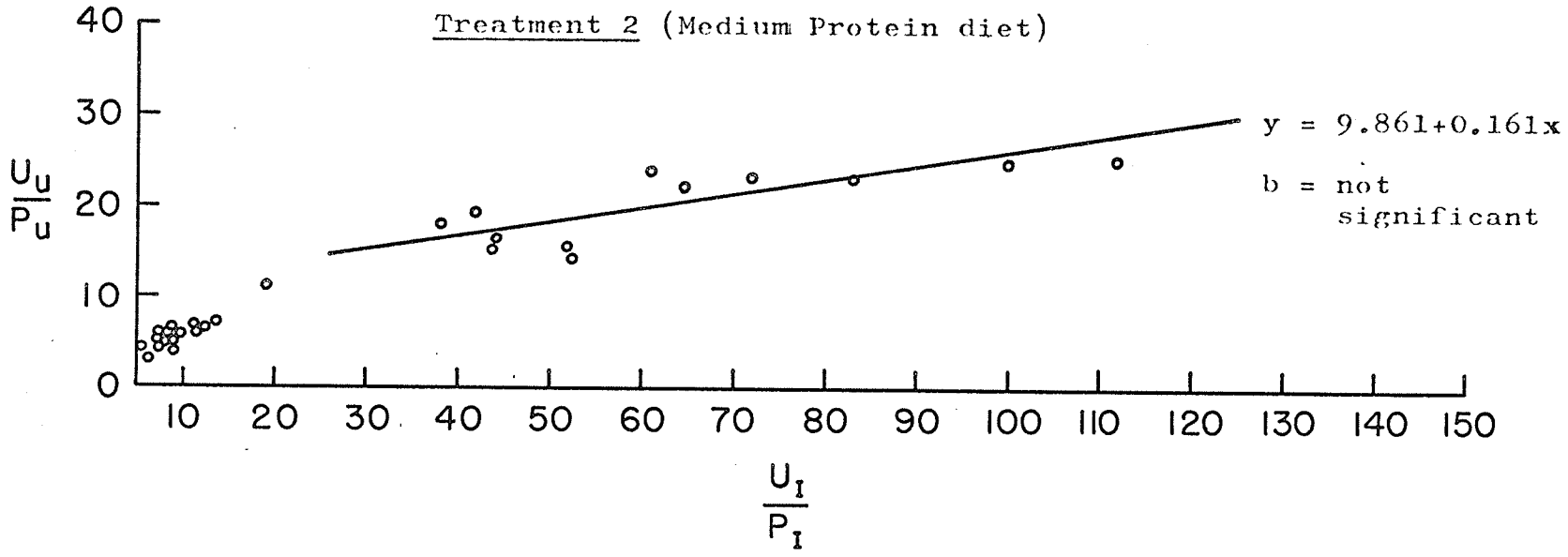


Fig.IVb The relationship between inulin $\frac{u}{p}$ ratio and urea nitrogen $\frac{u}{p}$ ratio during low urine flow rate

