ASPECTS OF NITROGEN METABOLISM IN THE KANGAROO ISLAND WALLABY *PROTEMNODON EUGENII* (DESMAREST)

by

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SUMMARY

Various aspects of digestion and utilization of nitrogen were investigated in the Kangaroo Island Wallaby *Protemnodon eugenii* (Desmarest). As the wallaby is thought to experience a seasonal shortage of nitrogen in the diet on Kangaroo Island, particular emphasis was placed upon the wallaby's response to a low nitrogen intake.

It was established that nitrogenous compounds are digested in a ruminant-like manner by this wallaby. Nitrogen supplied in the diet as urea is utilized as efficiently as that supplied as casein and ingested nitrogen undergoes extensive incorporation into microbial nitrogen in the forestomach.

Renal control of urea excretion in this wallaby also parallels that reported in ruminants. During periods of low nitrogen intake extensive renal retention of urea occurs. This is brought about by reabsorption of urea in the renal tubules, as glomerular filtration rate is independent of nitrogen intake and urea clearance is reduced. The renal response of this wallaby to injected urea is also similar to that of ruminants. Wallabies depleted of nitrogen retain 98% of injected urea and wallabies fed an adequate nitrogen diet retain only 30% of the injected urea.

Injected urea was also found to be recycled to the forestomach of the wallaby and incorporation of injected urea nitrogen into microbial nitrogen was more extensive in the nitrogen depleted wallabies than in the nitrogen sufficient wallabies. Whether this reflects a difference in the total amount of injected urea recycled to the forestomach in nitrogen sufficient and depleted wallabies was not established.

Renal retention of urea is enhanced in wallabies depleted of both nitrogen and water. Contrary to findings with cattle however, no improvement in nitrogen retention was noted in wallabies concurrently deprived of water and nitrogen. It was concluded, also, that the additional urea retained by these animals did not lead to an improvement in water balance.

The results of this study indicate that conservation and utilization of endogenous urea is a significant aspect of the nitrogen metabolism of the Kangaroo Island Wallaby. However, although utilization of endogenous urea may be of importance in the survival of the wallaby during periods of nitrogen depletion, this does not appear to be further enhanced by concurrent water restriction. These findings apply only to wallabies in captivity and fed laboratory diets.

DECLARATION

This thesis contains no material previously submitted by me for a degree in any University. I believe that it contains no material written or published by other people except when due reference is made in the text of this thesis.

ACKNOWLEDGEMENTS

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I am grateful to the CSIRO Division of Nutritional Biochemistry, Adelaide, for use of equipment and in particular to Mr. A.F. Pilgrim, Mr. B. Potter and Dr. C.W.S. Reid (DSIRO Division of Plant Chemistry, New Zealand) for advice and criticism of part of the manuscript. I would also like to thank Professor D.J.D. Nicholas of the Department of Agricultural Biochemistry, Waite Agricultural Research Institute, Adelaide, for the use of the Mass Spectrometer and Miss Marlene Short of the same department for technical assistance. Dr. S. Niven, of the Department of Biometry, Waite Agricultural Research Institute, advised on the use of statistical analyses for which I am indebted.

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I. INTRODUCTION

1. General

From a field study of the Kangaroo Island Wallaby, *Protemnodon eugenii* (Desmarest), Dr. S. Barker of the Zoology Department, University of Adelaide, considered that there might be a critical shortage of nitrogen in the diet of *Protemnodon eugenii* on Kangaroo Island towards the end of summer. The following investigation concerned the nitrogen metabolism of the Kangaroo Island Wallaby. In the light of field conditions, particular emphasis was placed upon the response and possible physiological adaptation of the wallaby to a lowered nitrogen intake.

2. Digestive physiology of macropod marsupials

Descriptions of the stomach anatomy of macropods published in the nineteenth century provided the first clue to their digestive physiology. In 1814, Home (cited, Barker, Brown and Calaby, 1963) described the macropod stomach as enlarged and sacculated. Later Owen (1839, cited Barker, Brown and Calaby, 1963) confirmed these findings and noted additionally that an oesophageal groove ran from the distal end of the oesophagus to the non-sacculated region of the stomach. These anatomical features prompted Owen to suggest that the enlargement of the macropod stomach was analogous to the enlargement of the ruminant stomach and was associated

with their herbivorous diet. No progress, however, was made in this field of research for the next one hundred years.

In the mid 1950's, Moir, Somers, Sharman and Waring (1954) and Moir, Somers and Waring (1956) suggested that the digestive physiology of the quokka was likely to resemble that of true ruminants. This suggestion was based on the finding that the quokka, like the ruminant, has an enlarged and sacculated forestomach with an oesophageal groove, together with "stearic rich" fat deposits and a relatively low blood sugar level which can be lowered still further by insulin treatment without apparent ill effect (Buttle, Kirk and Waring, 1952).

Later research into the digestive physiology of macropods has supported this suggestion and an enlarged and sacculated forestomach with an oesophageal groove has been found in ten species of macropod representing eight genera, _Macropus_, _Thylogale_, _Wallabia_, _Petrogale_, _Lagorchestes_, _Lagostrophus_, _Bettongia_ and _Setonix_ (Brown, 1959). Unlike the ruminant, the forestomach epithelium of the macropod is glandular (Schaffer and Williams, 1876; Moir _et al._, 1956; Griffiths and Barton, 1966), however the pre-gastric position of the sacculated region of the stomach is essentially a ruminant feature. Furthermore, the forestomach of the quokka is inhabited by a dense population of microorganisms consisting

of both bacteria and protozoa which are comparable in density (though not in diversity) to those in sheep (Moir, 1965; Waring, Moir and Tyndale-Biscoe, 1966). Similar micro-organisms have been described in the red kangaroo Megaleia rufa (Desmarest) by Harrop (1965).

Further evidence of similarities between macropods and ruminants was furnished by Brown (1959), who found that the ratio of stomach contents to total body weight in macropods, studied so far, falls within the range of values found for true ruminants (Elsden, Hitchcock, Marshall and Phillipson, 1946). The pH of the stomach contents of the quokka and euro Macropus robustus Gould, is also similar to that in true ruminants. In the quokka the pH of the stomach contents in the sacculated cardiac region is slightly alkaline (pH 7.05 - 7.95) whilst that in the non-sacculated region is highly acidic (Moir et al., 1956). Similar values were found for the euro, the pH of the fundus being as low as 2.4 (Ealey, 1962).

Cud chewing or rumination, which is possibly the most obvious feature of digestion in true ruminants, has also been reported in macropods on a number of occasions (Owen, 1839, cited Barker, Brown and Calaby, 1963; Wood-Jones, 1923; Moir et al., 1956; Calaby, 1958; Mollison, 1960) but observations of Barker et al. (1963) suggest that regurgitation

and remastication of food by macropods is an occasional occurrence and not truly analagous to rumination. In addition, the rate of passage of food residues through the alimentary tract of the quokka (Calaby, 1958) and euro (Ealey, 1962) is faster than in ruminants (Castle, 1956; Blaxter, Graham and Wainman, 1956) and also the quokka digests crude fibre less efficiently than ruminants but more efficiently than rabbits (Voris, Marcy, Thacker and Wainio, 1940). The ability of the quokka to digest crude protein (Calaby, 1958) is however within the range found in ruminants.

Studies of the carbohydrate metabolism of macropods, though few, indicate that this also is similar to that of ruminants. The fermentative action of the forestomach microbiota leads to the production of the same steam volatile fatty acids as in the ruminant (Barker, 1960, 1961). Barker (1960) confirmed also the relatively low blood sugar levels in the quokka, the range of which falls between levels found in ruminant and non-ruminant herbivores. Similarly low blood sugar levels have been reported in the red kangaroo (Harrop, 1965).

Thus the digestive physiology of macropod marsupials studied so far has many features in common with that of ruminants. It is well established that the microorganisms responsible for pre-gastric fermentation in ruminants confer

important survival advantages upon these animals with regard to cellulose utilization, vitamin synthesis and nitrogen metabolism. Moir *et al.* (1956) suggested that macropods with their ruminant-like stomach and pre-gastric microbiota might accrue similar nutritional advantages.

3. Ruminant nitrogen metabolism

Numerous studies arising from work started in the nineteenth century (Hagemann, 1891; Zuntz, 1891) have shown that ruminants can utilize non-protein nitrogen as a dietary nitrogen source for growth and the maintenance of body proteins (Hart, Bohstedt, Deobald and Wegner, 1939; Agrawala, Duncan and Huffman, 1953; Land and Virtanen, 1959; Williams and Tribe, 1957). From an ecological point of view, the capacity to utilize non-protein nitrogen could be an important factor in the survival of these animals during periods of nitrogen depletion, as a significant proportion of the nitrogen entering the stomach of the naturally grazing ruminant may be non-protein. Synge (1952) and Ferguson and Terry (1954) report that between 10 and 30% of nitrogen in herbage may be non-protein nitrogen, mainly in the form of free amino acids.

Endogenous nitrogen in the form of urea has also been shown to contribute to the nitrogen intake of ruminants.

Significant amounts of endogenous urea normally enter the stomach of ruminants via the saliva (Colin, 1886, cited Hungate, 1966; McDonald, 1948; McDougall, 1948; Bailey and Balch, 1961; Somers, 1961 a,b,c and d; Juhasz, 1965) and also directly from the blood stream across the rumen wall (Chalmers and Synge, 1954; Simmonet, Le Bars and Molle, 1957; Houpt, 1959; Decker, Hill, Gartner and Hornicke, 1960; Hogan, 1961; Egan, 1965; Weston and Hogan, 1967; Varady, Boda, Havassy, Bajo and Tomas, 1967; Houpt and Houpt, 1968).

It has been established that utilization of non-protein nitrogen by the ruminant depends upon the synthetic activities of the pre-gastric microbial population. The microorganisms in the rumen convert non-protein nitrogen to microbial protein (Agrawala _et al_., 1953) which, after digestion in the abomasum, is utilized by the ruminant for protein synthesis (Watson, Davidson and Kennedy, 1949; Ferrando, d'Acres and Communol, 1956). As the rumen microorganisms can synthesise all the essential amino acids (Loosli, Williams, Thomas, Ferris and Maynard, 1949; Hill, Decker, Hornicke, Holler and Gartner, 1962; Kosharov, Bensadoun, Breuer, Loosli, Morris, Reid and Legg, 1967), microbial protein synthesised from a non-protein source becomes an important addition to the dietary amino-acid pool of the ruminant (Moir, 1965). Thus the ability of the ruminant to utilize non-protein nitrogen as a dietary

nitrogen source indicates that extensive conversion of such nitrogen to microbial protein occurs. Weller, Pilgrim and Gray (1962) estimated that 80% of plant nitrogen ingested by sheep is converted to microbial nitrogen.

It is clear that recycling of endogenous urea to the rumen could be of considerable importance to the ruminant, particularly during periods of nitrogen depletion. The quantitative relationship, however, between the nitrogen status of the animal and the amount of urea recycled remains unresolved. The work of Somers (1961c and d) suggests that urea recycling is limited during periods of high nitrogen intake, but conflicting results leave this question unanswered (Gartner, Decker and Hill, 1961; Weston and Hogan, 1967; Houpt and Houpt, 1968).

Although the controlling factors in the movement of endogenous urea into the rumen have yet to be established, the role played by the kidney in the nitrogen economy of the ruminant is well documented. As pointed out by Schmidt-Nielsen (1958), the nitrogen depleted ruminant would be expected to retain urea rather than excrete it in the urine, since urea is a potential source of nitrogen for protein synthesis. Read (1925) first reported a reduction in the excretion of urea in the urine of camels which exhibit pre-gastric digestion similar to that found in

ruminants (Moir, 1965). Although this finding was disputed (Petri, 1927; Smith and Silvette, 1928), it was subsequently found that a reduction in the excretion of urinary urea was a feature of camels which were depleted of nitrogen (Schmidt-Nielsen, Schmidt-Nielsen, Houpt and Jarnum, 1957). These workers reported that urea excreted by the nitrogen depleted camel was significantly lower than that of non-ruminant mammals similarly depleted of nitrogen. They also found that glomerular filtration rate was independent of nitrogen intake and concluded that urea excretion was controlled by the renal tubules.

Sheep depleted of nitrogen, also retain urea through renal tubular reabsorption (Schmidt-Nielsen, Osaki, Murdaugh and O'Dell, 1958; Schmidt-Nielsen and Osaki, 1958; Schmidt-Nielsen and O'Dell, 1959). Livingston, Payne and Friend (1962) and Hill _et al._ (1962) demonstrated a similar reduction in the excretion of urea in the urine of nitrogen depleted cattle and goats respectively, as little as 5% of the urea filtered by the kidney being excreted in the urine of goats. It is not known if the reduction in urea excretion by nitrogen depleted cattle and goats is due to renal tubular reabsorption of urea or a reduction in the glomerular filtration rate.

Schmidt-Nielsen *et al.* (1957) and Schmidt-Nielsen and Osaki (1958) suggested that urea retained by the kidney of sheep and camels during periods of nitrogen depletion was subsequently recycled to the rumen and utilized for protein synthesis, thus supplementing the nitrogen intake and hence increasing the chances of survival of these animals. They further suggested that the extensive urea reabsorption by the renal tubules in these animals was stimulated by the lowered levels of urea circulating in the plasma during periods of low nitrogen intake. They found, however, that injecting urea, and thus raising the levels of plasma urea in nitrogen depleted sheep and camels to those found in nitrogen sufficient animals, did not necessarily lead to the expected increase in the amount of urea excreted in the urine. Injected urea was retained by the nitrogen depleted camels and urinary urea levels remained consistently low, although plasma urea levels did not fall to pre-injection level until eight days later. Schmidt-Nielsen and Osaki (1958) obtained contradictory results with sheep. Infused urea was not always retained and they attributed this difference in response to the nitrogen status of the experimental animals which was unknown as nitrogen retention was not measured. The ability of the nitrogen depleted sheep to retain injected urea and to recycle some of it, has since been confirmed (Houpt, 1959;

Somers, 1961d). Schmidt-Nielsen *et al.* (1957) and Schmidt-Nielsen and Osaki (1958) interpreted their findings to mean that the stimulus for the renal retention of urea by nitrogen depleted sheep and camels is associated with a lowered nitrogen intake rather than lowered plasma urea levels.

The work of Schmidt-Nielsen and O'Dell (1959) suggests that renal retention of urea in nitrogen depleted sheep involves active transport of urea across the renal tubular membrane. Recent work has supported the concept of uphill transport of urea in the mammalian kidney (Goldberg, Wojtczak and Ramirez, 1967; Ullrich, Rumrich and Schmidt-Nielsen, 1967).

Livingston *et al.* (1962) reported that renal retention of urea in nitrogen depleted cattle may be enhanced further if these animals were also allowed limited access to water. It was later found that nitrogen depleted cattle which were concurrently water restricted also exhibited an improvement in nitrogen retention (Livingston, Payne and Friend, 1964; Payne, 1964, 1965, 1966). Increased renal retention of urea has also been recorded in rabbits which were nitrogen depleted and water restricted (Houpt, 1963). Johnson, Javier, Hardison and Ordoveza (1966) reported that nitrogen retention was improved in cattle which were water deprived but receiving adequate nitrogen in the diet. It has been suggested from

the work of Houpt (1963) that renal retention of urea may not only contribute to nitrogen economy but also might improve water economy (Moir, 1965) by reducing water loss in the urine.

It is now clear that conservation and utilization of endogenous urea may make a significant contribution to the nitrogen economy of ruminants during periods of nitrogen depletion and water restriction. Since macropods have a ruminant-like digestion, endogenous urea may contribute in a similar manner to the nitrogen and water economy of these animals during periods of decreased nitrogen and water intake.

4. Nitrogen metabolism in macropod marsupials

This is a field in which there has been little research. The only comprehensive study of nitrogen metabolism in a macropod was that made by Brown (1964) of the euro. It was known that euros could persist and reproduce on pastures dominated by Spinifex (*Triodia* spp.) (Ealey and Richardson, 1960; Ealey, 1962) and that these animals under natural conditions were infrequent drinkers and could tolerate a considerable degree of dehydration (Ealey, Bentley and Main, 1965). Since it has been generally assumed that the euro, like the quokka (Moir *et al.*, 1956),

has a ruminant-like digestion, it was thought that it might also have some of the adaptive features characteristic of ruminant nitrogen metabolism and that these might enable it to survive in the field when water is scarce and the pasture fibrous. The findings of Brown (1964, 1969) that the euro could utilize diets in which nitrogen was supplied in the form of urea or casein equally well confirmed its ruminant-like digestion of nitrogen. Brown (1964) also demonstrated in the euro that urea was recycled to the forestomach both with the saliva and directly across the forestomach wall. It is apparent from his findings that, at least in this species of macropod, endogenous urea may form a significant proportion of the utilizable nitrogen entering the forestomach.

However little is known of the mechanism by which macropods control the excretion of urea in the urine. Clearly renal retention of urea is important in the nitrogen economy of the nitrogen depleted ruminant and the same could be inferred for macropods. Ealey (1962) demonstrated a seasonal reduction in urinary urea excretion by euros in the field and in addition Brown (1964) found that, in euros depleted of nitrogen, the excretion of total nitrogen in the urine was less than endogenous levels excreted by eutherian mammals of similar body weight. Brown (1964) also found that retention of injected urea by nitrogen depleted euros was

of the same order as that in ruminants. These findings indicate that the euro, like the ruminant, retains urea when nitrogen depleted and that the mechanism involved may be similar.

It has been suggested that the Kangaroo Island Wallaby possibly experiences a seasonal shortage of nitrogen and water in the field. If this wallaby, like the other macropods studied so far (Moir *et al.*, 1956; Brown, 1959, 1964), has a ruminant-like digestion, then renal retention of urea might be a feature of its nitrogen economy when nitrogen intake is low. During 1966 (Lintern and Barker, 1969) a preliminary study was made of the excretion of urea by this wallaby during periods of low and adequate nitrogen intake. It was found that nitrogen depletion resulted in a dramatic reduction in the excretion of urea in the urine and low values, similar to those found in nitrogen depleted ruminants, were recorded. Similar low values were also reported in the Western Australian race of the same species in the field during the summer months (Kinnear, cited Waring *et al.*, 1966). These findings taken in conjunction with those of Ealey (1962) and Brown (1964) suggest that nitrogen depleted macropods improve their nitrogen economy by conserving urea in a manner analogous to nitrogen depleted ruminants.

5. The present study

The study reported herein involved an investigation of the nitrogen metabolism of the Kangaroo Island Wallaby and in particular its response to a low nitrogen intake. Special emphasis was placed upon the control of urea excretion and its relevance to the nitrogen economy of this species, as renal retention of urea is of importance in the nitrogen economy of nitrogen depleted ruminants and possibly one other macropod. The first step, however, was to confirm that nitrogen digestion in this wallaby is ruminant-like. When this was established the major part of the project was continued. A series of experiments were carried out to investigate the role of the kidney in controlling urea excretion in nitrogen depleted and sufficient wallabies and a preliminary experiment was performed to establish whether nitrogen retention could be judged from easily measurable parameters. This was followed by three experiments designed to test whether this wallaby and ruminants are similar in the extent and site of control of renal retention of urea and whether differences in the excretion of the urea in the urine could be related to the utilization of endogenous urea by this wallaby.

Finally, as it has been claimed in true ruminants that concurrent water and nitrogen depletion bring about a

decrease in urinary urea excretion and improvement in nitrogen balance, experiments were carried out to determine if the wallaby responds to this treatment in the same way.

II. METHODS

1. Animals were obtained for this study from Kangaroo Island. Prior to experimental use they were kepteither in a stock colony maintained by the Waite Agricultural Research Institute or in animal yards at the Zoology Department.

2. Animal Husbandry

(a) Caging

In Experiments 1 - 6 animals were housed in individual outdoor pens (approximately 10 ft. x 3 ft.), with either concrete or gravel covered floors, each with its own shelter. In Experiment 7, animals were housed in a waterproof shed in individual pens (approximately 10 ft. x 3 ft.) with a sawdust floor and under natural lighting. Each animal had its own shelter.

(b) Diets

Between all experiments animals were fed kangaroo pellets made from grain concentrates supplemented with fruit, vegetables and bread. Maintenance diets fed to animals to accustom them to dry food and test diets fed to animals during feeding trials were made according to McDonald and Hall (1957), except that the chaff used was not alkali extracted. Both maintenance and test diets were made by mixing oaten chaff, starch, sugar

and minerals with molasses and water. Ground casein was added to adjust the nitrogen content to the desired concentration and the mixture was dried in a force draught oven at 185°F. A similar diet in which urea was used as the nitrogen source was mixed in the same way and fed to animals during Experiment 1.

(c) Medication

(i) Like the quokka (Kakulas, 1961, 1963), the Kangaroo Island Wallaby is susceptible to vitamin E deficiency. Throughout the study all experimental animals were given a prophylactic dose of 100 mg of vitamin E each week. This was supplied in the form of 50 mg alpha-tocopheryl tablets*.