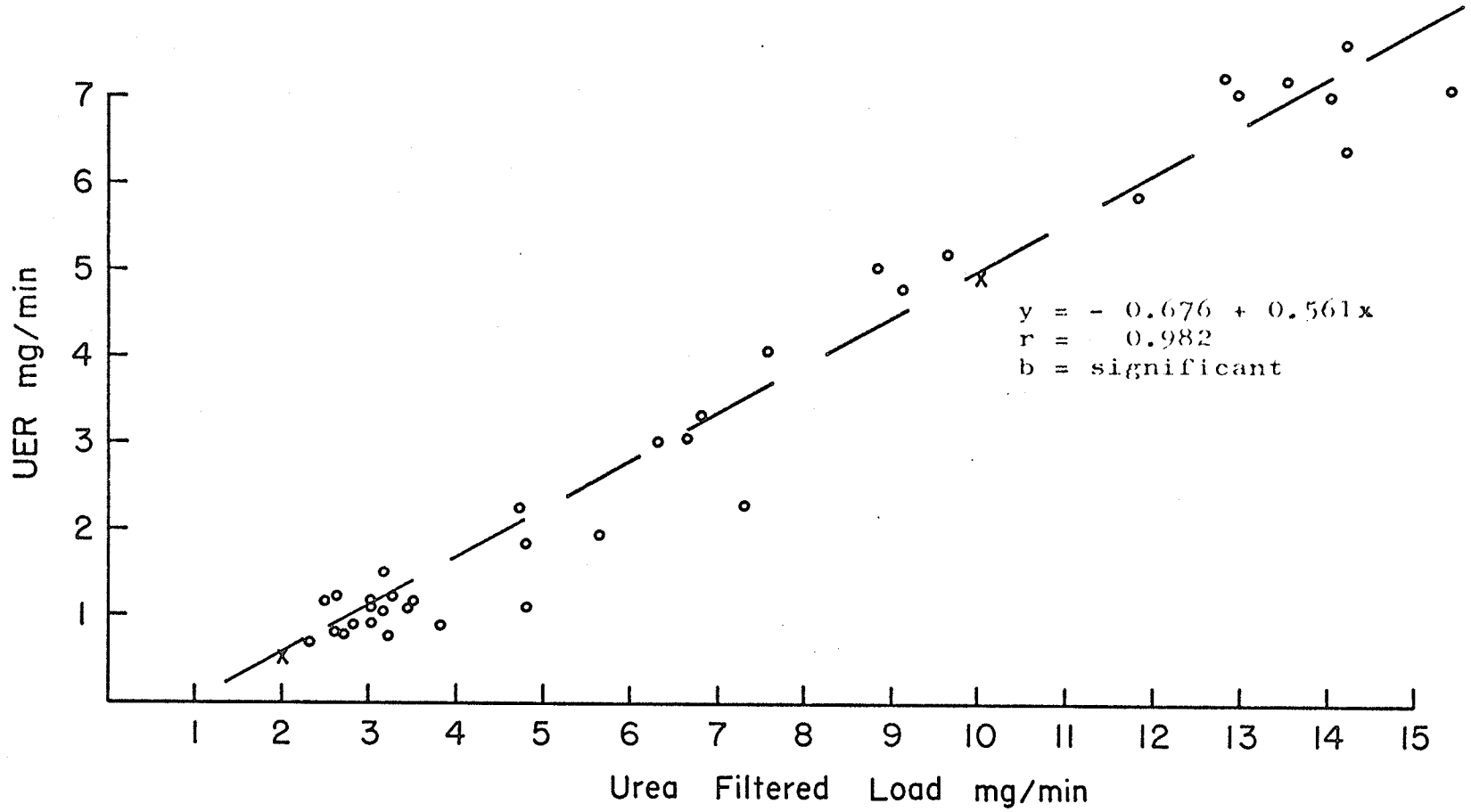


Fig.V The relationship between the amount of urea nitrogen excreted by the renal tubules and the amount filtered at the glomeruli during low urine flow rate

At high UFR, the relationships between $\frac{Cu}{C_I}$ and $\frac{U_I}{P_I}$ ratios in all three treatments respond similarly.

(b) The relationship between urea nitrogen filtered load and urea nitrogen excretion rate

Fig.V shows that, over a range of urea nitrogen filtered load varying from 2.3 mg. to 14.9 mg/min., the amount of urea nitrogen excreted increased as the amount of urea nitrogen filtered increased. The amount of urea nitrogen excreted is significantly related to $\dot{P}u$* but not to GFR or UFR when all the data from three treatments are considered.

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* $y = -1.003 + 0.5224\ x$
  $r = 0.951$

Discussion

The observations that Cu, $\frac{Cu}{C_I}$, and UER varied with the dietary protein imposed and were the lowest on the diet which produced the lowest Pu and that GFR was not significantly affected by the different levels of dietary protein intake are all in keeping with those data reported by Schmidt Nielsen et al. (1958); Gans (1966); McIntyre and Williams (1970); and Scott and Mason (1970).

The fact that GFR is not significantly affected by different levels of dietary protein intake suggests that urea reabsorption is not regulated at the glomerulus but at the tubular level. However, interpretation concerning dietary effects on Cu, $\frac{Cu}{C_I}$ and UER differs as to whether it may be explained by simple diffusion of urea as the result of changes in tubular permeability to urea (McIntyre and Williams, 1970) or by active tubular transport of urea (Schmidt-Nielsen et al., 1958). However, the experiment reported by McIntyre and Williams (1970) did not have any control on salt intake whereas that reported by Schmidt-Nielsen et al. (1958) involved abrupt change in diet which might change the salt and protein intakes.

If one should explain the effects of urine flow rate and dietary protein intake on urea excretion in sheep according to the permeability hypothesis, the findings that on a high protein diet the $\frac{Cu}{C_I}$ ratios does not change in the range of urine flow rate corresponding to $\frac{U_I}{P_I}$ ratios

between 30-140 in the present experiment, 10-600 in hydropenic sheep (Schmidt-Nielsen et al., 1958) and 100-500 in hydropenic sheep (Gans, 1966) lead one to conclude that the distal portion of the nephron in sheep kidneys on a high protein diet is not very permeable to urea. One must also conclude that the same portion of the nephron under the condition of low protein intake becomes so permeable to urea that equilibrium is almost reached between tubular fluid, surrounding tissue fluid and plasma in order to reduce the $\frac{Cu}{C_I}$ and $\frac{Uu}{Pu}$ ratios to a very low value. Furthermore, if this is likely the case, one also has to assume that there is little or no concentration gradient of urea in the interstitial fluid from the cortex to the medulla on a low protein diet.

Of course, it is reasonable to assume that the distal convoluted tubules have very low permeability to urea. If this portion of the nephron were very permeable to urea, it should not be possible to produce an osmotic diuresis by increasing the plasma urea concentration and urea could not be concentrated in the urine by any known mechanism. It has been shown frequently that urea administration produces an osmotic diuresis (Mudge et al., 1949; Cizek and Holmes, 1950; Anslow and Wesson, 1955). The present results and those obtained by McIntyre and Williams (1970) show that urea in urine in high protein diets can be concentrated to 70 times as high as that in plasma and 200

times in hydropenic sheep on a normal protein diet (Schmidt-Nielsen et al., 1958). Furthermore, analysis of tissue fluid urea concentration in sheep kidney slices reveals that renal interstitial fluid urea concentration gradient does exist in both low (2% D.P.) and high (7.5% D.P.) protein diets and that the $\frac{Tu}{Pu}$ ratio is even higher in the medullary tissue of the kidney in sheep fed low protein diet (average $\frac{Tu}{Pu}$ = 73) than those fed high protein diet (average $\frac{Tu}{Pu}$ = 47), (Schmidt-Nielsen and O'Dell, 1959). This evidence suggests that the distal convoluted tubules are only slightly permeable to urea on both low and high protein diets and that the permeability does not increase on low protein diet.

ADH can dilate the pore diameter of collecting ducts and distal convoluted tubules to 4Å whereas the molecular diameters of water and urea are 3Å and 3.5 Å respectively (Pitts, 1965). It has been demonstrated that the permeability of the collecting ducts and distal convoluted tubules can be enhanced by ADH in dogs (Jaenike, 1961) and in sheep (Cross et al., 1966). However, in the present experiment, the water and salt intakes of the sheep on all three diets were approximately the same and the urine flow rates were not significantly different so it can be suggested that the action of ADH was more or less to the same extent in all the dietary treatments. The present data indicate that the levels of

Cu, $\frac{Cu}{C_I}$ and $\frac{Uu}{Pu}$ are much lower in sheep fed low protein diets than those on high protein diets, showing that ADH cannot be an important factor in regulation of urea reabsorption in sheep. Consequently the permeability hypothesis is not satisfactory in explaining the present data.

Perhaps the present data of urea excretion in sheep fed the high protein diet can better be explained by counter-current mechanism set up by the active transport of sodium out of the thick ascending limbs of Loop of Henle. During water diuresis, when sheep were fed the high protein diet (treatment 1), about 64.5% of the filtered urea were excreted in the urine. Therefore, about 35.5% of the filtered urea were reabsorbed by the proximal convoluted tubules assuming that there was little or no reabsorption of urea in the distal portion of the nephron during diuresis. This value is within the range of those found by Scott and Mason (1970). With low urine flow, on the same diet, one can expect that 64.5% of the filtered urea passes through the proximal convoluted tubules. In transit through the descending limb of Loop of Henle, urea is added to the tubular fluid from the medullary interstitial fluid due to concentration gradient. A small amount of urea may be lost at the distal convoluted tubules as it is slightly permeable to urea. As the tubular fluid passes to the collecting duct, the concentration of

urea in the tubular fluid, after water reabsorption and addition of urea from the medullary interstitial fluid, is high enough to permit back diffusion of urea into the medullary interstitial fluid and hence creating a high concentration of urea in this part of the renal tissue fluid so that urea can recirculate into the descending limb of Loop of Henle. This counter diffusion of urea possibly is augmented by a similar pattern of urea diffusion in the vasa recta, and urea is returned to the blood by these capillaries.

The present data on urea excretion for the high protein diet seem to agree with the aforesaid hypothesis. According to Pitts (1965), if urea is reabsorbed passively, then the urea clearance varies with urine flow rate. This also means that when UFR is decreased (i.e. increase in $\frac{U_I}{P_I}$), the urea concentration in the tubular fluid increases and the urea concentration in the urine also increases. When U_u increases, the ratio $\frac{Uu}{Pu}$ also increases. In other words, when the ratio $\frac{U_I}{P_I}$ increases, the $\frac{Uu}{Pu}$ ratio also increases proportionally if urea reabsorption follows water reabsorption. Consequently, the ratio $\frac{Uu}{Pu} \Big/ \frac{U_I}{P_I}$ does not change significantly when plotted against $\frac{U_I}{P_I}$ (fig.IIIa), bearing in mind that $\frac{Cu}{C_I}$ is equal to $\frac{\frac{Uu}{Pu}}{\frac{U_I}{P_I}}$ (see terminology). Data shown in fig.IVa further support the argument that $\frac{Uu}{Pu}$ increases proportionally with increase in $\frac{U_I}{P_I}$ ratio.