(ii) Penicillin

Outbreaks of Nocardiosis (lumpy jaw) (Barker, Calaby and Sharman, 1963) had occurred among the experimental animals during the feeding trials performed by Barker (1968). Accordingly, prior to each feeding trial throughout the study, each animal was given

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* Ephynal. Roche Products Limited, Welwyn Garden City, England.

a prophylactic course of penicillin (1,000,000 units) intra-muscularly over five alternate days. The only outbreak of Nocardiosis occurred during period 4 of Experiment 3 when Animal 18 developed a jaw lump on day 3 of the balance period. However this jaw lump disappeared completely after administration of penicillin and the diaorrhea usually a feature of animals with lumpy jaw was not observed.

3. Procedures

(a) Anaesthesia

Wallabies were anaesthetised with Veterinary Nembutal (Abbott) (pentabarbitone sodium, 60 mg/ml) injected via a marginal ear vein. The dosage given was approximately 1 - 2 ml/kg body weight, but the response of individual wallabies varied greatly and Nembutal was injected until either the pedal or eye reflex disappeared; the recovery from deeper anaesthesia was poor (Croft, 1960). When necessary, animals were killed with an overdose of Nembutal injected through a marginal ear vein.

(b) Administration of urea and urea N^{15}

In Experiments 5 and 6 urea in a solution of 0.5% saline (after Houpt and Houpt, 1968) was injected into a marginal ear vein of each animal after the animal had been anaesthetised.

(c) Administration of inulin

A single injection of inulin was given subcutaneously in the shoulder area of each anaesthetised wallaby during Experiment 4 (Ramsay and Coxon, 1967).

(d) Experimental water diuresis

Water diuresis was induced during Experiment 4 by water loading each animal using a stomach tube with a syringe attachment. The method used was a modification of that used by Burn, Finney and Goodwin (1950) for rats. A graduated syringe was attached to a twelve inch length of stiff polythene tubing (3/8th inch bore). The animal was placed in a hessian bag with its head protruding and was held horizontally with ventral aspect uppermost. A round wooden gag with a hole bored through the centre was placed in the animal's mouth. The tubing attached to the charged syringe was pushed through the hole in the gag and manipulated so that its full

length passed down the animal's oesophagus. The water was then expelled from the syringe and the syringe refilled and the procedure repeated until the full dosage was given. As found by Barker (1960) the best position to hold the animal was with its head raised so that the buccal cavity was in a straight line with the oesophagus.

(e) Catheterising animals

Animals were catheterised during Experiments 4 and 6 using a bulb catheter. The catheter was lubricated with vaseline and inserted by digital manipulation via the urethra into the bladder. Urine was collected from polythene catheter tubing (pp 0.50 mm internal diameter, pp 0.98 mm external diameter). The bladder was emptied and washed with warmed distilled water using a syringe attachment.

(f) Administration of Evans Blue Dye (T1824)

Evans Blue Dye was injected during Experiment 7 part 2 through a patent cannula in the femoral vein. When successful cannulation was not achieved the dye was injected through a marginal ear vein.

4. Collection of Samples

(a) Blood samples

Throughout the investigation (except for Experiment 7 part 1) blood samples were withdrawn from a lateral tail vein using heparinised syringes with G21 needles. Blood samples during Experiment 7 part 1 were withdrawn by heart puncture. During blood volume determinations in Experiment 7 part 2, blood samples were withdrawn from a patent cannula in the femoral vein when possible and otherwise through a lateral tail vein. Plasma was obtained by low speed centrifugation in all cases.

(b) Collection and measurement of food and water intake and urinary and faecal excretion

All collections were made from animals held in metabolism cages (33 in. x 36 in. and 24 in. high) made from 1" square wire with fibre glass and stainless steel shutes and separators for the separate collection of urine and faeces (Barker, 1968). The animals were housed in metabolism cages in a temperature controlled animal house kept at 21°. When nitrogen balance trials were performed during Experiments 1, 3 and 7 and food intake trials (Experiment 2) collections were made according to Barker (1968). Individual twenty-

four hour urine collections during Experiments 1, 4, 5, 6 and 7 were made according to Lintern and Barker (1969). Throughout the study urine was collected in 25% H_2SO_4 except during Experiments 4, 5 and 7 part 2 when toluene was used. Addition of H_2SO_4 or toluene prevented bacterial breakdown of urine components.

(c) Collection and separation of forestomach digesta into component fractions (Experiments 2 and 6).

Immediately after death the enlarged forestomach of the wallaby was ligatured at the pyloric and fundic ends and excised. An incision was made along the whole of the lesser curvature of the forestomach and the contents removed.

Separation of a subsample of forestomach digesta for samples containing forestomach fluid, bacterial, protozoal, soluble and plant fractions was achieved using the method of Gray, Pilgrim and Weller (1958) with a modification suggested by Pilgrim (pers. com.). Pilgrim has found that, in sheep, a large proportion of the bacteria in the rumen (20-30%) were not separated from the plant fibres by simple washing and furthermore that these bacteria could be "shaken" from the plant fibres by stirring with a sonic probe*.

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* "Soniprobe". Dawe Instruments Ltd., London.

As this technique removed the necessity of determining bacterial contamination by analysing fractions for diaminopimelic acid - N (Weller, Gray and Pilgrim, 1958), it was incorporated when this method was used for the fractionation of forestomach digesta of the Kangaroo Island Wallaby into its component fractions. The steps in this fractionation process are summarised in Figure 1. Microscopical examination of the products at each step of the fractionation during a preliminary experiment confirmed that this method resulted in separation of the various fractions of forestomach digesta. However, as found in sheep (Pilgrim, pers. com.), some smaller protozoa and bacteria found their way into the bacterial and soluble fractions respectively. This may have been a small source of error.

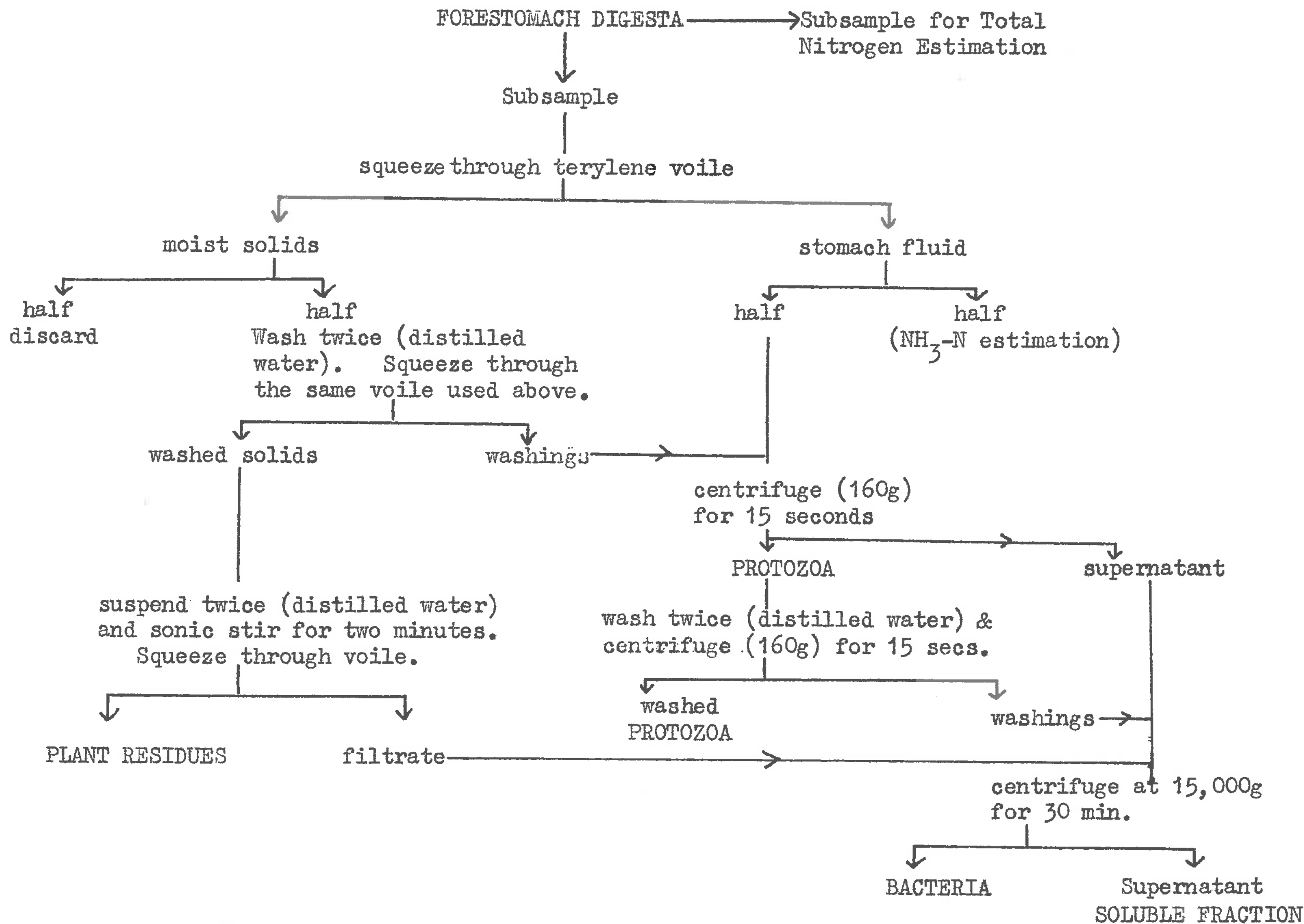
Remaining forestomach digesta, forestomach fluid and soluble and plant fractions were stored in polythene containers at -10° for subsequent analysis. Bacterial and protozoal fractions were transferred immediately to Kjeldahl flasks containing digest mixture and digested.

(d) Collection and treatment of caecal digesta

Immediately after death the caecum was ligatured and excised. A longitudinal incision was made along the

FIGURE I

Separation of forestomach digesta into protozoal, bacterial, plant and soluble fractions and the extraction of forestomach fluid.


FORESTOMACH DIGESTA
Subsample for Total Nitrogen Estimation
Subsample
squeeze through terylene voile
moist solids
stomach fluid
half
discard
half
Wash twice (distilled water). Squeeze through the same voile used above.
half
half
(NH_3-N estimation)
washed solids
washings
centrifuge (160g) for 15 seconds
PROTOZOA
supernatant
suspend twice (distilled water) and sonic stir for two minutes. Squeeze through voile.
wash twice (distilled water) & centrifuge (160g) for 15 secs.
PLANT RESIDUES
filtrate
washed PROTOZOA
washings
centrifuge at 15,000g for 30 min.
BACTERIA
Supernatant SOLUBLE FRACTION

whole length of the caecum and the contents removed and stored in polythene containers at -10° for subsequent analysis.

5. Chemical Analyses

(a) Urea

(i) Urea in the plasma and urine and caecal and forestomach digesta was determined by the micro-diffusion technique of Conway (1962) using the phosphate buffer of Wu and Wu (1951) and Dunning urease tablets. Potassium carbonate was used as the alkaline reagent.

(ii) Urea N^{15} in urine (Experiment 6) was determined using a modification of the microdiffusion technique of Conway (1962). No indicator was used in the boric acid solution and the resulting ammonium borate solution was brought to pH 1 with an excess of 5N H_2SO_4. N^{15} was then measured using a mass spectrometer* belonging to the Department of Agricultural Biochemistry, Waite Agricultural Research Institute.

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* A.E.I. Engineering Pty. Ltd. Mass spectrometer type M52 (No.110)

(b) Total Nitrogen

(i) Total nitrogen in food, food residues, faeces, urine and forestomach digesta (including bacterial, protozoal, plant and soluble fractions) and caecal digesta throughout the investigation (except Experiment 6) was determined by the microdiffusion technique of Conway (1962) after acid digestion using $SeO_2/CuSO_4$ catalyst. Potassium hydroxide was used as the alkaline reagent.

(ii) Determination of total nitrogen when N^{15} is present

In Experiment 6 total nitrogen was determined, after acid digestion with a $HgO/CuSO_4$ catalyst, by steam distillation into 4% boric acid using a solution of 40% sodium hyroxide and 5% sodium thiosulphate as the alkaline reagent. The resulting ammonium borate solution was titrated against N/50 HCl using a mixed indicator of bromocresol green and methyl red (Vogel, 1961).

(iii) Preparation of samples for the determination of N^{15}

This was the same as for the determination of total nitrogen when N^{15} is present except that no indicator was added to the resulting ammonium

borate solution and this solution was brought to pH 1 with an excess of 5N H_2SO_4. N^{15} was measured in a mass spectrometer.

(c) Ammonia

(i) Blood ammonia determinations were made according to Seligson and Seligson (1951). Potassium carbonate was used as the alkaline reagent.

(ii) Determinations of ammonia in the forestomach fluid during Experiments 2 and 6 were made by the microdiffusion technique of Conway (1962) with potassium metaborate as the alkaline reagent.

(iii) Preparation of samples containing ammonia nitrogen enriched with N^{15} for the determination of N^{15} was by steam distillation into 4% boric acid using magnesium oxide as the alkaline reagent. The resulting ammonium borate solution was brought to pH 1 with an excess of 5N H_2SO_4, and N^{15} determined using a mass spectrometer.

(d) Endogenous True Creatinine (ETC)

ETC was determined according to Hare (1950). Plasma ETC was determined, after protein precipitation, by adsorption onto Lloyd's reagent (Fuller's Earth)

and the colour then developed with alkaline picrate solution. Urinary ETC was determined in the same way but without initial protein precipitation as Albustix* gave no indication of protein in the urine. The samples containing the alkaline picrate solution were then brought to 36° in a water bath before reading at 520 mμ in a Beckman/Spinco spectrocolorimeter (Model 151).

(e) Inulin

Inulin in plasma and urine was determined during Experiment 4 by the anthrone method of Davidson and Sackner (1963). Samples were read at 620 mμ in a Beckman/Spinco spectrocolorimeter (Model 151).

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* Albustix - Ames Company, Division of Miles Laboratories, England.

III. EXPERIMENTAL

1. DIGESTION OF DIETARY NITROGEN BY THE KANGAROO ISLAND WALLABY

A. EXPERIMENT 1

(i) Introduction

It has generally been assumed from the work of Moir *et al.* (1956) and Brown (1959) that macropods and ruminants share features of digestion in common. This assumption is based upon the similarity in the anatomy of their alimentary tracts, as both groups of herbivores have enlarged forestomachs which house microbial populations (Moir *et al.*, 1956; Moir, 1957; Brown, 1959).

Through the synthetic activities of the pre-gastric microbiota, ruminants can utilize non-protein nitrogen for growth and maintenance of body proteins (Moir, 1957). It would be expected that macropods utilize non-protein nitrogen in a similar manner. To date, however, this has only been demonstrated in one species of macropod (Brown, 1969).

The Kangaroo Island Wallaby also would be expected to utilize non-protein nitrogen in a ruminant-like manner. The extensive renal retention of urea found in the wallaby during periods of nitrogen depletion suggests that, as in the ruminant (Houpt, 1959; Somers 1961a, b, c and d), urea may be recycled to the forestomach thus contributing to the

nitrogen intake of this species (Lintern and Barker, 1969). Accordingly the following experiment was carried out to determine whether the Kangaroo Island Wallaby can utilize non-protein nitrogen supplied in the diet in the form of urea, thereby establishing whether the urea recycling mechanism can operate in this species. In the reported experiment utilization of diets in which nitrogen was supplied in the form of urea and casein were compared. In a similar experiment with euros only 38% of the dietary nitrogen was in supplement form (Brown, 1969). As this may have minimised any differences in the utilization of casein or urea, 80% of the total nitrogen in the diet was presented in supplement form during the reported experiment.

(ii) Experimental Procedure

Four adult male Kangaroo Island Wallabies were used throughout these feeding trials. They were initially fed an excess of maintenance diet (1.80g N/100g dry weight) and allowed water *ad lib.* for four weeks. During this period they were periodically placed in metabolism cages in an attempt to reduce the effect of caging and handling on body weight (Lintern and Barker, 1969). The wallabies were then divided randomly into two groups with two animals in each group. One group was fed a diet supplemented with casein

(1.80gN/100g dry weight) and the other a diet supplemented with urea (1.81gN/100g dry weight). The nitrogen content of the basal ration to which either urea or casein was added was 0.38gN/100g dry weight.

Wallabies were fed an excess of test diets and allowed water *ad lib.* for four weeks. At the beginning of this period the wallabies were weighed, a blood sample taken and a twenty four hour urine sample collected. This was repeated after two weeks, three weeks, and four weeks. At the beginning of week three the wallabies were placed in metabolism cages and during the fourth week nitrogen balance was measured over a seven day period using the routine of Barker (1968). At the end of the four week experimental period the wallabies were returned to outdoor pens and fed the maintenance diet for four weeks. This was followed by a second four week experimental period during which the treatments were reversed. All blood samples were analysed for urea and ammonia and urine samples for urea and total nitrogen.

(iii) Results *+

(1) Total urinary nitrogen, urinary urea nitrogen, plasma urea, blood ammonia and body weight during both experimental periods are presented in Appendix 1 (A - G). Although all these parameters fluctuated during the experimental periods, these variations could not be attributed to the form in which nitrogen was supplied.

(2) Parameters measured during the nitrogen balance trials which are relevant to the discussion, are presented in Table 1 and variations due to period and treatment outlined below. The remainder of the data collected during the nitrogen balance trials is presented in Appendix 2A. Analysis of variations

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* Daily intake of dry matter and nitrogen and daily excretion of dry matter in faeces, nitrogen in faeces and urine and urea nitrogen in the urine have been related to the metabolic weight $kgW^{0.75}$ (Kleiber, 1961). Water turnover has been related to the power 0.8 of the body weight ($kgW^{0.8}$) after Richmond, Langham and Trujillo (1962).

\+ In presenting results of statistical analyses the following convention has been used to denote degrees of significance. NS = not significant: * = significant at the 5% level: ** = significant at the 1% level: *** significant at the 0.1% level.

in parameters, measured during the nitrogen balance trials, due to treatment and period are presented in Appendix 2B.

(a) Dry matter intake (DMI) did not differ significantly with either treatment or period (t_6 = 0.97 NS and t_6 = 0.38 NS respectively). Mean daily temperature during the outdoor prefeeding period prior to the two nitrogen balance trials was 17.8° and 16.9° respectively.

(b) Nitrogen balance was positive for all animals during both nitrogen balance trials. There was no significant correlation between nitrogen balance and nitrogen intake (r = +0.54 NS) and nitrogen balance did not differ significantly with either treatment or period (t_6 = 1.47 NS and t_6 = 0.16 NS respectively).

(c) Nitrogen intake was significantly correlated with dry matter intake (r = +0.95***) and this relationship is described by the regression equation

$$Y = 15.0X + 123.8$$

where Y = nitrogen intake ($mgN/kgW^{0.75}/day$)

X = dry matter intake ($g/kgW^{0.75}/day$)

TABLE 1

Daily dry matter and nitrogen intake and nitrogen excretion (expressed as a function of metabolic weight) and apparent dry matter and nitrogen digestibility of four wallabies fed diets supplemented with urea (U) and casein (C) during two nitrogen balance trials performed during Experiment 1.

Period	I				II			
Treatment	U	C	C	U	C	U	U	C
Animal	44	6	89	3	44	6	89	3
Nitrogen Intake*	663	552	733	613	753	608	615	725
Faecal Nitrogen*	213	173	224	197	221	183	195	224
Urinary Nitrogen*	398	324	449	350	470	376	374	431
Nitrogen Balance*	+52	+55	+60	+66	+62	+49	+46	+70
Urinary Urea Nitrogen*	324	250	336	312	386	318	298	344
% Urea Nitrogen in Urinary Nitrogen	81	77	75	89	82	85	80	80
Apparent Nitrogen Digestibility	68	69	69	67	71	70	73	69
Dry Matter Intake+	36.3	28.0	37.1	31.8	42.0	31.6	34.7	39.9
Apparent Dry Matter Digestibility	43	59	57	43	52	42	43	44
Body Weight (mean, $kgW^{0.75}$)	4.421	3.644	3.868	3.846	4.339	3.597	3.756	3.902

* $mgN/kgW^{0.75}/day$ + $g/kgW^{0.75}/day$

Because the nitrogen content of the feed consumed was different from that supplied (Appendix 3), as despite all precaution animals still selectively feed, the nitrogen intake from the supplement can only be estimated. In Table 2 the estimated intakes of supplemented nitrogen have been calculated from the difference between the total nitrogen intake and the nitrogen intake from the basal ration to which the supplement was added and this has been expressed as a percentage of the total nitrogen intake. Nitrogen balance was not correlated with the nitrogen intake from the supplement ($r = +0.56$ NS).

(d) Faecal nitrogen excretion was positively and significantly correlated with nitrogen intake ($r = +0.96^{***}$) and dry matter intake ($r = +0.92^{***}$). These relationships are given by the regression equations

$$Y = 0.26X + 32.8$$

where Y = faecal nitrogen ($mgN/kgW^{0.75}/day$)

X = nitrogen intake ($mgN/kgW^{0.75}/day$)

and

$$Y = 3.95X + 64.2$$

where Y = faecal nitrogen ($mgN/kgW^{0.75}/day$)

X = dry matter intake ($g/kgW^{0.75}/day$).

TABLE 2

Estimated intakes of supplementary nitrogen (mgN/kgW$^{0.75}$/day) from diets supplemented with urea (U)$^{+}$ or casein (C)$^{\emptyset}$ for each animal during nitrogen balance trials performed during Experiment 1*

Period	Treatment	Animal	Dry Matter Intake (g/kgW$^{0.75}$/day)	Total Nitrogen Intake (mgN/kgW$^{0.75}$/day)	Nitrogen Intake from supplement expressed as % of Total Nitrogen Intake	Nitrogen Balance (mgN/kgW$^{0.75}$/day)
I	U	44	36.3	663	79	+52
	C	6	28.0	552	81	+55
	C	89	37.1	733	81	+60
	U	3	31.8	613	80	+66
II	C	44	42.0	753	79	+62
	U	6	31.6	608	80	+49
	U	89	34.7	615	79	+46
	C	3	39.9	725	79	+70

* Nitrogen content of the basal ration was 0.38gN/100g dry weight

+ Diet supplemented with urea (1.81gN/100g dry weight)

∅ Diet supplemented with casein (1.80gN/100g dry weight)

(e) Total urinary nitrogen did not vary significantly with treatment (t_6 = 1.30 NS) or period t_6 = 0.90 NS) and was positively and significantly correlated with nitrogen intake (r = +0.98***). The relationship is given by the regression equation

$$Y = 0.68X - 51.0$$

where Y = total urinary nitrogen ($mgN/kgW^{0.75}/day$)

X = nitrogen intake ($mgN/kgW^{0.75}/day$)

Urinary urea nitrogen was also independent of the form in which nitrogen was supplied being positively and significantly correlated with nitrogen intake (r = +0.91***). The percentage of urea nitrogen excreted in the total urinary nitrogen during the nitrogen balance trials is presented in Table 1.

(f) Digestibility coefficients

The proportion of ingested material not appearing in the faeces is defined as the apparent digestibility of that material. Apparent digestibility of dry matter and nitrogen during the nitrogen balance trials are given in Table 1. Apparent digestibility of dry matter was not correlated with nitrogen intake (r = +0.09 NS)

or dry matter intake ($r = -0.06$ NS) and did not differ significantly with either treatment or period ($t_6 = 2.24$ NS and $t_6 = 1.06$ NS respectively). However when animals 44,6 and 89 received the nitrogen supplement in the form of urea, apparent digestibility of dry matter was consistently lower than when these animals received the diet supplemented with casein.

Apparent digestibility of nitrogen was not correlated with nitrogen intake ($r = +0.18$ NS) or dry matter intake ($r = +0.25$ NS) and did not differ significantly with treatment ($t_6 = 0.01$ NS). However apparent digestibility of nitrogen did differ significantly with period ($t_6 = 2.45$*).

B. EXPERIMENT 2

(i) Introduction

Like the ruminant, the euro (Brown, 1969) and the Kangaroo Island Wallaby (Experiment 1) can utilize non-protein nitrogen in the form of urea as a dietary nitrogen source. The ability of the ruminant to utilize urea depends on the extensive conversion of such nitrogen to microbial protein by the microorganisms in the rumen (Moir, 1957). A pre-gastric microbiota has been reported in the forestomach of the quokka

(Moir et al., 1956) and the red kangaroo (Harrop, 1965). As these microorganisms have been assumed to be present in the forestomach of all macropods, the ability of the euro and the Kangaroo Island Wallaby to utilize urea as a dietary nitrogen source implies that the conversion of dietary nitrogen by the pre-gastric microorganisms to microbial protein is as extensive as in the ruminant.

A preliminary examination of forestomach digesta from a wallaby fed a maintenance diet (1.5gN/100g dry weight) confirmed that in this species of macropod a pre-gastric microbial population, comprising both bacteria and protozoa, is present. No comprehensive study was made of numbers or range of types of bacteria and protozoa, however qualitative observations were made. In order to observe the types of free bacteria in the forestomach, preparations of forestomach fluid were obtained by squeezing forestomach digesta through terylene voile and 0.9% formal saline was added to the resulting fluid to fix bacteria present. Bacterial preparations were examined using phase contrast optics. Bacteria resembling oscillospiras, ovals, rods, large chain cocci and various small chain cocci were tentatively identified from descriptions of rumen bacteria from sheep (Smiles and Dobson, 1955). Numerous smaller bacteria also were observed. Protozoa were studied microscopically in fresh mounts and ciliates resembling

Isotricha sp. and Diplodinium sp. found in the rumen of sheep (Hungate, 1966) and described by Harrop (1965) in the red kangaroo were identified.

Having confirmed that a microbial population occurs in the forestomach of the Kangaroo Island Wallaby, the following experiment was carried out to investigate the role of the forestomach microorganisms of the wallaby in the digestion of dietary nitrogen. The changes in the distribution of nitrogen in the forestomach at intervals after feeding was measured, thereby estimating the incorporation of dietary nitrogen into microbial nitrogen (Weller et al., 1962).

(ii) Experimental Procedure

Five sexually mature female wallabies were fed a low nitrogen diet (0.42gN/100g dry weight) for five weeks. A low nitrogen diet was chosen as the experimental diet as the process used in the separation of forestomach digesta into its component fractions (see Methods) would have led to any supplemental nitrogen (urea or casein) being separated from the plant fibres, resulting in falsely high values for the nitrogen content of the bacterial, protozoal and soluble fractions of the forestomach digesta.

The animals were held in metabolism cages in a temperature controlled animal house (21°) for five weeks.

During the first three weeks they were allowed access to food for twenty four hours. During the final two weeks they were trained to consume most of their daily ration rapidly, by only presenting food for three hours each day in the morning. Daily dry matter intake was measured during the final three weeks of the experimental period.

On the last day of the experimental period collections for the measurement of nitrogen intake were made, the animals were weighed and then killed at three, five, eight, twelve and twenty four hours after commencement of feeding (Table 3). The contents of the forestomach were removed and a sub-sample separated into bacterial, protozoal, soluble, plant and forestomach fluid fractions. The total nitrogen content of the forestomach digesta and its component fractions was measured together with the ammonia content of the forestomach fluid.

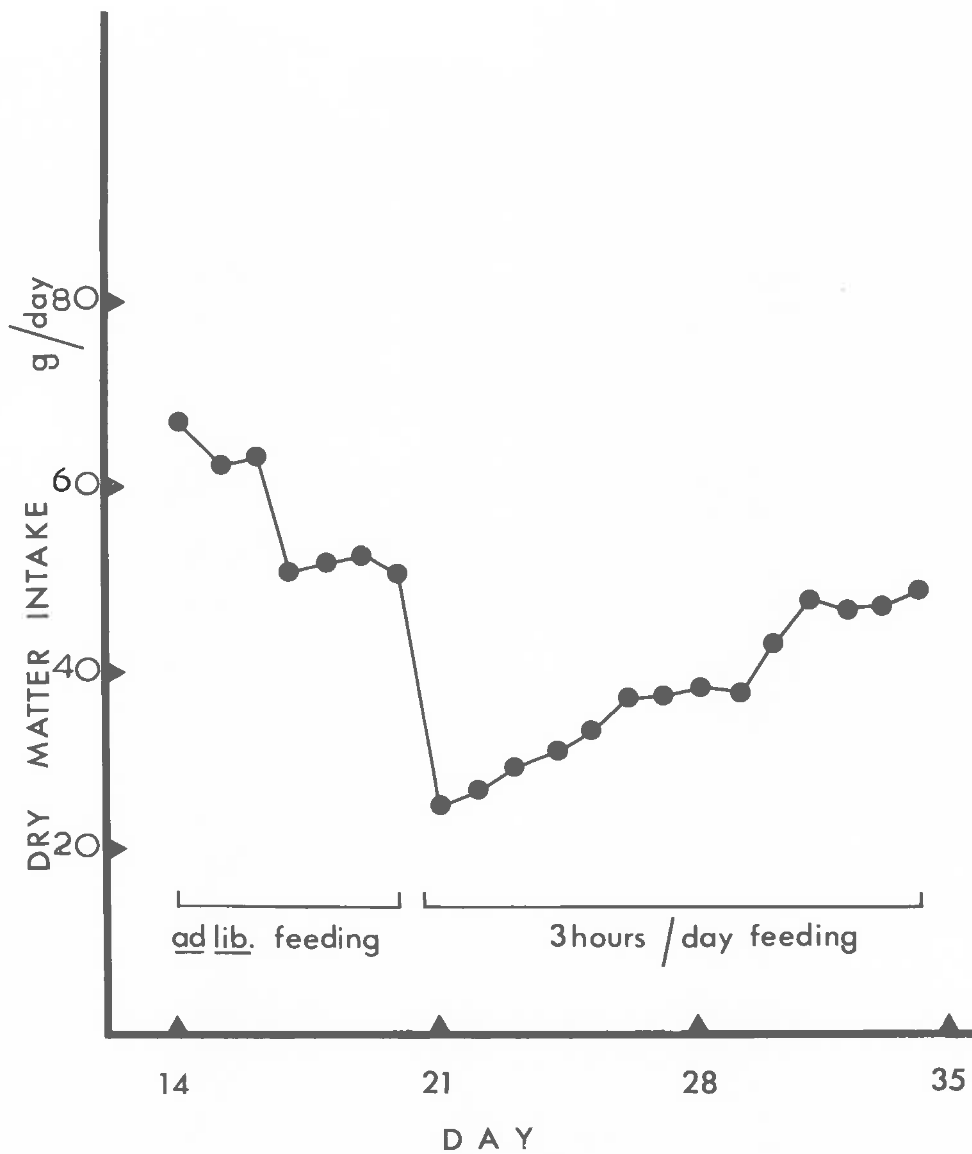
(iii) Results

(1) Dry matter intake (DMI) and nitrogen intake.

As can be seen from Figure 2, DMI declined during the initial period when feeding was allowed *ad lib*. This is a feature of Kangaroo Island Wallabies receiving a low nitrogen diet (Barker, 1968). A further decline in DMI occurred when feeding time was

FIGURE 2

Mean daily dry matter intake (g/day) of five wallabies during the final three weeks of Experiment 2. During the last two weeks of this period food was presented for only three hours each day.



DRY MATTER INTAKE g/day
80
60
40
20
ad lib. feeding
3 hours / day feeding
14
21
28
35
DAY

restricted to three hours each day. However, after two weeks of the restricted feeding regime, DMI was only slightly lower than at the end of the period when feeding was allowed _ad lib._ This final difference in DMI also may have been due to the feeding of a low nitrogen diet.

In Table 3 body weight, DMI, and nitrogen intake are presented for each wallaby on the last day of the experimental period. There were differences in these parameters between all the experimental animals. The aim of the experiment was to establish changes in the distribution of nitrogen in the forestomach of a single animal at different times after feeding, by extrapolating from the data collected from a number of different animals killed at progressive intervals after feeding. These initial differences in body weight, DMI and nitrogen intake between experimental animals may have led to a small error in the final result.

TABLE 3

Body weight (kg), DMI (g/day) and nitrogen intake (mg/day) for each wallaby on the last day of Experiment 2

Animal	Time killed after commencement of feeding (hours)	Body weight (kg)	DMI (g/day)	Nitrogen Intake (mg/day)
54	3	4.713	47	191
0	5	4.256	50	196
25	8	4.511	46	193
32	12	4.491	48	187
23	24	4.258	50	193

(2) Changes in the distribution of nitrogen throughout the various fractions of forestomach digesta at progressive intervals after feeding.

Changes in the nitrogen distribution throughout the fractions of forestomach digesta are presented in Figure 3. Although the points have been joined it must be remembered that values for each sampling time are derived from different animals. However as there was only a small difference in the nitrogen intake of the experimental animals (Table 3), combining the results in this way probably gives an indication of the trend of changes in the distribution of nitrogen in the forestomach of wallabies at progressive intervals after feeding.

It is clear from Figure 3 that microbial attack on plant nitrogenous compounds in the diet is extremely rapid. The forestomach probably contained less than 20 mg of plant nitrogen before feeding and within three hours after the ingestion of approximately 190 mg of new plant nitrogen only 70 mg were left, and at five hours only 41 mg. In considering the rate of attack, account must also be taken of the time needed to consume the ration, the bulk of which was consumed within ninety minutes after presentation, with a small amount being consumed during the remaining ninety

FIGURE 3

Distribution of nitrogen (g) in the forestomach of five wallabies at progressive intervals after commencement of feeding at time 0.

● Total Nitrogen

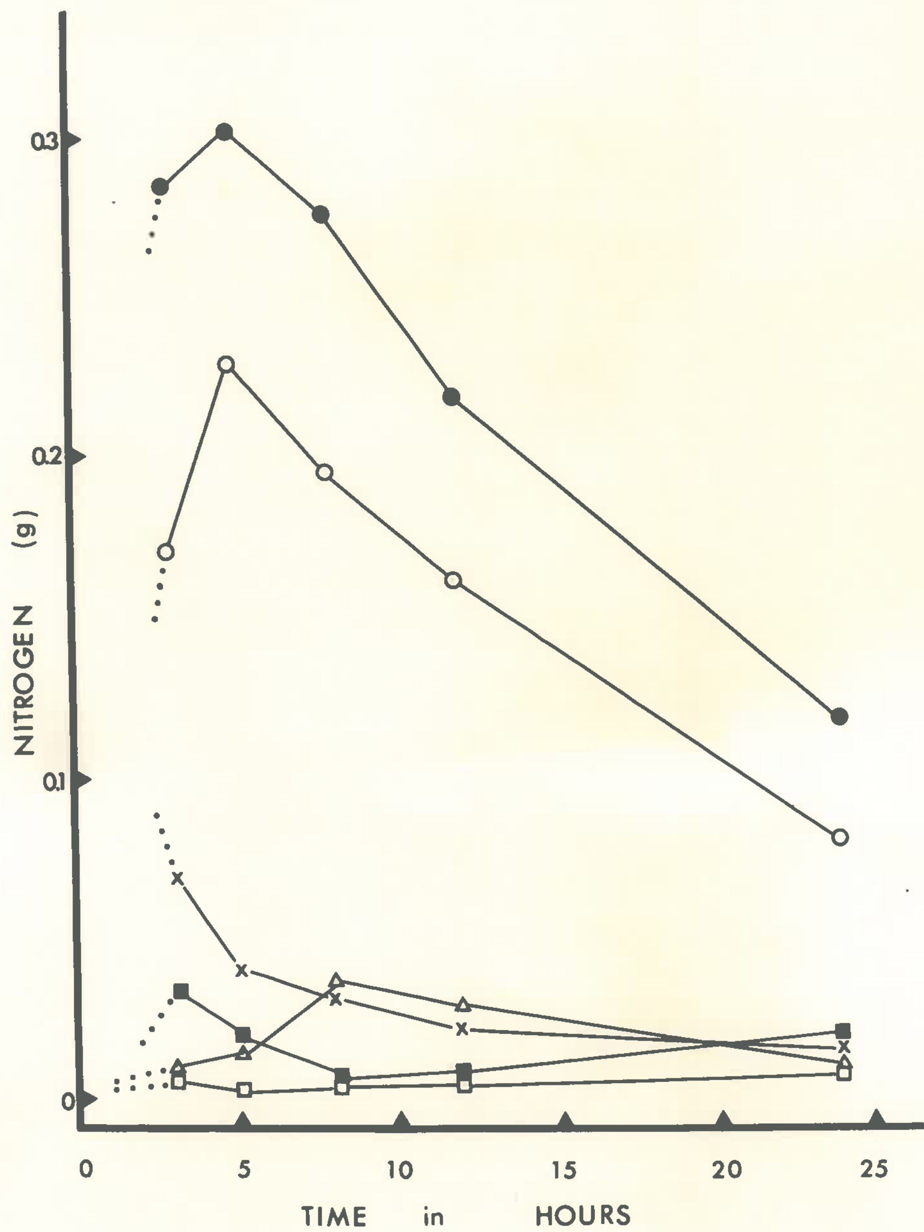
○ Bacterial Nitrogen

× Plant Nitrogen

△ Protozoal Nitrogen

■ Soluble Nitrogen

□ Ammonia Nitrogen



0.3
0.2
0.1
0
NITROGEN (g)
0
5
10
15
20
25
TIME in HOURS

minutes of the feeding period.

With the loss of plant nitrogen there was a corresponding increase in the bacterial, protozoal and soluble nitrogen fractions. Soluble nitrogen reached a maximum within three hours after the commencement of feeding and this was partly due to an increase in ammonia nitrogen (Figure 3). Soluble nitrogen gradually declined to a minimum eight hours after feeding and then increased again during the remaining sixteen hours. Bacterial nitrogen increased from about 80 mg before feeding to about 230 mg five hours later and protozoal nitrogen increased from about 10 mg before feeding to 35 mg eight hours later. Thus, although a little of the loss of plant nitrogen may have been due to onward passage of digesta to the smaller part of the stomach and no doubt part was also due to direct absorption of soluble nitrogenous compounds from the forestomach, by far the greatest loss was due to the incorporation of plant nitrogen into microbial nitrogen.

The changes in the concentration of nitrogen in the component fractions of the forestomach digesta are presented in Appendix 4 together with the weight of forestomach digesta. The proportions of bacterial

and protozoal nitrogen in the total microbial nitrogen fraction together with the percentage of microbial nitrogen in the total nitrogen in the forestomach at different times after feeding is presented in Table 4. It is clear that protozoal nitrogen did not at any time form more than a small part of the microbial nitrogen fraction. Weller *et al.* (1962) pointed out that if it can be assumed that all the nitrogen in the forestomach at any particular time is originally of plant origin then the proportion of microbial nitrogen in the total nitrogen in the forestomach is a measure of the extent of conversion of plant nitrogen into microbial nitrogen. It can be seen from Table 4 that between 64 - 85% of the total nitrogen in the forestomach of the wallaby was microbial. Hence the conversion of ingested plant nitrogen to microbial nitrogen in this wallaby fed this particular type of diet is 64 - 85% based on the assumption of Weller *et al.* (1962).

TABLE 4

Changes in microbial nitrogen at different times after feeding during Experiment 2

Animal	Time killed after commencement of feeding (hours)	% Bacterial nitrogen in Total microbial nitrogen	% Protozoal nitrogen in Total microbial nitrogen	% Microbial nitrogen in total nitrogen in forestomach digesta
54	3	94	6	64
0	5	94	6	80
25	8	85	15	85
32	12	86	14	84
23	24	89	11	74

2. RENAL RETENTION AND UTILIZATION OF UREA BY THE KANGAROO ISLAND WALLABY

A. EXPERIMENT 3

(i) Introduction

Lintern and Barker (1969) found that the Kangaroo Island Wallaby reduces the excretion of urea in the urine when depleted of nitrogen and low U/P urea ratios, similar to those obtained in nitrogen depleted ruminants (Schmidt-Nielsen et al., 1957, 1958), were recorded. Schmidt-Nielsen et al. (1957, 1958) have pointed out that renal retention of urea in nitrogen depleted ruminants is considerably greater than that recorded in monogastric mammals similarly depleted of nitrogen (Schmidt-Nielsen, 1958). They have suggested that extensive renal retention of urea in ruminants is an adaptive response to a lowered nitrogen intake, as urea retained in this manner may increase the amount of urea available for recycling to the rumen. This in turn may improve the nitrogen status of the animal and thereby increase the chance of survival of the ruminant when there is a critical shortage of nitrogen. This could also be inferred from the findings of Lintern and Barker (1969) for the Kangaroo Island Wallaby, as the results of Experiment 1 indicate that urea recycled to the forestomach could be utilized by the wallaby and such urea recycling has

been reported in the euro (Brown, 1964).

The following experiments were designed to establish if renal retention of urea in Kangaroo Island Wallabies depleted of nitrogen is a stimulated response to a lowered nitrogen intake or merely a reflection of a greater capacity of the kidney to retain urea regardless of nitrogen status.

In order to study any such effect of nitrogen status upon renal retention of urea in the Kangaroo Island Wallaby, it was found necessary to establish criteria whereby wallabies which are suffering a critical shortage of nitrogen can be distinguished from those that are not.

It appears, from studies arising from the work commenced by Boussingnault (1844, cited Munro, 1964) and Voit (1867), that once the minimum nitrogen requirement is satisfied, protein storage and hence nitrogen retention in mammals may be limited (Lusk, 1928; Swanson, 1959). It was considered that wallabies with complete protein stores, if protein storage is limited in this macropod, and receiving a diet containing sufficient nitrogen to maintain these stores, would not be experiencing a critical shortage of nitrogen. Therefore, the aim of the following experiment was to establish whether nitrogen retention is limited in wallabies with nitrogen intakes in excess of those required to maintain nitrogen equilibrium. Barker (1968) had already established the

nitrogen intake required to maintain nitrogen equilibrium in the wallaby by feeding both low and adequate nitrogen diets. As the wallabies used in the following experiment were those used by Barker (1968), it was hoped that, by combining the results from these two studies, criteria would emerge which would enable nitrogen retention to be judged from easily measurable parameters. These criteria could then be applied in the subsequent investigation of the extent and control of renal retention and recycling of urea.

(ii) Experimental Procedure

The four adult female wallabies used throughout this series of feeding trials had been in captivity for at least two years and had been cage trained and accustomed to experimental procedure during the previous twelve months, when they had been used by Barker (1968) in a similar 5 x 5 latin square experiment. The fifth animal used by Barker (1968) was no longer suitable for experimental use. In the reported 4 x 4 latin square experiment each diet was allotted to each animal using a table of random numbers. The nitrogen content of the four diets was A = 1.50gN/100g dry weight; B = 2.06gN/100g dry weight; C = 2.62gN/100g dry weight; D = 3.74gN/100g dry weight. The nitrogen content of each diet was adjusted using casein. As increases

in the casein content of each diet were not made at the expense of any energy supplement, the diets were not isocaloric; the diet containing the most casein (i.e. D) also had the highest energy content.