The above hypothesis is also supported by the findings by other investigators. Lassiter et al. (1961), using micropuncture technique, found that in the kidneys of rats fed a normal protein diet there was net loss of both water and solute from all segments of the nephron. In the Loop of Henle, water loss occurred primarily from the thin descending limb and solute loss (Na^+, Cl-) from the thick ascending limb by active transport of sodium. Urea was reabsorbed from the proximal and distal convoluted tubules as well as collecting ducts but was added to the tubular fluid in the descending limb of the Loop of Henle. Schmidt-Nielsen and O'Dell (1959) by tissue slice analysis method, found that in sheep kidney the renal tissue fluid urea concentration increases from the cortex to papilla and the urine urea concentration was usually higher than that in the renal interstitial fluid.

The above mentioned mechanism cannot satisfactorily explain the urea excretion in sheep fed low protein diet. Fig.IIIb and IIIc show that the $\frac{Cu}{C_I}$ ratio decreases with increase in $\frac{U_I}{P_I}$ ratio but $\frac{Uu}{Pu}$ ratio does not increase proportionally with $\frac{U_I}{P_I}$ ratio (figs.IVa and IVb). The average values of Cu, $\frac{Uu}{Pu}$, $\frac{Cu}{C_I}$ are much lower on low protein than high protein diet. It may be argued that the plasma urea concentration is lower in low protein diets and hence less urea is expected to be excreted (McIntyre and Williams (1970). However, the amount of salt and water intake were

approximately the same in all dietary treatments and the sheep in all experiments excreted alkaline urine, consequently one can expect that the counter-current mechanism should affect the $\frac{Cu}{C_I}$ and $\frac{Uu}{Pu}$ ratios to approximately the same extent in all dietary treatments and thus the above argument is not satisfactory.

The present data lead to the postulation of active transport of urea along the collecting duct. The tubular fluid traversed down the thin descending limb of Loop of Henle, carrying with it the urea which escaped passive reabsorption in the proximal convoluted tubules. But at the collecting duct, urea is transported from the tubular fluid to the medullary tissue fluid by both passive and active processes or by active transport alone. In this case, more urea is returned to interstitial fluid and then may be further carried away by the renal capillary vessels and urea is conserved. If active reabsorption does take place in the collecting ducts, one would also expect that the $\frac{Tu}{Pu}$ ratio in the medullary interstitial fluid is higher than the $\frac{Tu}{Pu}$ ratio on high protein diet because counter-current mechanism and active transport are taking place together, and also $\frac{Tu}{Pu}$ ratio decreases toward the tip of papilla. Anatomically, sheep have very long collecting ducts, so by the time the tubular fluid reaches the tip of papilla, the concentration of urea in the collecting duct urine and hence the fraction of filtered urea remaining can

drop to a low and steady value. This hypothesis seems to be supported by the experimental data that $\frac{Uu}{Pu}$ ratio does not increase proportionally with $\frac{U_I}{P_I}$ ratio and that $\frac{Cu}{C_I}$ ratio decreases with increase in $\frac{U_I}{P_I}$ ratio. The observation by kidney slice analysis that the $\frac{Tu}{Pu}$ ratio in renal medullary region in sheep is higher in low protein diet (average $\frac{Tu}{Pu}$ = 73) than in high protein diet (average $\frac{Tu}{Pu}$ = 47) and that the $\frac{Tu}{Pu}$ ratio decreases from the medulla to the papilla in low protein diet by Schmidt-Nielsen and O'Dell (1959) also lend further support to this hypothesis.

At high urine flows, the $\frac{Cu}{C_I}$ ratio increases towards unity in all three dietary treatments. Although observations concerning the movements of urea in the kidney during water diuresis is lacking, one may speculate that the $\frac{Tu}{Pu}$ gradient from cortex to papilla on all diets decreases due to rapid tubular flow and decrease in contact time. A large amount of filtered and tissue fluid urea is washed out and hence does not contribute to the set-up of $\frac{Tu}{Pu}$ gradient. It has been found that the concentrations of urea in the medullary interstitial fluid in dog kidney decreases during water diuresis (Levitin et al., 1962; Malvin and Wilde, 1959). Besides, the distal convoluted tubules and collecting ducts, during diuresis, are not permeable to water. Consequently any urea reabsorption would have resulted from proximal tubular reabsorption. The slightly lower average $\frac{Cu}{C_I}$ ratio

observed during diuresis in sheep fed low protein diet might also reflect the existence of active reabsorption of urea but at this stage, this active mechanism might have been largely overwhelmed or hindered by high urine flow rate.

If one is convinced that active reabsorption of urea does take place in the renal tubules on low protein diets, the most logical question that follows would be "What stimulates the active transport to take place?"

During low urine flows, over a range of filtered urea load varying from 2.5 to 15.5 mg/min, the amount of urea excreted increased as the amount of urea filtered increased (fig.V). The amount of urea excreted was not significantly related to GFR or UFR but significantly related to the concentration of plasma urea nitrogen. A similar relationship was also reported by McIntyre and Williams (1970); Scott and Mason (1970); and Thornton (1970c). However, one should not make a hasty attempt to conclude that active transport of urea is sensitive to the different levels of plasma urea nitrogen. It would give a better insight if control was made to regulate the rate of passage and reabsorption of protein in the digestive tract, and at the same time different levels of urea are infused intravenously while the animals were on low protein diet. In other words, factors such as nitrogen intake, or nitrogen metabolism should also be considered as the level of plasma urea is influenced by these factors.