Nitrogen retention was measured by nitrogen balance trials performed over a ten day period during each of the four experimental periods. The routine for feeding of animals and collection and analysis of samples was that of Barker (1968). In addition urea estimations were performed on urine samples collected during the reported series of feeding trials and the urine samples collected during periods 2 - 5 of the feeding trials of Barker (1968). Urine samples from period 1 of Barker's experiment were unavailable as they had been discarded. Blood samples were also collected on the last day of each ten day balance period in the reported experiment and analysed for urea. Blood samples were not collected from animals during the feeding trials performed by Barker (1968).

(iii) Results

The raw data from this experiment is presented in Appendix 5 (A - C). In Table 5 daily dry matter intake and nitrogen intake and excretion are related to the metabolic body weight, $kgW^{0.75}$ (Kleiber, 1961). Daily dry matter

excretion ($kgW^{0.75}$) and water turnover ($kgW^{0.8}$; after Richmond et al., 1962) are presented in Appendix 6. Analysis of variance calculated from the data given in Table 5 is presented in Table 6 (A - C) and that from data in Appendix 6 in Appendix 7. Correlation coefficients between paired measurements made during the experiment together with regression equations for significantly correlated parameters are given in Appendices 8 and 9.

(1) Main findings from analysis of variance and calculation of correlation coefficients from data obtained in the reported feeding trials.

(a) Dry matter intake (DMI) varied significantly with period and animal but not with treatment. There was a significant negative correlation ($r = -0.995^{**}$) between mean DMI and mean daily temperature during the outdoor prefeeding period (25.7°, 18.9°, 14.4° and 12.4° respectively for periods I - IV) prior to each experimental period. DMI was also positively and significantly correlated with dry faecal output, water intake and faecal water output.

(b) Nitrogen intake varied significantly with treatment and period but did not vary with animal. Nitrogen intake and total urinary nitrogen were

both significantly correlated with nitrogen intake.

(c) Total urinary nitrogen did not vary significantly with animal but did vary significantly with period and treatment and was positively and significantly correlated with nitrogen intake and nitrogen balance.

(d) Faecal nitrogen did not vary significantly with animal, period or treatment and was not correlated with any of the parameters measured during the nitrogen balance trials.

(e) Nitrogen balance varied significantly with treatment but not with period or animal and was positively and significantly correlated with nitrogen intake and total urinary nitrogen.

(f) Digestibility coefficients

Apparent digestibility of dry matter and nitrogen for this series of feeding trials are presented in Appendix 5 (A and B). There was no change in apparent digestibility of dry matter with changing nitrogen or dry matter intake. However there was a stepwise increase in apparent digestibility of nitrogen with increasing nitrogen intake and these two parameters were positively and significantly correlated ($r = +0.78^{***}$).

TABLE 5

Daily dry matter and nitrogen intake and partitioned nitrogen excretion measured during the nitrogen balance trials of Experiment 3 of four female wallabies expressed as functions of their metabolic weights

Period	Treatment	Animal	Body weight ($kgW^{0.75}$)	Dry Matter Intake*	Nitrogen Intakeø	Urinary Nitrogenø	Faecal Nitrogenø	Nitrogen Balanceø	Urinary urea Nitrogenø
I	A	2	2.925	9.9	161	105	75	-19	6
	B	16	3.476	11.4	322	163	87	+72	36
	C	1	3.767	23.5	667	389	220	+59	193
	D	18	3.313	18.3	723	391	225	+107	230
II	A	18	3.246	27.9	332	158	145	+28	60
	B	2	3.230	20.2	323	156	145	+22	62
	C	16	3.387	18.7	508	238	227	+44	107
	D	1	3.653	23.6	770	486	227	+57	311
III	A	16	3.283	25.0	433	150	225	+58	53
	B	1	3.617	28.5	486	226	216	+44	70
	C	18	3.200	35.6	907	648	178	+81	447
	D	2	2.906	24.1	927	707	149	+71	495
IV	A	1	3.574	29.0	335	172	148	+15	74
	B	18	3.148	33.2	694	418	215	+61	251
	C	2	2.902	26.0	778	473	222	+82	307
	D	16	3.226	27.2	1008	737	188	+83	575

* $g/kgW^{0.75}/day$ ø $mgN/kgW^{0.75}/day$

TABLE 6

Summary of analysis of variance of parameters measured during the nitrogen balance trials of Experiment 3 and presented in Table 6. Mean measurements are also presented.

A.

Parameter	Treatment				F	Statistical Significance
	A	B	C	D		
Dry Matter Intake+	23.0	23.3	25.9	23.3	1.11	NS
Nitrogen Intake°	315	456	715	857	34.14	***
Urinary Nitrogen°	147	241	437	580	12.97	**
Faecal Nitrogen°	148	166	212	197	1.16	NS
Nitrogen Balance°	+20	+49	+66	+80	6.00	**

+ $g/kgW^{0.75}/day$ ° $mgN/kgW^{0.75}/day$

TABLE 6 (cont.)

B.

Parameter	Period				F	Stastical Significance
	I	II	III	IV		
Dry Matter Intake^{+}	15.8	22.6	28.3	28.9	21.79	**
Nitrogen Intake^{o}	468	483	688	704	9.22	**
Urinary Nitrogen^{o}	262	260	433	450	3.74	*
Faecal Nitrogen^{o}	152	186	192	193	0.54	NS
Nitrogen Balance^{o}	+54	+37	+63	+61	1.22	NS

$^{+}$g/kgW$^{0.75}$/day $\quad$ ^{o} mgN/kgW$^{0.75}$/day

TABLE 6 (cont.)

C.

Parameters	Animal				F	Statistical Significance
	1	2	3	4		
Dry Matter Intake+	26.2	20.0	20.6	28.8	10.63	**
Nitrogen Intake°	564	547	568	664	1.59	NS
Urinary Nitrogen°	318	361	322	404	0.55	NS
Faecal Nitrogen°	203	148	182	191	0.78	NS
Nitrogen Balance°	+43	+38	+64	+69	2.05	NS

+ $g/kgW^{0.75}/day$ ° $mgN/kgW^{0.75}/day$

Apparent nitrogen digestibility was independent of DMI and apparent dry matter digestibility.

(2) Nitrogen intake, nitrogen balance and nitrogen excretion

(a) Nitrogen balance and nitrogen intake.

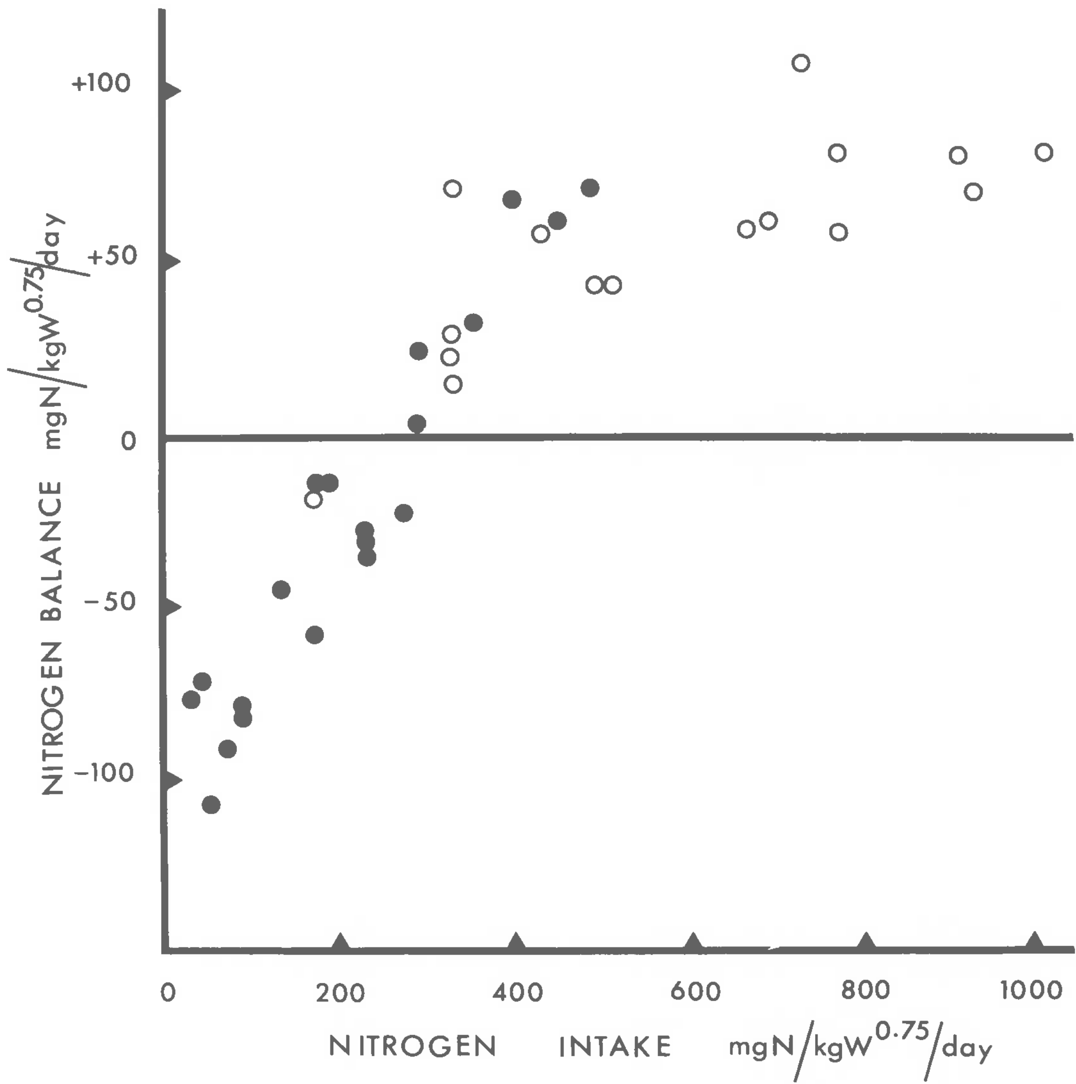
In Figure 4 nitrogen balance is plotted against nitrogen intake. Included are the data from the reported experiment together with the data of Barker (1968) for the same four animals given five treatments at lower nitrogen intakes. It is apparent that the relationship between nitrogen balance (retention) and nitrogen intake is not linear. At nitrogen intakes up to 400- 500 mgN/kgW$^{0.75}$/day nitrogen balance increases with nitrogen intake. However at higher nitrogen intakes ~~there is no corresponding increase in nitrogen balance and~~ the rate of nitrogen retention ~~is almost constant.~~ declines. Thus above and below nitrogen intakes of 400- 500 mgN/kgW$^{0.75}$/day there is a different relationship between nitrogen intake and nitrogen balance.

FIGURE 4

Changes in nitrogen balance (mgN/kgW$^{0.75}$/day) with increasing nitrogen intake (mgN/kgW$^{0.75}$/day) for four wallabies fed diets of differing nitrogen content during a 5 x 5 latin square experiment (Barker, 1968) and a 4 x 4 latin square experiment (this thesis).

● data obtained by Barker (1968)

○ data obtained by the author


+100
+50
0
−50
−100
NITROGEN BALANCE mgN/kgW$^{0.75}$/day
0
200
400
600
800
1000
NITROGEN INTAKE mgN/kgW$^{0.75}$/day

(b) Nitrogen **intake** and total urinary nitrogen excretion.

In Figure 5 total nitrogen and urea nitrogen excreted in the urine are plotted against nitrogen intake. Again data obtained in the reported experiment together with data for the same four animals obtained by Barker (1968) are included. In addition, values for urinary urea nitrogen excretion obtained by the author by analysing urine samples collected during periods 2 - 5 of Barker's experiment (Appendix 10) are presented. It can be seen that the pattern of nitrogen excretion in the urine, like nitrogen balance, differs above and below nitrogen intakes of 400 - 500 mgN/kg$W^{0.75}$/day. Below this nitrogen intake there is a slow and constant increase in the excretion of total urinary nitrogen. However above this nitrogen intake, total urinary nitrogen excretion increases rapidly.

This pattern of increasing total urinary nitrogen excretion with increasing nitrogen intake is brought about primarily by a similar pattern in the excretion of urea nitrogen. However the increase in total urinary nitrogen with increasing

nitrogen intake was also due to a slight increase in the excretion of non-urea nitrogen in the urine. There was a positive and significant correlation between non-urea nitrogen in the urine and nitrogen intake over the entire range of nitrogen intakes obtained in the reported experiment and that of Barker (1968) and this is illustrated in Figure 6. There was also a positive and significant correlation between the percentage of total urinary nitrogen excreted as urea nitrogen over the whole range of nitrogen intakes in the reported experiment and that of Barker (1968) (Figure 7).

(c) Faecal nitrogen and nitrogen intake

These two parameters were not significantly correlated in the reported experiment. In Figure 8 faecal nitrogen is plotted against nitrogen intake and includes values obtained in the reported experiment together with those obtained by Barker (1968). It can be seen that faecal nitrogen increased with increasing nitrogen intake up to a nitrogen intake of 400 - 500 $mgN/kgW^{0.75}/day$ and then remained unchanged up to a nitrogen intake of about 800 $mgN/kgW^{0.75}/day$. At higher nitrogen

FIGURE 5

Changes in total urinary nitrogen (mgN/kg$W^{0.75}$/day) and urinary urea nitrogen (mgN/kg$W^{0.75}$/day) with increasing nitrogen intake (mgN/kg$W^{0.75}$/day) for four wallabies.

Total urinary nitrogen

● Data obtained for these four wallabies fed five diets of differing nitrogen content by Barker (1968) during a 5 x 5 latin square experiment.

○ Data obtained for these four wallabies fed four diets of differing nitrogen content by the author during a 4 x 4 latin square experiment.

Urinary urea nitrogen

■ Data obtained for these four wallabies fed four diets of differing nitrogen content by Barker (1968) during four periods of a 5 x 5 latin square experiment. Urine samples from Barker's experiment were analysed for urea by the author and urine samples from period 1 of Barker's experiment were no longer available.

□ Data obtained for these four wallabies fed four diets of differing nitrogen content by the author during a 4 x 4 latin square experiment.

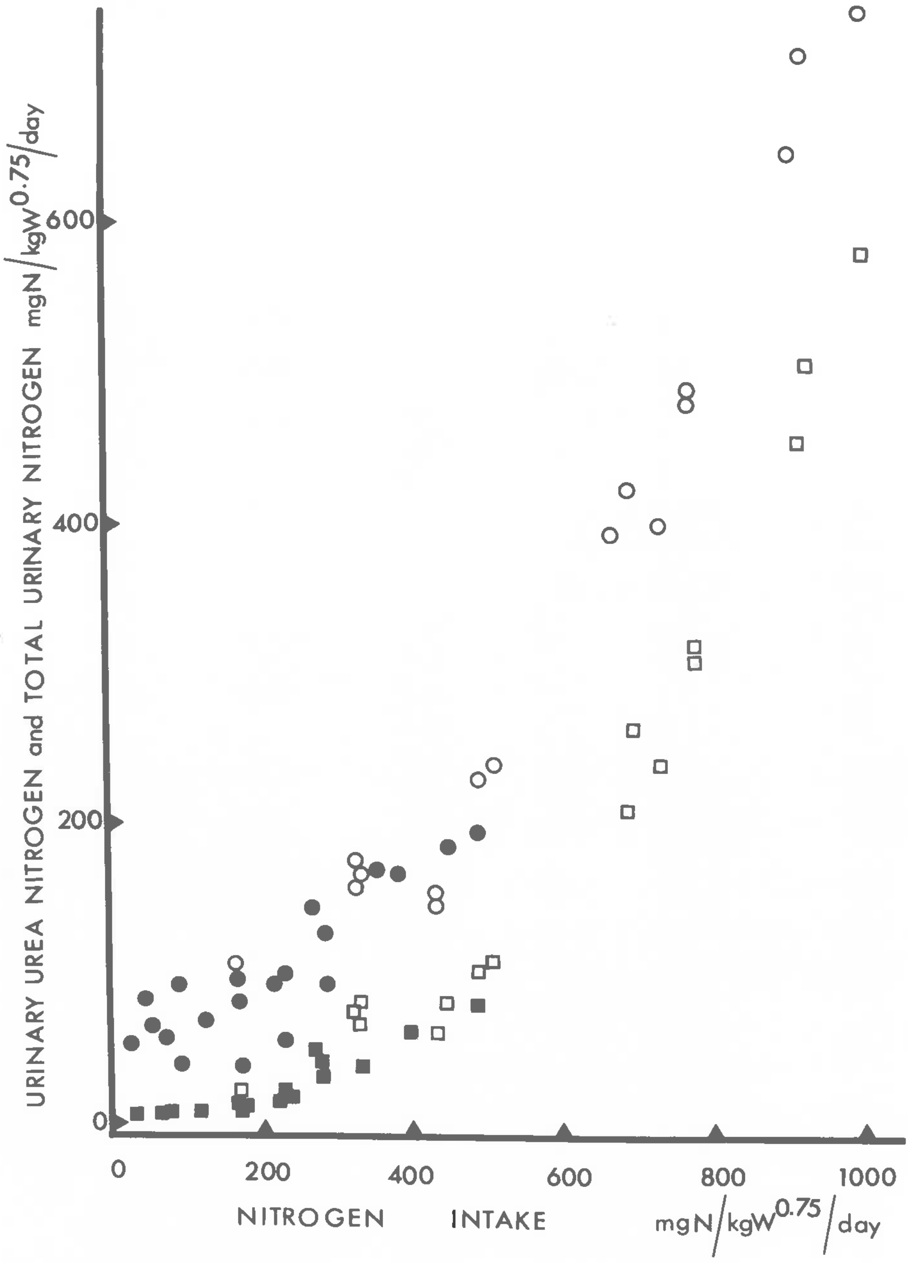

URINARY UREA NITROGEN and TOTAL URINARY NITROGEN mgN/kgW0.75/day
600
400
200
0
0
200
400
600
800
1000
NITROGEN INTAKE mgN/kgW0.75/day

FIGURE 6

Changes in urinary non-urea nitrogen (non-urea-N) (mgN/kg$W^{0.75}$/day) with increasing nitrogen intake (mgN/kg$W^{0.75}$/day) for four wallabies.

● Data obtained by reanalysis of urine samples from four periods of the 5 x 5 latin square experiment of Barker (1968).

○ Data obtained during the reported 4 x 4 latin square experiment.

Urinary non-urea nitrogen and nitrogen intake were positively and significantly correlated ($r = +0.92^{***}$); the regression equation is

$$Y = 0.17X + 39.1$$

where Y = urinary non-urea nitrogen (mgN/kg$W^{0.75}$/day)

X = nitrogen intake (mgN/kg$W^{0.75}$/day)

FIGURE 7

Changes in the percentage of urea nitrogen in the total urinary nitrogen (UUN % TUN) with increasing nitrogen intake (mgN/kg$W^{0.75}$/day).

● Data obtained by reanalysis of urine samples from four periods of the 5 x 5 latin square experiment of Barker (1968).

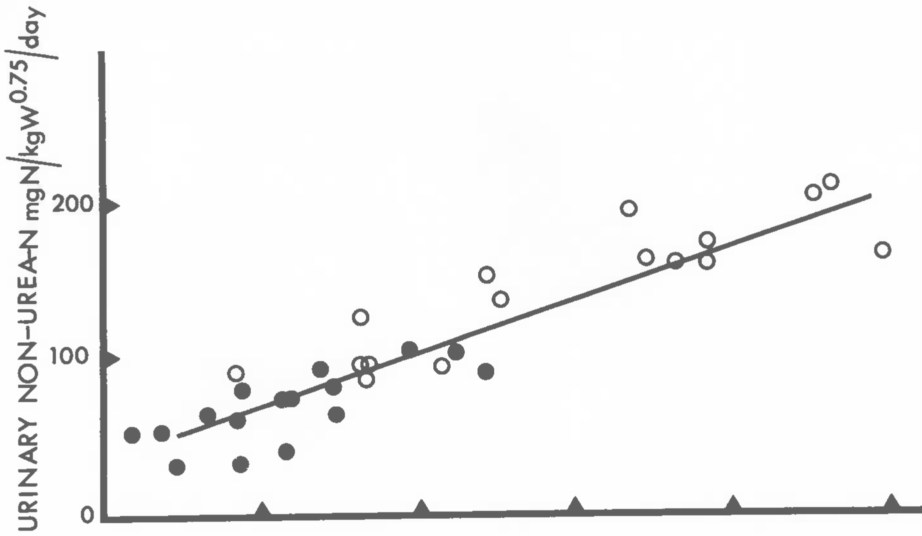
○ Data obtained during the reported 4 x 4 latin square experiment.

The percentage of urea nitrogen excreted in the total urinary nitrogen was positively and significantly correlated with nitrogen intake ($r = +0.96^{***}$); the regression equation is

$$Y = 0.08X + 3.9$$

where Y = the percentage of urea nitrogen excreted in the total urinary nitrogen.

X = nitrogen intake (mgN/kg$W^{0.75}$/day).


URINARY NON-UREA-N mgN/kgW$^{0.75}$/day
200
100
0

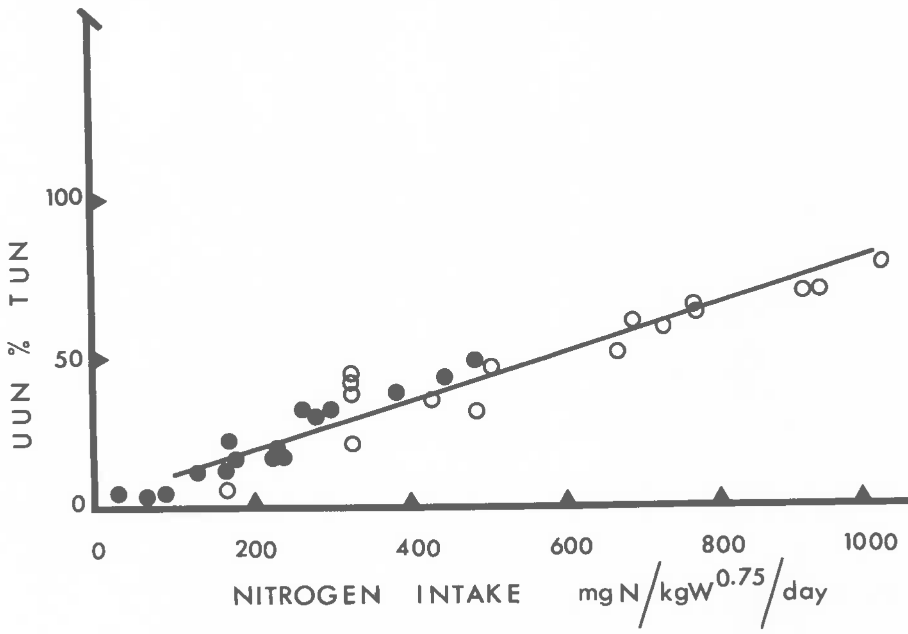
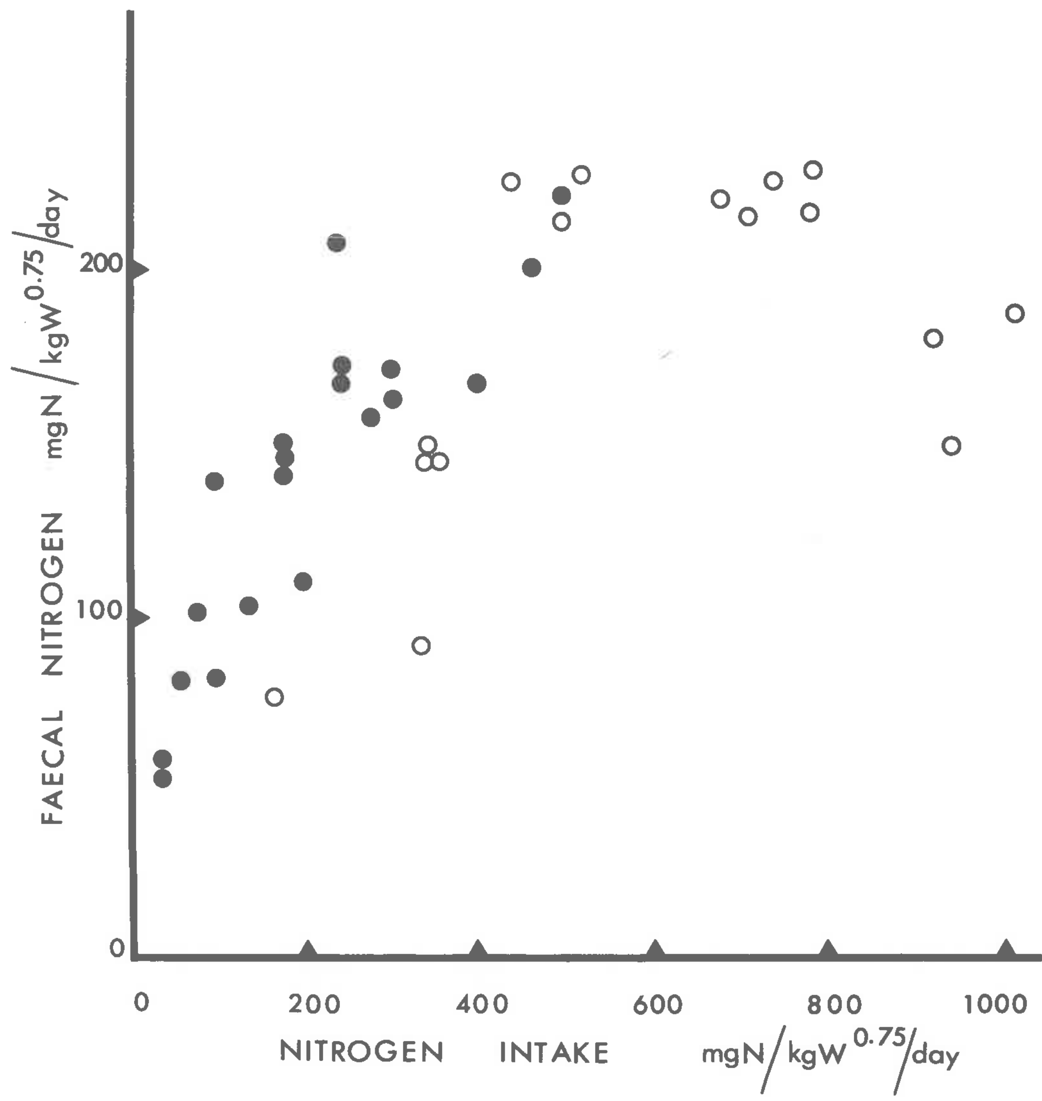

UUN % TUN
100
50
0
0
200
400
600
800
1000
NITROGEN INTAKE mgN/kgW$^{0.75}$/day

FIGURE 8

Changes in faecal nitrogen (mgN/kg$W^{0.75}$/day) with increasing nitrogen intake (mgN/kg$W^{0.75}$/day) for four wallabies fed nine diets of differing nitrogen content during a 5 x 5 latin square experiment (Barker, 1968) and a 4 x 4 latin square experiment (this thesis).

● data obtained by Barker (1968)

○ data obtained by the author



FAECAL NITROGEN mgN/kgW$^{0.75}$/day
200
100
0
0
200
400
600
800
1000
NITROGEN INTAKE mgN/kgW$^{0.75}$/day

intakes faecal nitrogen declined.

(3) Plasma urea and U/P urea ratios

In Figure 9 plasma urea concentration is plotted against nitrogen intake. These two parameters were positively and significantly correlated. Urea U/P ratios were also positively and significantly correlated with nitrogen intake and this is illustrated in Figure 10.

(4) Conclusion

The results of the reported experiment indicate that the rate of nitrogen retention in the Kangaroo Island Wallaby fed this particular type of diet is limited at nitrogen intakes in excess of 400mgN/kg$W^{0.75}$/day. The nitrogen contents of the presented diets which resulted in maximum rate of nitrogen retention were greater than 1.5gN/100g dry weight. A maximum rate of nitrogen retention was associated with levels of urea nitrogen in the urine which formed more than 50 - 60% of the total urinary nitrogen and U/P urea ratios of greater than 50. At the lowest levels of nitrogen retention obtained by Barker (1968) for the same wallabies urea nitrogen formed less than 15% of the total nitrogen in the urine. Corresponding U/P urea ratios cannot be determined because plasma urea was not measured in the experiment of Barker (1968).

FIGURE 9

Changes in plasma urea (mg/100 ml) with increasing nitrogen intake ($mgN/kgW^{0.75}/day$) for four wallabies fed four diets of differing nitrogen content during a 4 x 4 latin square experiment. Plasma urea was positively and significantly correlated with nitrogen intake ($r = +0.71^{**}$); the regression equation is:

$$Y = 0.03X + 28.2$$

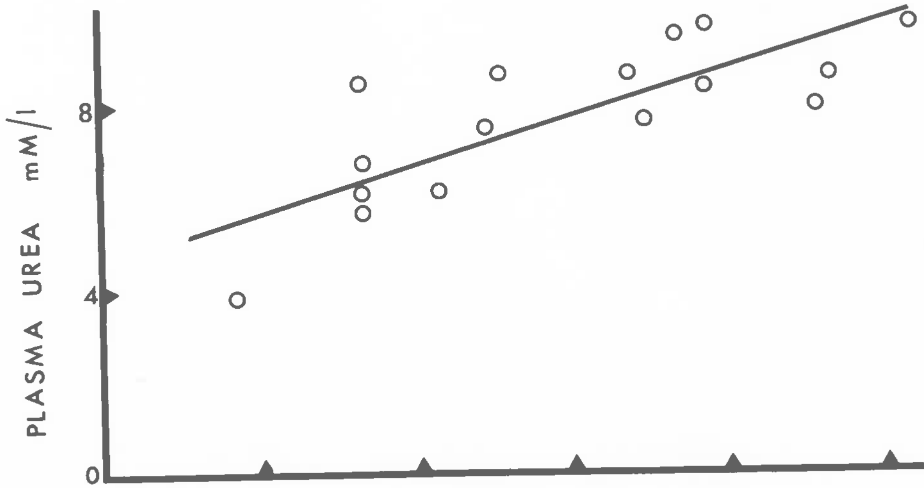
where Y = plasma urea (mg/100 ml)
X = nitrogen intake ($mgN/kgW^{0.75}/day$)

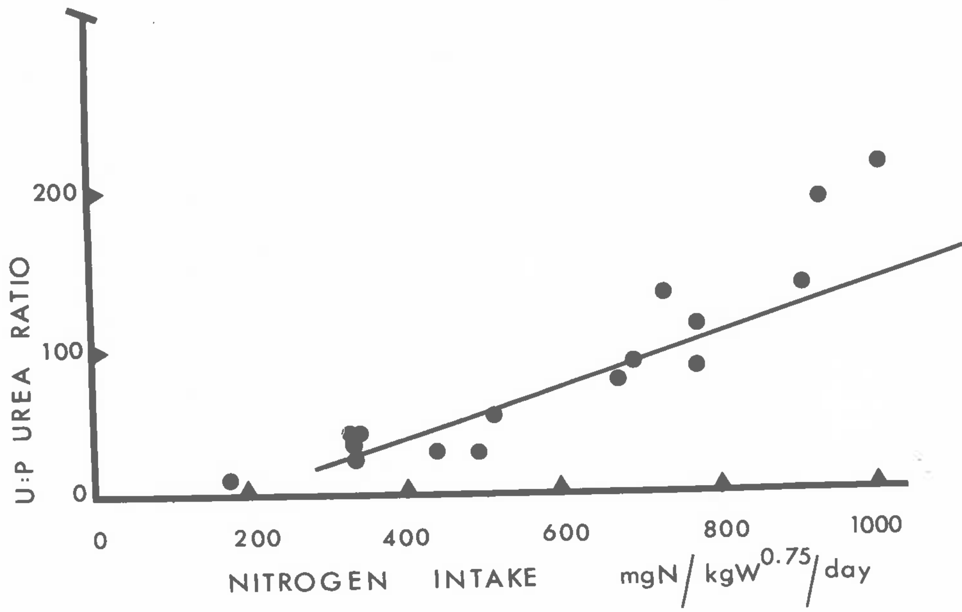
FIGURE 10

Changes in the ratio: urea Urine/Plasma (U/P urea) with increasing nitrogen intake ($mgN/kgW^{0.75}/day$) for four wallabies fed four diets of differing nitrogen content during a 4 x 4 latin square experiment. Urea U/P ratios were positively and significantly correlated with nitrogen intake ($r = +0.92^{***}$); the regression equation is:

$$Y = 0.22X - 46.3$$

where Y = U/P urea
X = nitrogen intake ($mgN/kgW^{0.75}/day$)


PLASMA UREA mM/l
8
4
0


U:P UREA RATIO
200
100
0
0
200
400
600
800
1000
NITROGEN INTAKE mgN/kgW0.75/day

However Lintern and Barker (1969) found that male wallabies, fed a low nitrogen diet and in negative nitrogen balance similar to that obtained by Barker (1968) for female wallabies, had U/P urea ratios of 11 to 12. Therefore in the following three experiments wallabies fed diets of nitrogen content greater than 1.5gN/100g dry weight and with U/P urea ratios greater than 50 and excreting more than 50% of the total urinary nitrogen in the form of urea nitrogen have been defined as having a maximum rate of nitrogen retention. Similarly wallabies fed a diet with the lowest nitrogen content possible (i.e. one in which no supplemental nitrogen has been added) have been defined as having a minimum rate of nitrogen retention when their U/P urea ratios are 10 or less and urea nitrogen forms less than 15% of the total urinary nitrogen.

B. EXPERIMENT 4

(i) Introduction

The following experiment was carried out to further the investigation of the control and extent of renal retention of urea in the Kangaroo Island Wallaby during periods of nitrogen depletion.

Lintern and Barker (1969) had already established that the reduction in the excretion of urea in the urine of the nitrogen depleted wallaby was similar to that recorded in nitrogen depleted sheep (Schmidt-Nielsen _et al._, 1958; Schmidt-Nielsen and O'Dell, 1959) and camels (Schmidt-Nielsen _et al._, 1957) and considerably less than that recorded in man and dog similarly nitrogen depleted (Schmidt-Nielsen, 1958). As previously pointed out, this difference in the excretion of urea in the urine between nitrogen depleted monogastric mammals and ruminants and wallabies is thought to reflect an adaptation of the ruminant and wallaby kidney to periods of nitrogen depletion. Extensive renal retention of urea in these mammals may enhance their nitrogen economy by increasing the amount of urea available for recycling to and utilization in the forestomach.

Schmidt-Nielsen _et al._ (1957, 1958) have established that renal retention of urea in camels and sheep is due to extensive reabsorption of urea in the renal tubule, as glomerular filtration rate (GFR) is independent of nitrogen intake whilst urea clearance is closely related to nitrogen intake. They also found that renal tubular reabsorption of urea in nitrogen depleted sheep and camels is extremely efficient, as only 1 - 5% of the urea filtered by the kidney was subsequently excreted in the urine.

Kidney slice analysis, similar to that performed on sheep (Schmidt-Nielsen and O'Dell, 1959), suggested that renal regulation of urea excretion in the Kangaroo Island Wallaby also occurs at the renal tubular level. However as nitrogen depletion in rats and dogs results in a reduction in urinary urea excretion which is associated with a reduction in GFR (Shannon, 1936a; Dicker, Heller and Hewer, 1946; Dicker, 1949), the possibility that this could also account for the reduction in urinary urea excretion in wallabies depleted of nitrogen could not be disregarded.

Accordingly the following experiment was carried out to investigate the site of control of renal retention of urea in the Kangaroo Island Wallaby, and in addition, if this were found to be due to renal tubular reabsorption, to establish whether renal tubular reabsorption of urea in nitrogen depleted wallabies is as extensive as it is in nitrogen depleted ruminants.

As there is no published account of measurement of GFR in a macropod marsupial, it was necessary to find a suitable reference substance (Smith, 1956) the clearance of which could be used for this purpose. As the wallaby responds adversely to extensive handling (Lintern and Barker, 1969), a reference substance of endogenous origin was considered to be the ideal index of GFR, since measurement of the clearance of such a

substance would not require handling the animal except for the collection of blood samples.

Although clearance of endogenous true creatinine (ETC) has been widely used as an index of GFR in mammals fed a creatinine-free diet, comparisons of ETC clearance with a standard reference substance, such as inulin, have revealed that ETC clearance is only a valid measure of GFR in some mammals. It is valid for the camel (Schmidt-Nielsen et al., 1957), sheep (Shannon, 1937; Schmidt-Nielsen et al., 1958), rabbit, seal and cat (Smith, 1956) but not the dog (Hare and Hare, 1954), rodents (Schmidt-Nielsen, 1954), cattle (Campbell and White, 1967) and man (Hare, Goldstein, Barnett, McNamara and Hare, 1949; Pitts, 1969; Mandel, Jones, Willis and Cargill, 1953; Miller and Winker, 1938; Mattar, Barnett, McNamara and Lauson, 1952; Taggart, 1950; Miller, Leaf, Mamby and Miller, 1952). In the case of man the results have been somewhat conflicting since McQueen, Morrison and Wong (1960) have found ETC clearance to be a valid measure of GFR.

In the light of this evidence the validity of ETC clearance as a measure of GFR was tested in the Kangaroo Island Wallaby using inulin clearance as a standard of reference.

(ii) Experimental Procedure

(1) The validity of ETC clearance as a measure of GFR using inulin clearance as a standard of reference.

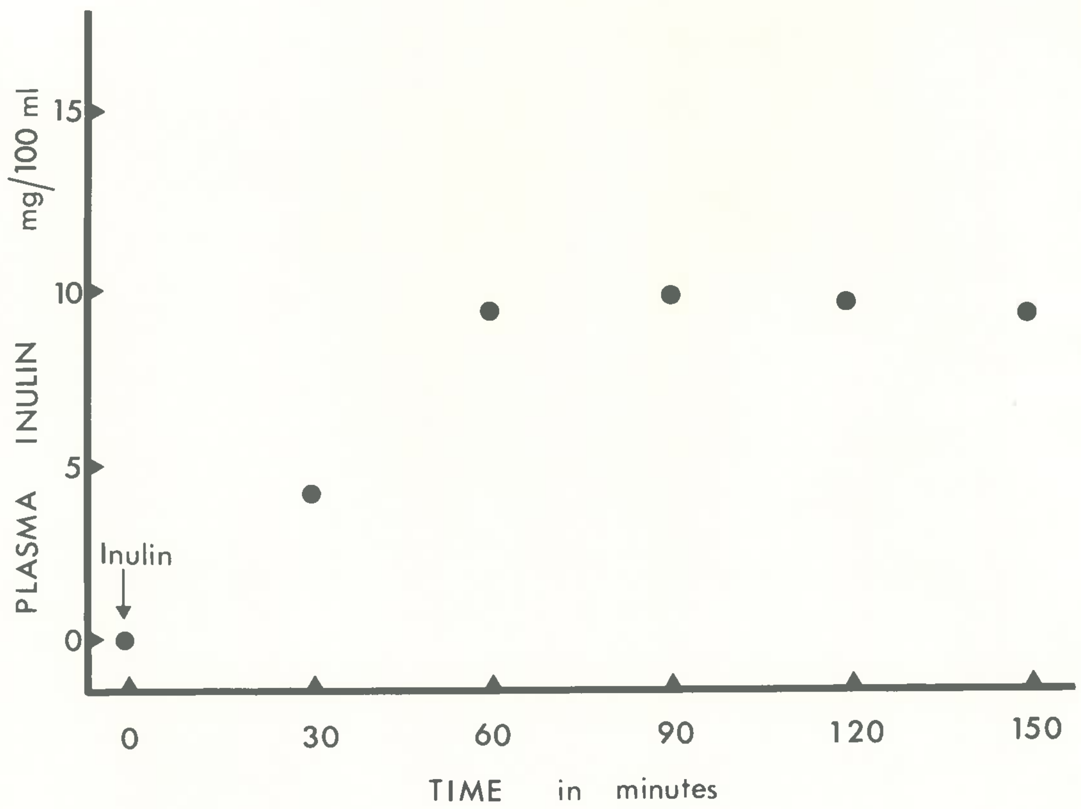
Four adult male Kangaroo Island Wallabies, which had been housed in individual outdoor pens in the Zoology Department animal yards and fed a maintenance diet of 1.5gN/100g dry weight and allowed water *ad lib.*, were used throughout this experiment.

Ramsay and Coxon (1967) found that a single subcutaneous injection of inulin (2 ml/kg body weight of an aqueous solution of 20g inulin/100 ml) could be used to achieve a constant plasma inulin level for a minimum period of one hour in dogs. In order to test whether this dosage was applicable to the Kangaroo Island Wallaby each animal was anaesthetised with nembutal via a marginal ear vein, and a blood sample taken at thirty minute intervals for one hundred and fifty minutes. Plasma from these samples was then analysed for inulin. Mean plasma inulin concentrations for the four wallabies are presented in Figure 11.

A constant plasma inulin concentration lasting for sixty to ninety minutes and commencing sixty minutes after the injection was achieved for each animal. These results agree with those of Ramsay and Coxon (1967).

FIGURE 11

Changes in mean plasma inulin concentrations (mg/100ml) for four male wallabies before and after a subcutaneous injection of inulin (2ml/kg body weight of an aqueous solution 20g inulin/100ml) during Experiment 4.



PLASMA INULIN mg/100 ml
15
10
5
0
Inulin
0
30
60
90
120
150
TIME in minutes

Having found that this method was suitable for the Kangaroo Island Wallaby, two thirty minute clearance trials were carried out to compare the clearance of inulin and ETC in animals fed a maintenance diet. Each animal was fasted overnight and the following morning weighed and water loaded with approximately 50 ml of water per kilogram body weight to induce water diuresis and hence a high urine flow rate (Ramsay and Coxon, 1967). Thirty minutes after water loading the animals were anaesthetised and given a subcutaneous injection of inulin (2 ml/kg body weight of an aqueous solution 20g/100 ml) in the shoulder area.

Ninety minutes after water loading and sixty minutes after dosage with inulin two thirty minute clearance periods were taken. Immediately prior to the commencement of the clearance periods a polythene bulb catheter was introduced via the urethra into the bladder and the bladder emptied. Urine was collected from the bladder during the clearance periods from the catheter. A blood sample was collected at the mid point of each clearance period. Penicillin was administered as a routine at the end of the clearance period and the animals returned to outdoor pens and feeding of the maintenance diet continued.