A philosophical but reasonable question posed by the present results and discussion and those of other researchers, is the purpose of this increased urea reabsorption by sheep kidney when dietary intake of nitrogen is low. Schmidt-Nielsen et al. (1958) suggested that it may be of survival value, arguing that the return of urea to the rumen by way of rumen wall or saliva may lead to its incorporation into microbial protein of subsequent use to the host. Besides, in arid and semi-arid environments where rainfall is low, the nitrogen content of the forages is generally poor. Also, forages low in nitrogen content are often associated with low potassium content. This decreases dietary salt intake and hence decreases the renal excretory load as dietary potassium is mainly excreted by the kidneys. Consequently ruminants living under such conditions may be conserving water and urea at the same time. While such argument may be valid, it does not follow that active renal conservation is necessarily related to this urea cycle since the results obtained by micropuncture in dog (Goldberg et al., 1967) and the rat (Lassiter et al., 1966; Clapp, 1966) indicate that tubular active transport of urea also occurs. Scott and Mason (1970) suggest that active tubular reabsorption of urea on low protein diet may be a feature of mammalian kidney in general and rather specifically a feature of ruminants alone. However, one must not forget that the

Loop of Henle and collecting duct are much longer in sheep than those in the monogastric animals. Because of this anatomical modification, sheep can conserve water and urea more efficiently than many monogastric animals. From this point of view, it can be concluded that sheep kidney is more adapted for water and urea reabsorption than monogastric animals although the mechanism involved in urea reabsorption may be the same.

SUMMARY AND CONCLUSION

1.

Water diuresis on a long term basis results in a very marked rise in the urine flow rate and free water clearance, with only a slight rise in osmolar clearance. Inulin and para-aminohippuric acid clearances are not significantly changed, indicating renal response to water diuresis depends on alteration of tubular reabsorption.

2.

The GFR shows no consistent change with different levels of dietary protein intake. Therefore the renal regulation of urea excretion in sheep is unlikely to be at the glomerular level.

3.

During low urine flow rate, on high protein intake, renal reabsorption of urea is probably by passive reabsorption. Counter-current mechanism could have contributed to creating urea concentration gradient in the tubular fluids in the distal portion of the nephron.

4.

The regulation of urea excretion on low protein intake during low urine flow rates in sheep could be brought about through a change in the tubular permeability to urea or through an active urea transport and counter-current mechanism. The data obtained from both high and low

protein diets during low urine flow rate with control of salt and water intakes make the first possibility highly unlikely.

5.

The amount of filtered urea excreted increases as the concentration of urea in the plasma increases. The mechanism which stimulates the increase in reabsorption of urea on low protein diet is not known. Further investigation of the effect of nitrogen intake, rate of nitrogen reabsorption and nitrogen metabolism is suggested.

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A P P E N D I X

TABLE 1

Experiment Date: 12/8
Sheep No.: 103
Diet: Treatment 1 (High Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	1.5	806	293	4.2	-2.7
2	3.0	433	302	4.3	-1.3
3	2.5	580	302	4.8	-2.3
4	2.5	536	298	4.5	-2.0
5	10.0	131	290	4.5	5.5
6	7.0	181	287	4.4	2.6
7	8.0	174	290	4.8	3.2
8	5.8	198	289	4.0	1.8
9	6.0	203	290	4.2	1.8
10	7.3	162	289	4.1	3.2

TABLE 2

Experiment Date: 11/9
Sheep No.: 164
Diet: Treatment 1 (High Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	0.5	1924	283	3.4	-2.9
2	1.1	1096	286	4.1	-3.0
3	1.0	1108	284	3.9	-2.9
4	1.0	1055	285	3.7	-2.7
5	10.0	137	298	4.6	5.4
6	11.0	127	299	4.2	6.8
7	7.7	154	296	4.0	3.7
8	8.8	134	300	4.0	4.8
9	10.0	133	300	4.4	5.4
10	9.3	132	300	4.1	5.2

TABLE 3

Experiment Date: 2/10
Sheep No.: 50
Diet: Treatment 1 (High Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	0.9	1470	289	4.6	-3.7
2	0.8	1806	289	5.0	-4.2
3	1.6	961	290	5.2	-3.6
4	1.0	1416	289	4.9	-3.9
5	9.3	167	295	5.3	4.0
6	7.7	199	300	5.1	2.6
7	11.7	144	300	5.6	6.1
8	8.3	192	303	5.3	3.0
9	14.0	125	301	5.8	8.2
10	11.0	147	300	5.4	5.6

TABLE 4

Experiment Date: 11/8
Sheep No.: 50
Diet: Treatment 2 (Medium Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	2.7	427	285	4.0	-1.3
2	1.5	696	290	3.6	-2.1
3	1.3	741	290	3.4	-2.1
4	0.6	1680	288	3.5	-2.9
5	7.5	100	290	3.8	3.1
6	7.8	90	291	3.6	4.2
7	7.3	96	291	3.6	3.7
8	7.5	103	290	3.8	3.7
9	11.0	91	295	4.4	6.6
10	11.0	83	291	4.3	6.7

TABLE 5

Experiment Date: 10/9
Sheep No.: 103
Diet: Treatment 2 (Medium Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	2.5	464	290	4.0	-1.5
2	1.7	695	290	4.0	-2.3
3	0.8	1172	293	4.2	-2.4
4	1.5	735	290	3.8	-2.3
5	9.0	178	288	4.0	5.0
6	8.3	127	287	3.7	4.6
7	8.7	109	287	3.3	5.4
8	8.8	102	289	3.1	5.7
9	7.3	137	286	3.5	3.8
10	8.0	132	286	3.7	4.3

TABLE 6

Experiment Date: 29/9
Sheep No.: 164
Diet: Treatment 2 (Medium Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	2.5	486	289	4.2	-1.7
2	2.7	456	290	4.2	-1.5
3	1.9	601	290	4.0	-2.1
4	1.2	943	290	4.0	-2.8
5	9.0	139	291	4.3	4.7
6	8.5	131	291	3.8	4.7
7	7.7	130	292	3.5	4.2
8	9.2	124	291	3.9	5.3
9	9.2	124	291	3.9	5.3
10	7.8	150	294	4.0	3.8

TABLE 7

Experiment Date: 17/9
Sheep No.: 50
Diet: Treatment 3 (Low Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	1.8	591	285	3.8	-2.0
2	2.3	488	284	4.0	-1.7
3	1.6	692	286	3.8	-2.2
4	1.4	700	286	3.5	-2.1
5	7.0	141	281	3.5	3.5
6	8.4	121	282	3.6	4.8
7	10.0	112	280	4.0	6.0
8	11.0	102	280	4.0	7.0
9	9.0	115	280	3.7	5.3
10	8.5	122	281	3.7	4.8

TABLE 8

Experiment Date: 24/9
Sheep No.: 103
Diet: Treatment 3 (Low Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	2.0	554	283	3.9	-1.9
2	2.0	573	282	4.1	-2.1
3	2.8	464	283	4.1	-1.3
4	2.5	458	282	4.1	-1.6
5	10.0	122	293	4.2	5.8
6	8.7	138	293	4.1	4.8
7	8.5	134	288	4.0	4.5
8	10.3	126	290	4.4	5.9
9	8.0	144	289	4.0	4.0
10	10.0	127	290	4.4	5.6

TABLE 9

Experiment Date: 4/11
Sheep No.: Y
Diet: Treatment 3 (Low Protein)

Clearance Period	UFR (ml/min)	Uosm (mOsm)	Posm (mOsm)	Cosm (ml/min)	C_{H_2O} (ml/min)
1	2.0	580	283	4.1	-2.0
2	1.8	634	283	4.1	-2.3
3	1.2	851	283	3.7	-2.5
4	1.2	895	283	3.7	-2.5
5	6.8	187	278	4.6	2.2
6	11.0	109	278	4.3	6.7
7	10.3	133	279	4.9	5.4
8	11.0	121	278	4.8	6.2
9	11.0	114	279	4.5	6.5
10	11.9	117	279	5.0	6.9

TABLE 10

Animal No. and Date of Experiment	UFR (ml/min.)	C_{PAH} (ml/min.)	Mean
	1.5	482	
50	2.7	546	
	1.3	457	
11/8	0.6	496	495 ± 38 *
	7.5	510	
	7.8	542	
Trt 2	7.3	450	
	7.5	506	
	11.0	489	
	11.0	500	500 ± 30
	0.5	458	
164	1.1	562	
	1.0	474	
11/9	1.0	522	504 ± 47
	10.0	520	
	11.0	514	
Trt 1	7.7	526	
	8.8	600	
	10.0	469	
	9.3	526	526 ± 42
	2.0	566	
103	2.0	538	
	2.8	562	
24/9	2.5	522	547 ± 21
	10.0	522	
	8.7	514	
Trt 3	8.5	582	
	10.3	508	
	8.0	544	
	10.0	526	532 ± 27
	2.0	559	
Y	1.8	533	
	1.2	634	
2/10	1.2	565	573 ± 43
	6.8	571	
	11.0	571	
Trt 3	10.3	594	
	11.0	571	
	11.0	681	
	11.9	636	604 ± 45

* Standard deviations of observations

TABLE 11

Experiment Date: 12/8
Sheep No.: 103
Diet: Treatment 1 (High Protein)