Inulin and ETC in plasma and urine samples were determined and from the results presented in Appendix 11 it is clear that the ratio (ETC U/P/ Inulin U/P) is not significantly different from 1 indicating that ETC clearance, under the conditions of this experiment, is a valid measure of GFR in the Kangaroo Island Wallaby.

(2) Fluctuations in plasma urea and ETC concentrations over a thirty six hour period.

In order to measure ETC and urea clearance over a twenty four hour period during which only one blood sample was taken it was necessary to determine if plasma urea and ETC concentrations fluctuated during this time. Accordingly the four wallabies (still fed a maintenance diet) were placed in metabolism cages and blood samples collected at six hourly intervals over a thirty six hour period. The animals during this period were allowed food and water ad lib.

Plasma ETC and urea concentrations are presented in Appendix 12A where it can be seen that plasma ETC remained fairly constant over the entire thirty six hour period, with the exception of animal 56. This animal was particularly agitated when caged and the fluctuations in plasma ETC may have been due to activity (Beard, 1943). Plasma urea concentrations fluctuated

in a uniform manner reaching maximum values at 11 a.m. and 11 p.m. daily.

Somers (1961c) attributed similar diurnal fluctuations in plasma urea concentrations in sheep to the feeding pattern of these animals, as plasma urea concentrations reached a maximum approximately nine hours after feeding and five hours after ruminal ammonia levels had reached a maximum. It was considered that the uniform fluctuation in plasma urea recorded in the Kangaroo Island Wallaby may also be due to the feeding pattern. Wallabies in captivity have been observed to feed mainly at dawn and dusk, regardless of when food is presented and in Experiment 2 it was demonstrated that feeding resulted in a rise in forestomach ammonia levels which reached a maximum some three hours after feeding. It would be expected therefore that this would result in raised plasma urea levels as occurs in sheep.

Accordingly six hourly blood sampling was repeated, the animals were deprived of food and water during this thirty six hour period and also eight hours prior to the collection of the first blood sample. From Appendix 12B it can be seen that fluctuations in plasma urea and ETC were less evident under these conditions. Since urea levels remained at the minimum value obtained

during the previous thirty six hour collection period (Appendices 12 A and B) this was evidence to support the suggestion that the peak in plasma urea levels was postprandial in origin. Thus it was now possible to create conditions in which a single blood sample was representative of 24 hour plasma urea and ETC levels.

(3) Measurement of GFR and the fraction of filtered urea excreted in wallabies fed a high or low nitrogen diet.

The wallabies were then divided randomly into two groups. Animals 392r and 394r were assigned to a low nitrogen diet (0.3gN/100g dry weight) and animals 10 and 56 a high nitrogen diet (3.7gN/100g dry weight) and these diets were fed with water ad lib. for one month. One week prior to the end of the prefeeding period the animals were placed in metabolism cages in a temperature controlled animal house (21°). The animals were weighed and a twenty four hour urine sample and a blood sample collected on each of three alternate days. The animals were deprived of food and water during each twenty four hour collection and for eight hours prior to the commencement of each collection period. Depriving the animals of water ensuredthat urine flow rate was low, a condition necessary for this experiment, as

Schmidt-Nielsen (1958) has reported "wash out" of urea from kidney tissue of some nitrogen depleted mammals with high urine flow rates. Depriving the animals of food ensured that the plasma urea levels would be constant over a twenty four hour period.

Meyer, Weir and Smith (1955) found that sheep, which had been fed an adequate nitrogen diet and then deprived of food and water for thirty six hours, show a small reduction in body weight, urine volume and the excretion of nitrogen in the urine. Although depriving wallabies of food and water for thirty two hours may have had a similar effect in the reported experiment, both groups of wallabies were treated in the same way.

Urine was collected every three hours and residues remaining on the shutes were washed down with distilled water. Bulked samples of urine and washings were immediately frozen at -10°. This was to reduce any decomposition of creatinine which occurs at room temperature (Van Niekerk, Bensadoun, Paladines and Reid, 1963).

It should be pointed out that in order to use ETC clearance as a measure of GFR in this study the basic assumption had to be made that renal handling of ETC was the same under conditions of either high urine flow

(water diuresis) or low urine flow. O'Connor (1962) points out that examination of papers in which ETC clearance and inulin clearance have been reported as not significantly different, reveals that the urine flow has frequently been raised by osmotic or water diuresis and therefore the validity of extrapolating from this to conditions of minimum urine flow is questionable and conflicting results leave this unresolved (Shannon, 1936b; Chelsey, 1938; Ladd, Liddle and Gagnon, 1956). Before clearance of ETC and inulin are accepted categorically as a measure of GFR in the Kangaroo Island Wallaby the clearance of these substances should be investigated at varying plasma concentrations and urine flow rates.

Plasma and urine samples were analysed for ETC and urea and in addition urine samples were analysed for total nitrogen. Samples from the first collection day were analysed before collections were made on the subsequent two collection days to ensure that U/P urea ratios in high nitrogen animals were greater than 50 and that urinary urea formed more than 50% of the total nitrogen in the urine and similarly that low nitrogen wallabies had U/P urea ratios of less than 10 and that urea formed less than 15% of the total urinary nitrogen.

Following the three day collection period a comparison of inulin and ETC clearance was performed, as described before, to test whether ETC is a valid measure of GFR in wallabies fed high and low nitrogen diets.

The animals were then returned to outdoor pens and fed a maintenance diet for one month. Animals 10 and 56 were then fed the low nitrogen diet and animals 392r and 394r the high nitrogen diet for a subsequent month. Following one week of cage training during the last week of the prefeeding period, twenty four hour collections were made on three alternate days as before. This was again followed by a comparison of ETC and inulin clearance.

(iii) Results

The results from the three alternate day collections during Periods I and II are presented in Table 7(A and B). Results for individual daily collections are presented in Appendix 13(A, B, C and D). It can be seen that on day one of each collection period wallabies fed the high nitrogen diet had U/P urea ratios greater than 50 and urea nitrogen formed more than 50% of the total urinary nitrogen. Similarly, wallabies fed the low nitrogen diet had U/P urea ratios of less than 10 and urea nitrogen formed less than 15% of the total urinary

nitrogen. Therefore the criteria established in Experiment 3 were satisfied.

Statistical analyses of the results indicates urine volume, plasma ETC, urinary ETC, GFR and urea retained by the kidney do not differ significantly with the nitrogen content of the diet and hence nitrogen intake. However plasma urea, urinary urea and U/P urea ratios differed significantly with the diet. None of the parameters measured varied significantly with period (Table 8).

A comparison of inulin and ETC clearance after Periods I and II indicated that ETC clearance is a valid measure of GFR when the animals are fed a high or low nitrogen diet (Appendix 14A and B).

TABLE 7A

Mean values for parameters measured on three alternate days during period I of Experiment 4 for four wallabies fed a high or low nitrogen diet

Diet	Low Nitrogen		High Nitrogen	
Animal	392r	394r	10	56
Body weight (kg)	4.013	6.061	7.158	6.456
Urine Volume (ml/day)	72	59	89	63
Plasma Urea (mM/l)	2.0	1.8	8.4	7.1
Plasma ETC (mg/100 ml)	1.2	1.3	1.0	1.0
Urinary Urea (mg/day)	14	17	6360	5114
Urinary ETC (mg/day)	89.0	128.8	153.1	138.8
U/P Urea Ratio	2	3	143	189
Creatinine coefficient*	22.18	21.26	21.39	21.50
GFR (ml/day)	7380	9984	15310	13880
Renal retention of urea (mg/day)	884	1091	1361	840
% filtered urea excreted	1.5	1.6	82.1	86.1
Urinary Nitrogen (mg/day)	86	100	3356	2897
% Urea Nitrogen in Urinary Nitrogen	8	8	89	83

* ETC excreted in the urine expressed in mg/kg/day

TABLE 7B

Mean values for parameters measured on three alternate days during period II of Experiment 4 for four wallabies fed a high or low nitrogen diet

Diet	High Nitrogen		Low Nitrogen	
Animal	392r	394r	10	56
Body Weight (kg)	4.724	6.602	6.308	5.610
Urine Volume (ml/day)	63	75	116	53
Plasma Urea (mM/1)	7.5	9.1	1.5	1.7
Plasma ETC (mg/100 ml)	1.4	1.4	0.9	0.9
Urinary Urea (mg/day)	2577	4707	28	17
Urinary ETC (mg/day)	104.8	140.6	136.2	123.2
U/P Urea Ratio	94	120	3	3
Creatinine coefficient*	22.18	21.30	21.60	21.95
GFR (ml/day)	7521	10067	15191	13839
Renal retention of urea (mg/day)	821	777	1370	1423
% filtered urea excreted	75.9	85.9	2.0	1.2
Urinary Nitrogen (mg/day)	1638	2852	145	118
% Urea Nitrogen in Urinary Nitrogen	74	79	9	7

* ETC excreted in the urine expressed in mg/kg/day

TABLE 8

Variations of parameters presented in Tables 7A and 7B with diet and period during the two experimental periods of Experiment 4

Parameter	Period t_6	Diet t_6
Urine Volume (ml/day)	0.40 NS	0.17 NS
Plasma Urea (mM/l)	0.05 NS	13.42***
Plasma ETC (mg/100ml)	1.20 NS	0.85 NS
Urinary Urea (mg/day)	0.85 NS	5.95***
Urinary ETC (mg/day)	0.31 NS	1.02 NS
U/P urea	0.51 NS	6.61***
GFR (ml/day)	0.01 NS	0.04 NS
Creatinine coefficient	0.64 NS	0.58 NS
% filtered urea excreted	0.05 NS	35.97***
Renal retention of urea (mg/day)	0.24 NS	1.24 NS
Urinary Nitrogen (mg/day)	0.38 NS	6.99***
% Urea Nitrogen in Total Urinary Nitrogen	0.51 NS	16.3 ***

C. EXPERIMENT 5

(i) Introduction

The results of Experiment 4 indicated that the Kangaroo Island Wallaby, like the ruminant (Schmidt-Nielsen *et al.*, 1957, 1958) reduces the fraction of filtered urea excreted in the urine during periods of nitrogen depletion and that this is brought about by extensive reabsorption of urea from the filtrate in the renal tubule. However, no statistical difference was found in the amount of urea retained by the kidney of the nitrogen depleted wallabies and those in positive nitrogen balance, although the former consistently retained more urea than the latter.

It has been demonstrated that camels (Schmidt-Nielsen *et al.*, 1957) and sheep (Schmidt-Nielsen and Osaki, 1958; Houpt, 1959; Somers, 1961d) retain injected urea when they are depleted of nitrogen although circulating plasma urea levels, at least for a limited period, may be elevated to levels found when these animals are receiving an adequate nitrogen diet. It was further found that camels (Schmidt-Nielsen *et al.*, 1957) and sheep (Somers, 1961d) receiving an adequate nitrogen diet excrete much of the injected urea in the urine. Retention of injected urea has also been demonstrated in goats (Houpt, 1959) and rabbits (Houpt, 1963) depleted of nitrogen. These results suggest that a particular mechanism for the reabsorption of

urea operates in the kidney of these animals during nitrogen depletion and is stimulated by a depressed nitrogen status but unaffected by temporary rises in circulating plasma urea levels. It was considered that as the Kangaroo Island Wallaby parallels the ruminant in other aspects of renal handling of urea, the kidney of this wallaby might also retain injected urea during periods of nitrogen depletion. This was investigated in the following experiment.

(ii) Experimental Procedure

Eight adult male Kangaroo Island Wallabies were used. They were fed a maintenance diet (1.5gN/100g dry matter intake) and allowed water *ad lib.* for four weeks. During this time they were periodically placed in metabolism cages to accustom them to experimental routine. At the end of this period the animals were divided randomly into two groups of four. One group (wallabies 39r, 392r, 89 and 394r) was fed a low nitrogen diet (0.30gN/100g dry weight) and the other (wallabies 39, 7, 1 and 4) a high nitrogen diet (2.60 gN/100g dry weight).

Twenty four hour urine samples were collected together with a blood sample immediately prior to the commencement of feeding of test diets and again three weeks later. The wallabies were then held in metabolism cages for one week for cage training. After the wallabies had been fed test diets for

one month, twenty four hour urine samples together with blood samples were collected on three consecutive days. At the end of the three day collection period each animal was injected with 700mg of urea (30g/100ml in 0.5% saline after Houpt and Houpt, 1968). Since these animals are particularly difficult to handle, each animal was anaesthetised immediately prior to the injection of urea to ensure that the total dose was introduced. The amount of urea given was designed to raise the plasma urea concentration in the nitrogen depleted wallabies to within the range found in nitrogen sufficient wallabies (5 - 10mM/l) and in the nitrogen sufficient wallabies to raise the plasma urea concentration no higher than values previously recorded in this species (Lintern and Barker, 1969; this thesis).

Blood samples were taken immediately prior to the urea injection, after ten, thirty and two hundred and seventy minutes and then at twenty four hour intervals after the injection of urea until plasma urea levels approached pre-injection values. Twenty four hour urine samples were collected following the injection of urea and also continued until plasma urea levels approached pre-injection values. Urea was estimated in the plasma and urine and total nitrogen in the urine. ETC clearance was measured on the three days before and after the injection of urea and on these days urine was collected three hourly and immediately frozen (Van Niekerk _et al_., 1963).

(iii) Results

(1) Changes in body weight, plasma and urinary urea and total urinary nitrogen during the prefeeding period.

(a) Body weight.

During the first three weeks of feeding of test diets (the prefeeding period) the wallabies fed the high nitrogen diet maintained their body weight. However when these animals were removed to metabolism cages they lost weight and this continued throughout the remainder of the experiment for animals 1 and 4, animals 39 and 7 recovering some of their initial weight loss by the end of the experimental period. This weight loss may have been due to a decline in appetite arising from stress caused by the change in holding conditions. The mean weight loss for the animals fed the high nitrogen diet at the end of the experiment was 5%. The wallabies fed the low nitrogen diet lost weight continually throughout the prefeeding and experimental periods (18%) and thus any effect of caging on body weight in this group of wallabies would have been masked.

Changes in body weight for both groups of wallabies during the prefeeding and experimental periods are

presented in Appendix 15.

(b) Plasma and urinary urea and total urinary nitrogen*. These parameters increased during the prefeeding period in the wallabies fed the high nitrogen diet and decreased in those fed the low nitrogen diet (Appendices 16, 17 and 18). These changes were reflected in corresponding changes in U/P urea ratios and the percentage of urea nitrogen excreted in the total urinary nitrogen (Tables 9 and 10). The response of wallaby 39r differed from that of the other wallabies fed the same diet. In this wallaby plasma urea levels did not decrease and the decline in urinary urea and total urinary nitrogen was less than that recorded in the other three wallabies. From Tables 9 and 10 it can be seen that at the end of the four week prefeeding period U/P urea ratios and percentage of urea nitrogen in the total urinary nitrogen were greater than 50 and 50% respectively in the wallabies fed the high nitrogen diet and less than 10 and 15% respectively

---

* When urinary urea (mg/day) and total urinary nitrogen (mg/day) are given for a particular day, e.g. day 31, this is the twenty four hours commencing on day 30 and ending on day 31. Urea U/P ratios have been calculated from plasma urea values estimated from blood samples collected at the end of each twenty four hour urine collection.

in the wallabies fed the low nitrogen diet. In animal 39r, although the U/P urea ratio was less than 10 at the end of the prefeeding period, urea nitrogen formed 30% of the total nitrogen excreted in the urine. However, with the exception of animal 39r, the values obtained for U/P urea ratios and the percentage of urea nitrogen in the total urinary nitrogen comply with the criteria set down in Experiment 3 for detecting wallabies with maximum and minimum rates of nitrogen retention. It is clear from Appendices 16, 17 and 18 that caging of the wallabies receiving the high nitrogen diet not only resulted in a decline in body weight but also was reflected in a decline in plasma and urinary urea, total urinary nitrogen and hence U/P urea ratios and the percentage of urea nitrogen in the total urinary nitrogen (Tables 9 and 10). This supports the hypothesis that caging causes an initial depression of appetite and hence a decline in dry matter and nitrogen intake. However, after one week of caging these parameters had stabilised at a slightly lower level. Caging may have had a similar effect on the wallabies fed the low

nitrogen diet. However since feeding a low nitrogen diet resulted in a continual decline in plasma and urinary urea and total urinary nitrogen during the prefeeding period, any such effect was masked.

(2) Plasma and urinary urea, total urinary nitrogen and derived parameters following an intravenous injection of urea.

Changes in plasma urea following the injection of 700mg of urea in each group of wallabies (Appendix 17) have been expressed as means and illustrated in Figures 12 and 13. Data from animal 39r has not been included owing to the atypical response of this animal to a low nitrogen diet (Appendix 17). In both groups the injection of urea resulted in an initial increase in plasma urea and this was followed by a rapid decline which was complete thirty minutes after the injection of urea. This initial rapid decline in plasma urea was no doubt due to the mixing of injected urea throughout the total body water available for urea dilution (Painter, 1940).

After mixing of injected urea was complete, a difference was found in the pattern of decline of plasma urea in the two groups of wallabies. In the wallabies

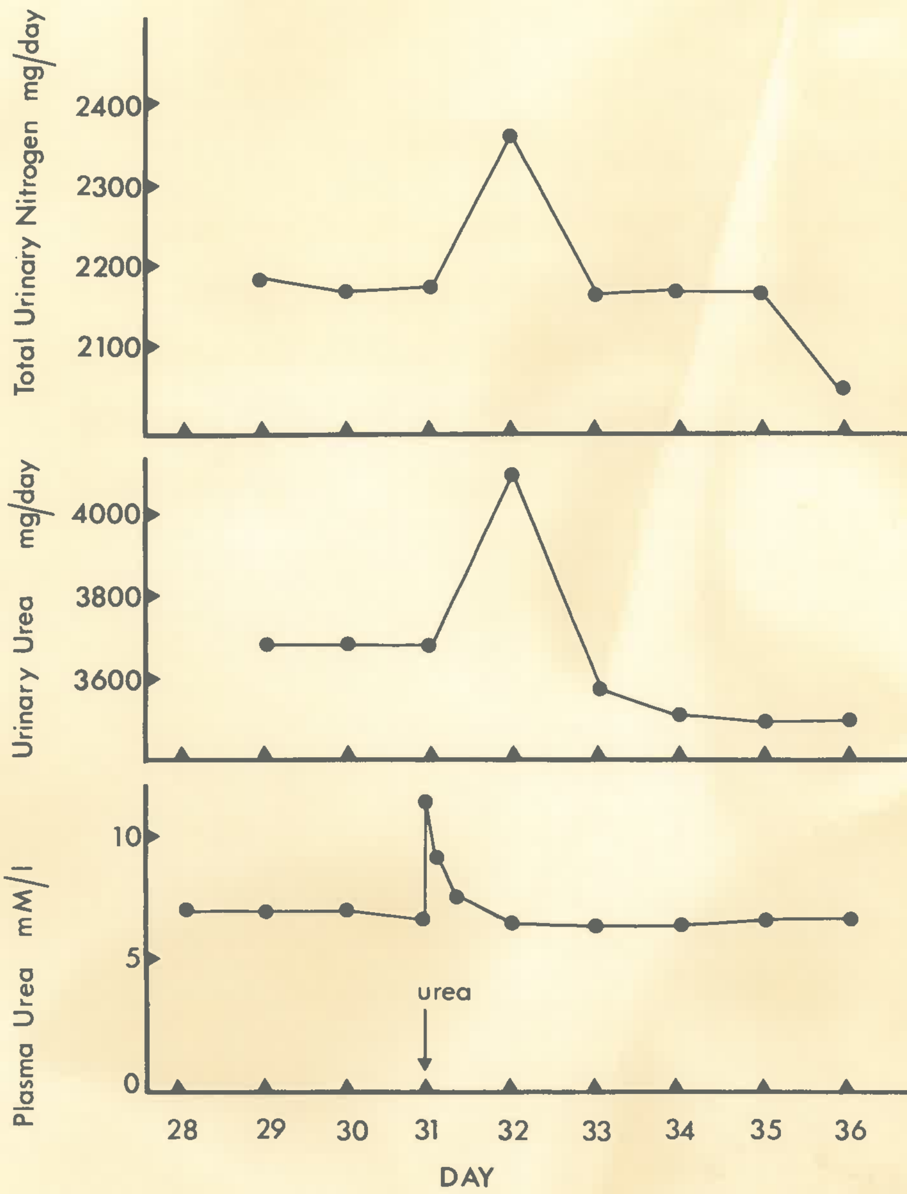
fed the high nitrogen diet plasma urea levels had returned to pre-injection level within twenty four hours after the injection of urea (Figure 13). However, in the wallabies fed the low nitrogen diet the decline in plasma urea levels was much slower and plasma urea levels were greater than the pre-injection level five days later (Figure 13).

The rapid decrease in plasma urea, and hence the rapid disappearance of injected urea from the total body water available for urea dilution, (Painter, 1940) during the twenty four hours following the injection of urea was accompanied by an increase in urinary urea and hence total urinary nitrogen in the wallabies fed the high nitrogen diet (Figure 12). U/P urea ratios showed a corresponding increase (Table 9) as did the percentage of urea nitrogen excreted in the total urinary nitrogen (Table 10). Urinary urea and total urinary nitrogen then remained at pre-injection level or slightly lower for the subsequent four days of the experimental period (Figure 12).