Clearance Period	UFR ml/min	U_I mg%	P_I mg%	C_I ml/min	Uu mg%	Pu mg%	Cu ml/min	$\frac{Cu}{C_I}$	$\frac{Uu}{Pu}$	$\frac{U_I}{P_I}$
1	1.5	2937	40	110	469	12.8	55.0	0.500	36.6	73.4
2	3.0	1394	40	104	240	13.0	55.3	0.532	18.4	34.7
3	2.5	1408	36	97	289	13.2	54.8	0.565	21.9	38.8
4	2.5	1648	40	103	282	12.5	56.3	0.547	22.5	41.2
5	10.0	369	41	90	67	12.0	56.0	0.622	5.6	9.0
6	7.0	510	43	83	88	12.4	50.0	0.600	7.1	11.9
7	8.0	429	43	80	76	12.5	50.0	0.610	6.1	10.0
8	5.8	717	38	110	136	12.2	65.0	0.591	11.1	18.7
9	6.0	633	38	100	115	12.0	58.2	0.582	9.7	16.7
10	7.3	560	42	97	98	12.3	58.3	0.601	8.0	13.2

TABLE 12

Experiment Date: 11/9
Sheep No.: 164
Diet: Treatment 1 (High Protein)

1	0.5	5989	43	69	964	13.2	36.5	0.527	73.0	138.0
2	1.1	2784	44	68	470	13.0	38.7	0.569	36.2	63.6
3	1.0	3075	41	75	516	12.8	40.3	0.537	40.3	75.0
4	1.0	2571	43	58	415	13.0	31.0	0.535	32.0	59.8
5	10.0	309	39	80	66	11.1	59.0	0.737	5.9	8.0
6	11.0	267	38	77	52	11.7	48.9	0.675	4.4	7.0
7	7.7	310	39	61	69	12.1	43.7	0.717	5.7	8.0
8	8.8	360	43	74	69	12.5	48.8	0.650	5.5	8.4
9	10.0	328	41	80	65	13.0	50.3	0.629	5.0	8.0
10	9.3	360	46	73	64	13.0	46.0	0.630	4.9	7.8

TABLE 13

Experiment Date: 2/10
Sheep No.: 50
Diet: Treatment 1 (High Protein)

1	0.8	4446	41	90	777	15.8	40.9	0.454	49.2	108.4
2	0.9	4565	42	99	789	15.6	45.6	0.461	50.6	110.0
3	1.6	2365	38	99	488	14.3	53.6	0.541	34.1	63.1
4	1.0	3150	38	84	587	14.0	42.0	0.493	42.0	84.0
5	9.3	446	43	98	98	13.1	70.0	0.714	7.5	10.5
6	7.7	416	43	75	84	13.1	50.0	0.660	6.4	9.8
7	11.7	310	40	90	71	13.1	63.5	0.710	5.4	7.7
8	8.3	207	42	85	80	12.8	52.0	0.650	6.2	8.0
9	14.0	273	43	90	60	12.8	66.0	0.730	4.7	6.4
10	11.0	353	49	80	46	13.0	47.2	0.590	4.3	7.3

TABLE 14

Experiment Date: 11/8
Sheep No.: 50
Diet: Treatment 2 (Medium Protein)

Clearance Period	UFR ml/min	U_I mg%	P_I mg%	C_I ml/min	Uu mg%	Pu mg%	Cu ml/min	$\frac{Cu}{C_I}$	$\frac{Uu}{Pu}$	$\frac{U_I}{P_I}$
1	2.7	1107	47	63	56.4	5.0	30.1	0.478	11.3	23.6
2	1.5	1942	45	65	80.3	5.0	24.1	0.371	16.1	43.3
3	1.3	2209	50	59	85.1	5.1	22.2	0.376	16.7	44.4
4	0.6	3983	48	50	119.4	5.1	14.0	0.280	23.3	83.3
5	7.5	371	46	60	22.2	5.1	32.6	0.544	4.4	8.0
6	7.8	498	51	76	30.5	5.1	46.9	0.609	5.9	9.7
7	7.3	449	48	68	28.2	5.1	40.6	0.597	5.5	9.3
8	7.5	404	52	58	25.0	5.0	39.0	0.672	5.0	7.7
9	11.0	259	53	72	21.3	5.2	45.0	0.625	4.1	4.8
10	11.0	357	58	68	20.8	5.2	44.0	0.648	4.0	6.2

TABLE 15

Experiment Date: 10/9
Sheep No.: 103
Diet Treatment 2 (Medium Protein)

1	2.5	1414	38	94	89.8	5.0	44.9	0.478	18.0	37.6
2	1.7	2606	40	108	116.1	5.2	37.3	0.345	22.3	64.6
3	0.8	4218	38	90	137.8	5.3	20.1	0.223	26.0	112.4
4	1.5	2380	39	92	125.5	5.2	36.2	0.393	24.1	61.3
5	9.0	366	39	85	30.8	5.1	54.4	0.640	6.0	9.4
6	8.3	454	38	100	34.1	5.1	55.7	0.557	6.7	12.0
7	8.7	353	41	75	25.8	5.0	44.8	0.597	5.2	8.6
8	8.8	391	41	84	29.6	5.2	50.4	0.600	5.7	9.5
9	7.3	625	46	100	35.8	5.0	52.5	0.525	7.2	13.6
10	8.0	492	43	92	32.0	5.1	50.3	0.547	6.3	11.5

TABLE 16

Experiment Date: 29/9
Sheep No.: 164
Diet: Treatment 2 (Medium Protein)

1	1.2	3402	47	90	188.0	8.1	28.5	0.427	23.0	73.1
2	2.5	1307	43	76	119.0	8.3	35.7	0.470	14.3	52.2
3	2.7	1383	45	83	122.3	8.2	39.8	0.480	14.9	51.7
4	1.9	2023	48	81	157.6	8.2	37.1	0.458	19.2	42.0
5	9.0	388	42	83	48.4	8.1	53.8	0.648	6.0	9.2
6	8.5	396	50	68	42.0	7.8	45.8	0.674	5.4	8.0
7	7.7	498	46	84	53.0	7.9	51.5	0.613	6.7	11.0
8	9.2	447	50	82	46.5	8.0	53.3	0.650	5.8	8.9
9	9.2	318	47	62	41.6	8.3	46.0	0.740	5.0	6.8
10	7.8	431	46	74	50.2	8.4	46.8	0.632	6.0	9.4

TABLE 17

Experiment Date: 17/9
Sheep No.: 50
Diet: Treatment 3 (Low Protein)

Clearance Period	UFR ml/ Min	U_I mg%	P_I mg%	C_I ml/ min	Uu mg%	Pu mg%	Cu ml/ min	$\frac{Cu}{C_I}$	$\frac{Uu}{Pu}$	$\frac{U_I}{P_I}$
1	1.8	1967	48	75	47.1	3.9	22.1	0.294	12.1	41.0
2	2.3	1580	48	77	48.5	3.9	29.0	0.377	12.4	33.9
3	1.6	2194	49	70	48.4	3.8	20.0	0.286	12.7	44.6
4	1.4	2022	48	60	47.2	3.9	17.3	0.288	12.1	42.1
5	7.0	352	47	53	15.3	3.8	28.1	0.530	4.0	7.7
6	8.4	351	49	60	13.9	3.6	32.4	0.540	3.9	7.1
7	10.0	321	49	66	14.0	3.9	36.0	0.545	3.6	7.6
8	11.0	395	50	70	13.1	3.7	38.8	0.554	3.5	7.8
9	9.0	418	51	74	15.8	3.6	39.6	0.535	4.4	8.2
10	8.5	363	51	60	13.4	3.6	31.7	0.528	3.7	7.1

TABLE 18

Experiment Date: 24/9
Sheep No.: 103
Diet: Treatment 3 (Low Protein)

1	2.0	1811	40	91	45.1	3.1	29.1	0.383	17.5	45.5
2	2.0	2418	43	113	57.7	3.1	36.6	0.324	18.9	56.5
3	2.8	1328	43	87	44.7	3.0	36.5	0.422	12.9	30.7
4	2.5	1490	43	86	46.4	2.9	40.0	0.433	16.0	33.9
5	10.0	396	41	97	14.3	2.6	55.0	0.564	5.5	9.7
6	8.7	341	33	90	14.9	2.8	46.2	0.513	5.3	10.4
7	8.5	464	44	90	16.3	2.8	49.4	0.544	5.8	10.6
8	10.3	398	44	94	14.0	2.9	50.0	0.532	4.8	9.1
9	8.0	557	48	92	16.9	3.0	45.0	0.489	5.6	11.5
10	10.0	442	48	92	15.2	3.0	50.5	0.540	5.1	9.2

TABLE 19

Experiment Date: 4/11
Sheep No.: Y
Diet: Treatment 3 (Low Protein)

1	2.0	1841	41	90	54.1	3.5	30.9	0.340	15.5	45.0
2	1.8	2373	44	98	60.2	3.5	31.5	0.321	17.2	53.6
3	1.2	3947	46	106	63.9	3.6	21.5	0.319	17.5	86.2
4	1.2	3216	45	84	63.5	3.8	21.0	0.203	16.9	71.8
5	6.8	588	38	106	17.8	3.5	34.8	0.467	5.1	15.5
6	11.0	400	40	110	11.7	3.3	39.1	0.527	3.5	10.1
7	10.3	410	40	106	12.9	3.6	36.9	0.5oo	3.6	10.3
8	11.0	383	41	102	12.8	3.7	38.2	0.528	3.5	9.3
9	11.0	379	46	91	10.5	3.7	31.1	0.530	2.8	8.7
10	11.9	333	40	99	9.7	3.6	32.1	0.540	2.7	8.3