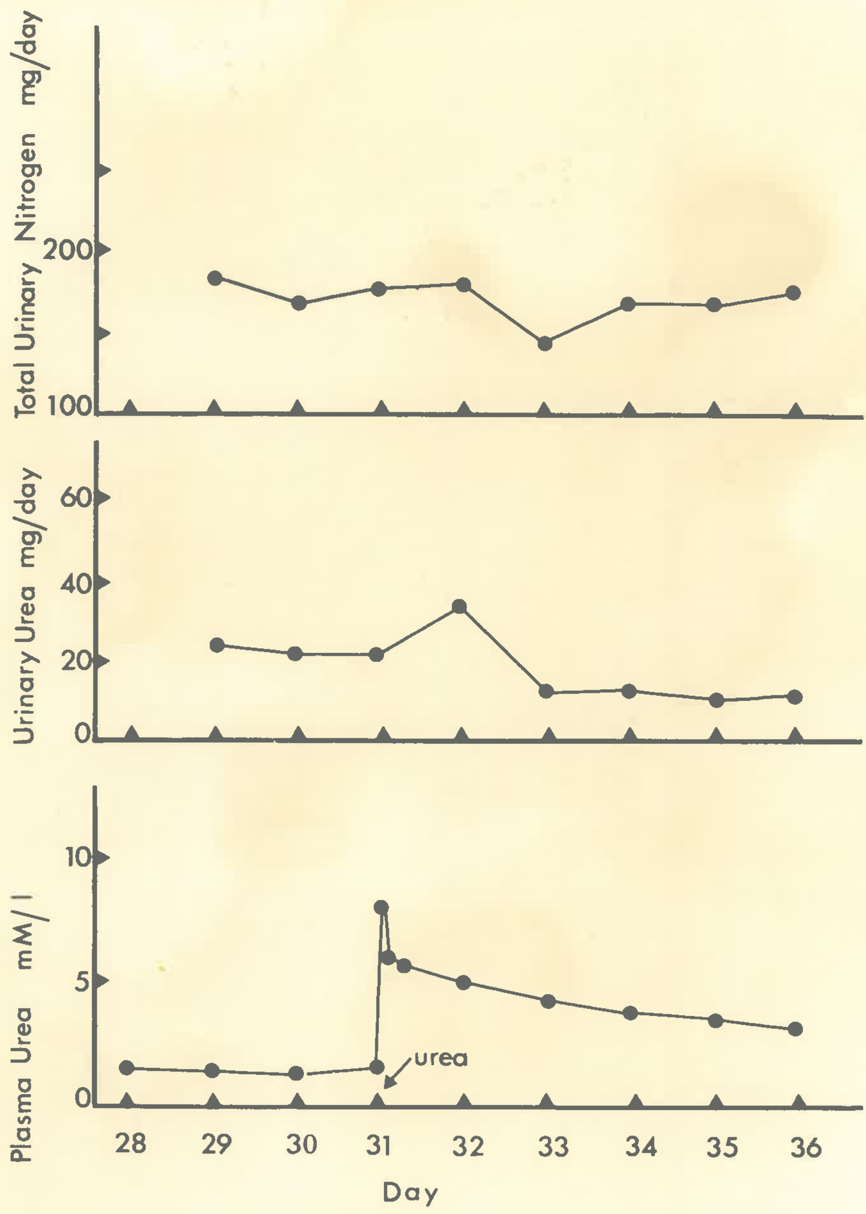
The injection of urea did not result in a similar increase in urinary urea and total urinary nitrogen in the wallabies fed the low nitrogen diet (Figure 13). Urinary urea excretion increased slightly in wallabies

FIGURE 12

Mean plasma urea (mM/l), urinary urea (mg/day) and total urinary nitrogen (mg/day) before and after an intravenous injection of urea for four male wallabies fed a high nitrogen diet during Experiment 5.



Total Urinary Nitrogen mg/day
2400
2300
2200
2100
Urinary Urea mg/day
4000
3800
3600
Plasma Urea mM/l
10
5
0
urea
28
29
30
31
32
33
34
35
36
DAY



Total Urinary Nitrogen mg/day
200
100
Urinary Urea mg/day
60
40
20
0
Plasma Urea mM/l
10
5
0
urea
28
29
30
31
32
33
34
35
36
Day

FIGURE 13

Mean plasma urea (mM/l), urinary urea (mg/day) and total urinary nitrogen (mg/day) before and after an intravenous injection of urea for three male wallabies (392r, 89 and 394r) fed a low nitrogen diet during Experiment 5.

TABLE 9

U/P urea ratios during the prefeeding period and the experimental period of Experiment 5 when an intravenous injection of urea was given to eight wallabies fed on low or high nitrogen diet. The wallabies were removed to metabolism cages on day 23 and on day 31 were injected with urea.

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
1	53	59	77	66	89	98	81	32
23	56	74	72	78	29	9	10	15
29	45	74	93	79	12	6	4	1
30	56	70	83	108	11	5	5	3
31	73	89	95	119	8	6	4	4
Urea Injection (700mg/ animal)								
32	93	136	105	155	14	7	1	2
33	63	103	111	119	13	1	1	1
34	54	105	155	189	7	1	1	0.9
35	90	118	145	119	10	2	1	1
36	71	98	123	124	10	1	0.8	1

TABLE 10

Urinary urea nitrogen expressed as a percentage of total urinary nitrogen during the prefeeding period and the experimental period of Experiment 5, when an intravenous injection of urea was given to eight wallabies fed a low or high nitrogen diet. The wallabies were removed to metabolism cages on day 23 and on day 31 they were injected with urea.

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
1	61	65	72	63	50	69	72	54
23	80	81	84	78	52	24	27	30
29	71	84	81	86	55	10	6	5
30	80	79	70	78	25	12	9	5
31	78	79	82	79	30	7	7	4
Urea Injection (700mg/ animal)								
32	81	86	83	81	41	17	4	15
33	79	76	79	77	41	17	4	4
34	73	78	79	76	31	4	4	4
35	73	76	79	77	29	4	4	2
36	71	82	79	77	29	4	4	3

392r and 394r but declined to an even lower value in wallaby 89. Wallaby 39r responded to the injection of urea by increasing the excretion of urinary urea during the forty eight hours following the urea injection. Urinary urea levels were lower than pre-injection levels (with the exception of 39r) for the remainder of the experimental period in the wallabies fed the low nitrogen diet (Figure 13, Appendix 15). This was accompanied by corresponding changes in total urinary nitrogen excretion (Figure 13) and hence U/P urea ratios (Table 9) and the percentage of urea nitrogen excreted in the total urinary nitrogen (Table 10). On two occasions during the post-injection period U/P urea ratios of less than unity were recorded (Table 9). It must be pointed out that post-injection U/P urea ratios are not true values for each twenty four hour period following the injection of urea as plasma urea values, which were estimated from the blood samples collected at twenty four hour intervals, have been used to calculated this parameter. It is clear from Figures 12 and 13 that plasma urea levels declined during the twenty four hours following the injection of urea in the wallabies fed the high nitrogen diet, thus the U/P urea ratios calculated for this period

of the post-injection period are too high.
Similarly U/P urea ratios during the entire post-injection period in the wallabies fed the low nitrogen diet are too high for the same reason. Thus, although U/P urea ratios of less than unity were recorded on only two occasions, if the mean plasma urea level for each twenty four hours of the post-injection period is used to calculate the U/P urea ratio for each day, then this is less than unity in the wallabies depleted of nitrogen for much of the post-injection period.

The percentage of injected urea not appearing in the urine for both groups of wallabies has been calculated in Table 11 from the following equation:

$$U_R = \frac{U_I - (U_A - U_B)}{U_I} \times \frac{100}{1}$$

where U_R = the percentage of injected urea not appearing in the urine during the twenty four hour post-injection period.

U_A = urea excreted in the urine during the twenty four hour post-injection period (mg/day).

U_B = mean urea excretion (mg/day) in the urine on the three days prior to the injection of urea.

U_I = Urea injected (mg)

The amount of urea which would have been excreted in the urine on the day of the experiment if urea had not been injected has been estimated by calculating the mean daily excretion of urea in the urine from the daily urinary urea excretion on the three days immediately before the experiment. It can be seen from Appendix 16 that the excretion of urea in the urine by each animal on these three days fluctuated only slightly.

The wallabies fed the high nitrogen diet excreted 62% of the injected urea in the urine within the twenty four hours after the injection of urea. However, the wallabies fed the low nitrogen diet excreted only 3% of the injected urea in the urine during the same period.

In Table 12 ETC clearance for both groups of wallabies on the three days before and three days after the injection of urea is presented. It can be seen that ETC clearance, and hence GFR (Experiment 4) was unaffected by the injection of urea.

TABLE 11

The percentage of injected urea* not appearing in the urine of two groups of wallabies, one of which was fed a high nitrogen diet and the other a low nitrogen diet during Experiment 5

Animal	Diet	U_B °	U_A ∅	U_R +
39		3336	3797	34
7	High	3457	3904	36
1	Nitrogen	4573	4983	42
4		3390	3808	40
				$\bar{X}$ = 38
39r		260	368	85
392r	Low	28	69	94
89	Nitrogen	28	14	100
394r		14	26	98
		$\bar{X}$ (392r, 89, 394r)		= 97

* 700mg of urea injected intravenously into each wallaby

°U_B = Mean urea excretion (mg/day) in the urine of each wallaby on the three days prior to the experiment

∅U_A = urea excreted (mg/day) in the urine during the twenty four hour post-injection period

+U_R = the percentage of injected urea not appearing in the urine during the twenty four hour post-injection period.

TABLE 12

ETC clearance (ml/day) measured on three days before and three days after the injection of urea for each wallaby during Experiment 5

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
29	11,715	13,366	15,723	12,119	13,076	7,605	13,431	9,960
30	11,980	13,366	15,746	12,153	12,980	7,487	13,386	10,024
31	11,894	13,407	15,725	12,142	12,980	7,518	13,493	10,001
	Urea Injection (700mg/animal)							
32	11,868	13,383	15,762	12,159	13,012	7,574	13,427	9,894
33	11,804	13,401	15,698	12,187	13,090	7,436	13,427	10,016
34	11,911	13,401	15,723	12,126	12,994	7,583	13,399	9,935

D. EXPERIMENT 6

(i) Introduction

Although Colin (1886, cited Hungate, 1966) first reported the presence of urea in ruminant saliva, it was McDonald (1948) and Somers (1961a, b, c and d) who demonstrated that salivary urea entering the rumen formed a significant and utilizable portion of the nitrogen intake in these animals. The theory that endogenous urea might also enter the rumen directly from the blood stream was later advanced by Chalmers and Synge (1954) and in 1957 Simmonet _et al._, using an isolated rumen, demonstrated that this occurs. It has been established that the major portion of endogenous urea entering the rumen comes directly from the blood stream (Houpt, 1959; Decker, Hill, Gartner and Hornicke, 1960), only a small fraction arising from salivary urea (Somers, 1961a, b, c, and d).

Brown (1964) suggested that, since the euro had a ruminant-like digestion, endogenous urea might be recycled to its forestomach and that this additional nitrogen source would be of particular importance during periods of lowered nitrogen intake. In a subsequent experiment Brown (1964) demonstrated that urea is recycled to the forestomach of the euro via the saliva and directly across the forestomach wall from the blood stream. Furthermore, like sheep and goats, most of the urea recycled to the forestomach came directly

from the blood stream.

Previous experiments reported herein have indicated that the Kangaroo Island Wallaby could utilize urea if it were recycled in the way outlined above. In addition the marked renal conservation of urea by nitrogen depleted wallabies, similar to that found in sheep (Schmidt-Nielsen _et al._, 1958), goats (Hill _et al._, 1962) and camels (Schmidt-Nielsen _et al._, 1957) fed low nitrogen diets, suggests that endogenous urea contributes to the nitrogen economy of this macropod via urea recycling. This suggestion is supported by the results of the previous experiment. Injected urea gradually disappeared from the total body water available for urea dilution of the nitrogen depleted wallaby, however this urea did not appear in the urine and therefore presumably entered the digestive tract (Houpt, 1959).

The following experiment was designed to investigate the quantitative aspects of the renal response of nitrogen depleted and sufficient Kangaroo Island Wallabies to injected urea. The aim of this experiment was to establish whether renal retention of injected urea occurred concurrently with urea recycling to the forestomach. In addition it was considered that the difference in the renal response to injected urea by nitrogen depleted and sufficient wallabies might be a reflection of a difference in the amount of injected

urea recycled to and utilized in the forestomach, as utilization of endogenous urea would be expected to be of greater importance in the nitrogen economy of nitrogen depleted wallabies than in those with an adequate nitrogen intake. This was investigated by measuring the extent of incorporation of injected urea into microbial nitrogen in the forestomach.

During a preliminary examination of digesta from various regions of the digestive tract of a wallaby fed a maintenance diet (see Experiment 2), bacteria and living protozoa were found not only in the forestomach but also in the caecum and adjacent regions of the intestine. Therefore the possibility that urea is recycled to the hindgut was also investigated. The region chosen was the caecum because it is clearly defined and easily recognisable.

The movement of injected urea into the forestomach and caecum of the wallaby was measured directly using N^{15} labelled urea ($N^{15}H_2CON^{15}H_2$*). Movement of injected urea into the entire digestive tract was also calculated indirectly using the urea dilution technique of Painter (1940) as applied to sheep and goasts by Houpt (1959) for this purpose.

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* N^{15} labelled urea obtained from ONIA (Office National Industriel de L'Azote), Toulouse, France.

(ii) Experimental Procedure

It was originally planned to use eight experimental animals, however, due to under delivery of N^{15} labelled urea, the number of experimental animals had to be reduced to five. These wallabies were used in the previous experiment and therefore were accustomed to experimental routine. They were fed a maintenance diet (1.5gN/100g dry weight) for one month and during this time they were allowed water *ad lib.* They were then divided into two groups. Animals 392r and 4 were fed a high nitrogen diet (2.60gN/100g dry weight) and animals 39, 1 and 7 a low nitrogen diet (0.34gN/100g dry weight). In this experiment the feeding of test diets was extended to six weeks instead of the customary four weeks. This was due to the late delivery of the N^{15} labelled urea. Body weight, plasma and urinary urea and total urinary nitrogen were measured immediately prior to the commencement of feeding of test diets and after eighteen, thirty two and thirty seven days.

The experiment was performed over a three hour period on the last day of the six week prefeeding period and the following routine was employed. Eleven hours prior to the experimental period the wallabies were fed. After three hours, remaining food was removed and the experiment commenced

eight hours later*. At the beginning of the experimental period each animal was anaesthetised, a blood sample taken and the bladder catheterised, emptied and washed. Urine and washings were discarded. An intravenous injection of N^{15} labelled urea in 0.5% saline (Houpt and Houpt, 1968) (14.643 atom percent excess N^{15}) was then given to each animal in the dosage of 90mg urea/kg body weight. Blood samples were taken at ten minutes, thirty minutes, one hour, two hours and three hours after the injection of urea. The wallabies were anaesthetised throughout the experimental period. Urine spontaneously voided during this period was collected from the catheter into 25% H_2SO_4. At the end of the experimental period the bladder was emptied and washed and urine and washings bulked.

Each wallaby was then killed and the forestomach, caecum and bladder ligatured and excised. The small amount of fluid in the bladder was added to the urine and washings collected during the experimental period and total urine and washings stored at -10° for later analysis. The forestomach

---

* This treatment was to ensure that plasma urea levels remained unaltered during the collection of urine (Experiment 4) and that ingested food was at the same stage of digestion in the forestomach within each group of wallabies (Experiment 2). As the wallabies were fed at dawn, most of the ration was consumed (Experiment 4).

was then removed and a subsample separated into bacterial, protozoal, soluble and forestomach fluid fractions. Bacterial and protozoal fractions were then immediately digested for total nitrogen and N^{15} determinations. The forestomach fluid, soluble fraction of forestomach digesta and remaining forestomach digesta were stored at -10° for subsequent analysis. Digesta removed from the caecum was also stored at -10° for later analysis.

Urea estimations were performed on plasma, urine and caecal and forestomach digesta. Total nitrogen in the forestomach digesta and associated fractions and in caecal digesta was also determined together with the ammonia content of the forestomach fluid. The atom percent excess N^{15} in the forestomach digesta and associated fractions, caecal digesta and N^{15} excreted as urea nitrogen in the urine were determined.

(iii) Results

(1) Changes in body weight, plasma and urinary urea and total urinary nitrogen during the prefeeding period are presented in Appendix 19. From Tables 13 and 14 it can be seen that by day thirty seven U/P urea ratios were less than 10 in the wallabies fed the low nitrogen diet and greater than 50 in those fed the

high nitrogen diet. Similarly, the percentage of urea nitrogen in the total urinary nitrogen was 15% or less in the nitrogen depleted wallabies and greater than 50% in the wallabies fed the high nitrogen diet. Thus the criteria set down in Experiment 3 of this thesis for detecting wallabies with maximum and minimum nitrogen retention were satisfied.

(2) Basal plasma urea concentrations during the experimental period for each animal could not be estimated by N^{15} determinations, as extremely large blood samples would have been needed. Accordingly, the plasma urea concentration estimated immediately prior to the experimental period was used as an estimate of basal plasma urea concentration during the experimental period. This value agreed well with plasma urea concentrations measured at the same time on the three days prior to the experiment (Table 15).

Plasma urea concentrations before and after the injection of N^{15} labelled urea for both groups of wallabies are presented in Figures 14 and 15. As found in the previous experiment, the mixing of injected urea throughout the total body water available for urea dilution was complete thirty

TABLE 13

U/P urea ratios during the prefeeding period of Experiment 6 for five wallabies

Animal	Diet / Day	0	18	32	37
39	Low Nitrogen	82	6	5	8
7		88	7	5	7
1		100	6	4	2
392r	High Nitrogen	71	37	100	115
4		73	71	70	79

TABLE 14

Urinary urea nitrogen expressed as a percentage of total urinary nitrogen during the prefeeding period of Experiment 6 for five wallabies

Animal	Diet	0	18	32	37
39	Low Nitrogen	80	23	19	12
7		75	20	18	15
1		72	17	7	7
392r	High Nitrogen	66	71	80	82
4		70	75	74	79

FIGURE 14

Changes in plasma urea concentration (mM/l) following an intravenous injection of N^{15} labelled urea for wallabies 39 (●), 7 (○) and 1 (×) fed a low nitrogen diet during Experiment 6.

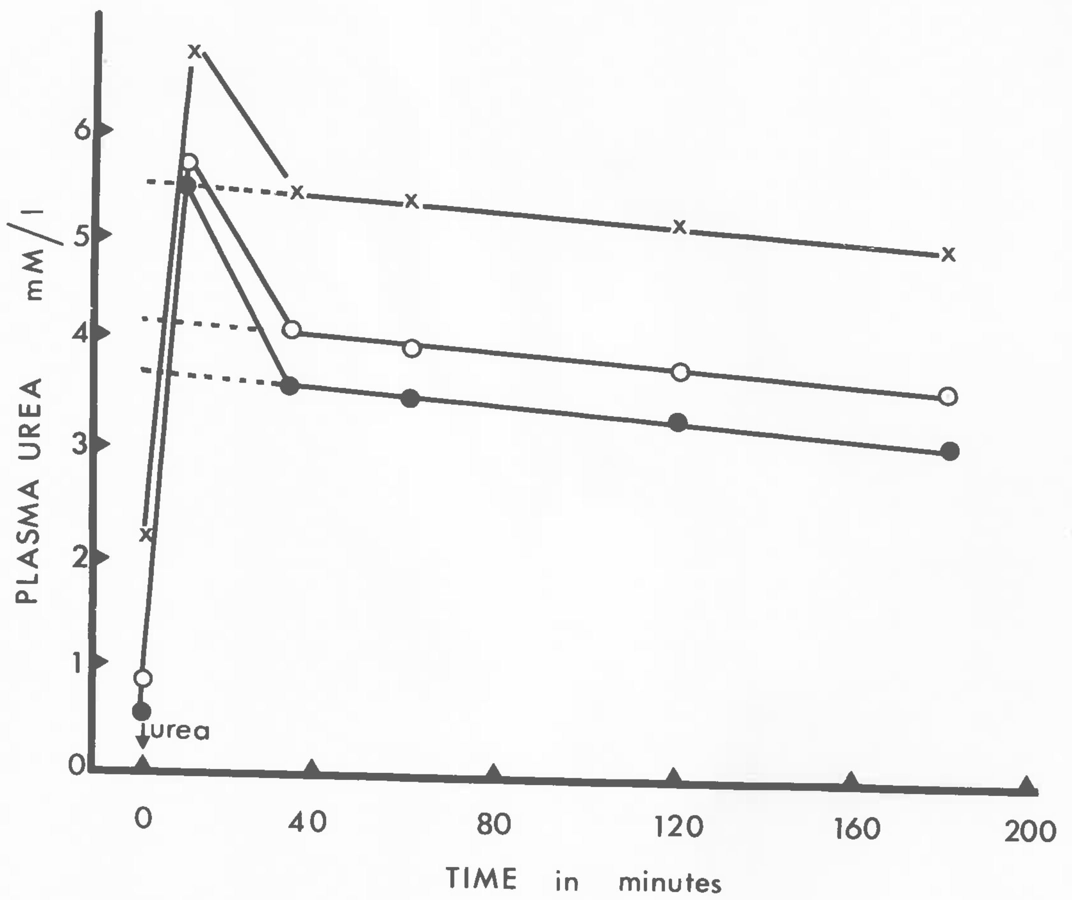
PLASMA UREA mM/l
0
1
2
3
4
5
6
urea
0
40
80
120
160
200
TIME in minutes

FIGURE 15

Changes in plasma urea concentration (mM/l) following an intravenous injection of N^{15} labelled urea for wallabies 392r (○) and 4 (●) fed a high nitrogen diet during Experiment 6.

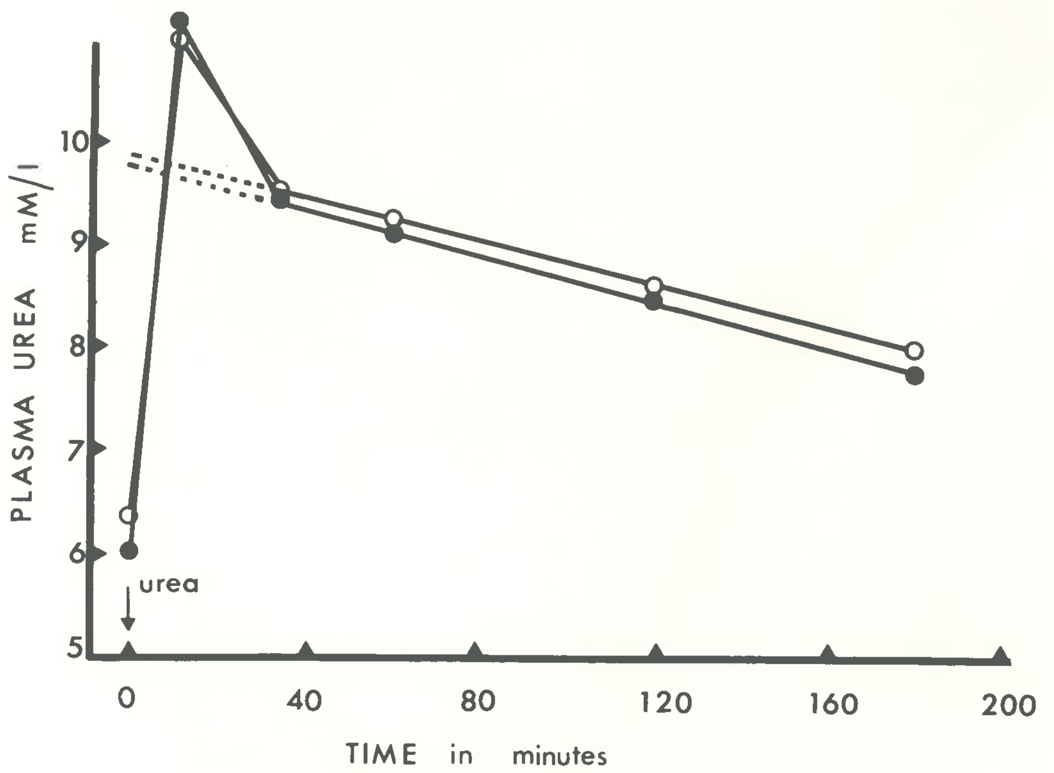
PLASMA UREA mM/l
10
9
8
7
6
5
urea
0
40
80
120
160
200
TIME in minutes

TABLE 15

Plasma urea (mM/l) for five wallabies on the three days prior to Experiment 6 and also on the day of Experiment 6 immediately prior to the injection of N^{15} labelled urea

Diet	High Nitrogen		Low Nitrogen		
Animal / Day	392r	4	39	7	1
1	6.6	6.4	0.5	0.9	2.1
2	6.4	6.1	0.7	0.8	2.0
3	6.6	6.2	0.5	1.0	2.2
4*	6.4	6.1	0.8	0.9	2.3

* Day of experiment

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minutes after the injection of urea. Similarly, the disappearance of injected urea from the blood (and hence the urea dilution volume) was more rapid in the wallabies fed the high nitrogen diet (Figure 15) than in those fed the low nitrogen diet (Figure 14). The decline in plasma urea in both groups of wallabies was linear once initial mixing of injected urea was complete.

(3) The excretion of injected urea in the urine during the experimental period has been calculated from the atom percent excess N^{15} in the urea nitrogen excreted in the urine during this period (Appendix 20). The wallabies fed the high nitrogen diet excreted 44.5% of the injected urea in the urine during the three hours following the injection of urea, whereas the wallabies fed the low nitrogen diet excreted only 0.3% of the injected urea during the same period (Table 16). These results agree with those obtained in Experiment 5.

(4) Estimation of the total amount of injected urea present in the digestive tract at the end of the experimental period using the indirect method of Houpt (1959).

Houpt (1959), working with sheep and goats, stated that injected urea lost from the total body water

available for urea dilution, which does not appear in the urine, enters the digestive tract. The amount of injected urea present in the digestive tract at the end of the experimental period is calculated from the difference between the total amount of injected urea which has disappeared from the total body water available for urea dilution and the amount of injected urea appearing in the urine. The total body water available for urea dilution can be calculated by the urea dilution technique of Painter (1940). Values for total body water available for urea dilution in the wallabies of the reported experiment have been calculated and presented in Table 17. It appeared that total body water available for urea dilution formed a smaller percentage of the body weight in the wallabies fed the high nitrogen diet than in those fed the low nitrogen diet. However this difference was not statistically significant.

The amount of injected urea which disappears from the total body water available for urea dilution (I_{UDT}) is calculated from the relationship:

$$I_{UDT} = P_{ID} \times V \qquad (1)$$

where P_{ID} = total amount of injected urea which has disappeared from the plasma (mg/l) during

TABLE 16

The percentage of injected urea excreted in the urine of five wallabies during Experiment 6

Animal	Diet	Urea injected (mg)	Injected Urea excreted in the urine (mg/3 hours)	% of Injected Urea excreted in the urine
39	Low Nitrogen	463	2	0.3
7		471	2	0.4
1		604	2	0.3
392r	High Nitrogen	471	214	45.2
4		532	245	43.8

the experimental period. This has been calculated for each animal from Figures 14 and 15 using the equation:

$$P_{ID} = (P_{UO} - P_B) - (P_{UT} - P_B)$$

where P_{UO} = plasma urea (mg/1) immediately after the injection of urea if mixing were complete at this time and estimated by extrapolation of the linear part of the curve (Figures 14 and 15) back to the time when urea was injected. Painter (1940) has shown this to be a valid procedure.

P_B = plasma urea (mg/1) immediately before the injection of urea.

P_{UT} = plasma urea at the end of the experimental period.

V = total body water available for urea dilution (1).

I_{UDT} = total injected urea (mg) which disappears from the body water available for urea dilution during the experimental period.

Therefore the amount of injected urea which is present in the entire digestive tract at the end of the experimental period (I_{UR}) is given by the equation:

$$I_{UR} = I_{UDT} - U_{IE} \qquad (2)$$

where I_{UDT} = total injected urea (mg) which disappears from the total body water available for urea dilution during the experimental period.

U_{IE} = Injected urea (mg) excreted in the urine during the experimental period.

This has been calculated for wallabies in the reported experiment and presented in Table 18.

It is clear from Table 18 that injected urea was present in the digestive tract of both groups of wallabies at the end of the experimental period. Significantly more injected urea was present in the digestive tract of the wallabies fed the low nitrogen diet than in those fed the high nitrogen diet.

(5) Direct estimation of the amount of injected urea in the forestomach and caecum at the end of the experimental period using N^{15} labelled urea.

(a) Injected urea present in the forestomach.

N^{15}, which could only have arisen from urea N^{15} introduced into the blood stream, was detected in the forestomach digesta of both groups of wallabies and part of the N^{15} was in the form of microbial nitrogen (Appendix 21). The amount of injected urea nitrogen in the forestomach digesta at the

TABLE 17

Calculation of total body water available for urea dilution of five wallabies during Experiment 6 using the urea dilution technique of Painter (1940)+

Animal	Diet	Body Weight (kg)	U_{IR} (mg)	P_{UIO} (mg/l)	V (l)	V% Body Weight
39	Low Nitrogen	5.148	463	192	2.20	42.6
7		5.232	471	210	2.23	42.8
1		6.711	604	204	2.96	44.1
						$\bar{X}$ = 43.1
392r	High Nitrogen	5.233	471	222	2.12	40.4
4		5.908	532	223	2.38	40.2
						$\bar{X}$ = 40.3
						t_3 = 0.46 NS

+ Total body water available for urea dilution (V) = Urea Injected (U_{IR}) / Concentration of injected urea in plasma (P_{UIO}) if mixing were complete immediately after the injection of urea

TABLE 18

The amount of injected urea in the entire digestive tract of five wallabies at the end of Experiment 6 using Equations 1 and 2

Animal	Diet	Injected urea lost from the plasma (mg/l) (P_{ID})	Injected urea (mg) lost from the total body water available for urea dilution (I_{UDT})	Injected urea (mg) appearing in the urine (U_{IE})	Injected urea present in the digestive tract (mg) (I_{UR})	Percentage of Injected urea present in the digestive tract
39	Low Nitrogen	32	70	2	68	15
7		32	71	2	69	15
1		36	73	2	71	11
392r	High Nitrogen	108	229	214	15	3
4		116	276	245	31	6

$t_3 = 7.23^{**}$

end of the experimental period has been calculated from Equation 3 and these values are presented in Table 19.

Injected urea nitrogen in the forestomach at the end of the experimental period (mg)

$$= \frac{N^{15}(mg)}{14.643^{*}} \times \frac{100}{1} \qquad (3)$$

*(atom percent excess N^{15} in injected urea)

The amount of injected urea which must have entered the forestomach to give rise to this quantity of N^{15} detected at the end of the experimental period also is presented in Table 19 and was calculated by dividing Equation 3 by 0.47, the fraction of the urea molecule comprised of nitrogen.

It can be seen from Table 19 that at the end of the experiment, more injected urea nitrogen was present in the forestomach digesta of the wallabies fed the low nitrogen diet than in those fed the high nitrogen diet. This trend followed that found when injected urea present in the entire digestive tract was measured indirectly (Table 18) and the quantities involved were similar.

The distribution of injected urea nitrogen throughout the bacterial protozoal and soluble fractions also

differed in the two groups of wallabies (Table 20). In those fed the low nitrogen diet, injected urea nitrogen was equally distributed between bacterial and soluble nitrogen, only 1 - 2% being in the form of protozoal nitrogen. In the wallabies fed the high nitrogen diet however, injected urea nitrogen was found predominantly in the nitrogen of the soluble fraction (81%), the remaining 19% being incorporated as microbial nitrogen, 14.5% in the bacterial fraction and 4.5% in the protozoal fraction. Of the injected urea nitrogen in the soluble fraction of forestomach digesta, 55% was in the form of ammonia nitrogen. This contrasted with the wallabies fed the low nitrogen diet. Only 25% of the injected urea nitrogen in the soluble nitrogen fraction was in the form of ammonia nitrogen (Table 21). No urea was detected in the forestomach digesta of either group of wallabies. This was probably due to extensive hydrolysis of urea to ammonia by the forestomach microbiota (Experiment 2).

(b) Injected urea present in the caecum.

N^{15} arising from injected urea was detected in the caeca of both groups of wallabies and the amount

of injected urea required to give rise to the quantity of N^{15} measured is presented in Table 22. As found for the forestomach, more injected urea nitrogen was present in the caecal digesta of the wallabies receiving the low nitrogen diet than in those receiving the high nitrogen diet. It appears that injected urea nitrogen in the caecum arose from movement of urea directly from the blood stream, as no excess N^{15} was detected in the digesta taken from the distal end of the duodenum.

TABLE 19

The total amount of nitrogen (mg), arising from injected urea, present in the forestomach of five wallabies three hours after a single injection of N^{15} labelled urea (Equation 3) during Experiment 6. The total amount of injected urea (mg) which must have entered the forestomach to give rise to the amount of N^{15} detected is also presented.

Animal	Diet	N^{15} (mg) in forestomach	Nitrogen in forestomach (mg) arising from injected urea	Injected urea (mg) which entered forestomach
39	Low Nitrogen	4.260	29	62
7		4.292	29	63
1		4.063	28	59
392r	High Nitrogen	1.101	8	16
4		1.283	9	19

TABLE 20

The distrubtion of injected urea nitrogen (mg) in the forestomach fractions (calculated from Equation 3) and the percentage of the total injected urea nitrogen present in the forestomach in the various forestomach fractions of five wallabies three hours after a single injection of N^{15} labelled urea during Experiment 6

Animal	Diet	Forestomach Fraction								
		Bacterial			Protozoal			Soluble		
		N^{15+}	TN°	%*	N^{15+}	TN°	%*	N^{15+}	TN°	%*
39	Low Nitrogen	2.429	16.6	57	0.039	0.2	1	1.802	12.3	42
7		1.929	13.2	45	0.088	0.6	2	2.276	15.5	53
1		1.535	10.5	38	0.092	0.6	2	2.412	16.5	60
392r	High Nitrogen	0.129	0.9	12	0.024	0.2	3	0.991	6.8	86
4		0.215	1.5	17	0.075	0.5	6	0.941	6.4	77

+ N^{15}(mg) in each forestomach fraction at the end of the experimental period.

° Total nitrogen arising from injected urea present in each forestomach fraction at the end of the experimental period.

* Percentage of total injected urea nitrogen in forestomach in the various forestomach fractions at the end of the experimental period.

TABLE 21

The amount of injected urea nitrogen (mg) in the forestomach digesta of five wallabies three hours after an intravenous injection of N^{15} labelled urea (calculated from Equation 3) in the form of ammonia nitrogen (NH_3-N) together with the percentage of injected urea nitrogen in the soluble fraction of the forestomach digesta in the form of NH_3-N during Experiment 6

Animal	Diet	NH_3-N (mg) in forestomach digesta	Atom % excess N^{15} in NH_3-N	N^{15} (mg) in NH_3-N	NH_3-N (mg) arising from injected urea	% of soluble nitrogen arising from injected urea nitrogen in the form of NH_3-N
39	Low Nitrogen	15	2.734	0.410	2.8	23
7		7	7.029	0.492	3.4	22
1		10	7.360	0.736	5.0	31.
392r	High Nitrogen	33	1.734	0.572	3.9	58
4		69	1.438	0.480	3.3	51

TABLE 22

The amount of injected urea nitrogen (mg) present in the caecal digesta of five wallabies three hours after an intravenous injection of N^{15} labelled urea (calculated from Equation 3) together with the amount of injected urea which would have to move into the caecum to give rise to the detected amount of N^{15} during Experiment 6

Animal	Diet	Nitrogen (mg) in caecal digesta	Atom % excess N^{15} in caecal nitrogen	N^{15} (mg) in caecal digesta	Caecal nitrogen (mg) arising from injected urea	Injected urea (mg) in caecal digesta
39	Low Nitrogen	9.8	1.056	0.103	0.70	1.49
7		13.4	0.992	0.133	0.91	1.94
1		17.5	0.664	0.116	0.79	1.60
392r	High Nitrogen	20.2	0.256	0.052	0.27	0.62
4		25.2	0.200	0.050	0.27	0.62

3. THE EFFECT OF CONCURRENT WATER AND NITROGEN DEPLETION ON RENAL RETENTION OF UREA AND NITROGEN RETENTION IN THE KANGAROO ISLAND WALLABY

A. EXPERIMENT 7

(i) Introduction

Livingston *et al.* (1962) reported a reduction in the excretion of urea in the urine of nitrogen depleted cattle, obtaining values similar to those found in nitrogen depleted sheep (Schmidt-Nielsen *et al.*, 1958; Schmidt-Nielsen and Osaki, 1958; Schmidt-Nielsen and O'Dell, 1959) and camels (Schmidt-Nielsen *et al.*, 1957). They also found that if these cattle were subsequently water restricted, urinary urea excretion decreased further and this was accompanied by a decrease in the percentage of urea nitrogen in the total urinary nitrogen. Houpt (1963) found that rabbits responded in a similar way to water restriction. Rabbits totally deprived of free water utilized injected urea more efficiently than when free access to water was allowed, and, if urea was not injected, their urinary urea excretion was less than control rabbits. Moir (1965) suggested that renal retention of urea by nitrogen depleted animals might contribute not only to their nitrogen economy but also their water economy.

An additional effect of water restriction on nitrogen depleted cattle was reported by Payne and his colleagues

(Livingston et al., 1964; Payne, 1964, 1965, 1966). They found, from the results of a single experiment, that nitrogen depleted cattle, which were subsequently water restricted, exhibited improved nitrogen balance, in two cases from negative to positive. Johnson et al. (1966) have also reported that water restriction improved nitrogen retention in three breeds of cattle fed an adequate nitrogen diet.

No experimental work has been published on the nitrogen metabolism of macropods under conditions of water depletion. However, Holsworth (1967) speculated that the quokka might improve its nitrogen retention when water intake is low at the end of summer, in a manner analogous to cattle. From a field study on Kangaroo Island, Dr. S. Barker suggested that the Kangaroo Island Wallaby also experiences a combined shortage of nitrogen and water in the field towards the end of summer. This is supported by the finding of a large relative renal medullary thickness in this wallaby which indicates a comparatively high renal concentrating ability (Appendix 22).

Accordingly the following experiments were carried out to investigate whether the effect of water and nitrogen depletion on nitrogen retention and renal retention of urea in this wallaby was similar to that reported in cattle. Incidental to the main purpose of the experiments, the suggestion of Moir (1965) that renal retention of urea in

water restricted animals may lead to improved water balance was tested.

(ii) Experimental Procedure

(1) Experiment 7.1

Twelve sexually mature male wallabies were used. They were fed a maintenance diet (1.3gN/100g dry weight) for eighteen days. During this period water and dry matter intake were measured daily and the animals placed in metabolism cages for several days at a time for cage training. The twelve animals were then divided into two groups of six animals. One group was fed a low nitrogen diet (0.3gN/100g dry weight) and the other a high nitrogen diet (1.3gN/100g dry weight). The two groups were then further divided into sub-groups of three animals. One sub-group was given water *ad lib.* and the others restricted to 160 ml each per day. This was calculated to approximate 50% of the unrestricted water intake measured prior to the commencement of the experiment. The following is their designation.

Group 1 Low nitrogen, water *ad lib.*
Group 2 " " , " restricted.
Group 3 High nitrogen, " "
Group 4 " " , " *ad lib.*

The food and water intake of each wallaby was measure daily and body weight was measured at the end of each week. Twenty four hourly collections of urine and faeces were made from wallabies held in metabolism cages and without air conditioning and a blood sample taken immediately before the commencement of treatments and at the end of three, six, ten and thirteen weeks. Nitrogen balance was measured over a ten day period after nine weeks of treatment. Between collections animals were returned to pens. Moisture content of faecal samples, plasma and urinary urea and total urinary nitrogen were determined on all twenty four hour urine and faecal samples.

Through animal losses, the results of this experiment were inconclusive, although they indicated differences between animals that had a restricted water intake and those that were allowed unlimited access to water. Accordingly, the experiment was repeated with a greater number of animals and particular emphasis was placed upon the response of nitrogen depleted wallabies to water restriction.

(2) Experiment 7.2

A number of sexually mature male wallabies were obtained from Kangaroo Island. Because of severe

seasonal conditions on the Island they were in very poor condition. Twelve wallabies were selected for experimental purposes, placed in individual pens and fed a maintenance diet with bread and vegetable supplements and allowed water ad lib. The remainder were kept in the department stock colony where they were fed grain concentrates and allowed free access to water. Exceptional animal losses occurred due to death or general deterioration of health and these animals had to be replaced. Some replacements were obtained from animals in the stock colony and others from animals used in previous experiments. They were kept in individual pens and fed a maintenance diet (1.3gN/100g dry weight) and allowed water ad lib. As in Experiment 7.1, water and dry matter intake were then measured daily for eighteen days. The animals were then divided into two groups of six animals in each group. Both groups were fed a low nitrogen diet (0.3gN/100g dry weight). The control group were allowed water ad lib. and the experimental group were given 170 ml of water each day which was calculated to be approximately 50% of the unrestricted water intake.

All collections were made from animals held in metabolism cages in a temperature controlled animal

house (21°). Collection of twenty four hour urine and faecal samples together with a blood sample were made immediately before commencement of treatments and then at four weeks. Nitrogen balance was estimated over a ten day period at the end of seven weeks and at the end of this period a final collection of urine and faeces excreted over twenty four hours was made together with a blood sample. The animals were then returned to their pens and treatments were continued for another week at the end of which an estimation of their circulating plasma volume was made. During the course of this experiment one control animal died and one was removed from the experiment, as it rapidly lost weight and became weak.

In addition to the analyses made in Experiment 7.1 plasma volume was estimated by the dye dilution method using Evans Blue Dye (T.1824).

(iii) Results

(1) Experiment 7.1

The progressive changes in body weight of all groups of wallabies are presented in Appendix 23 (A - E). Group 1 wallabies, which were nitrogen depleted but allowed water *ad lib.* showed a steady weight loss which

was 20% of their initial body weight at the end of the experiment. In constrast, Group 2 wallabies (nitrogen depleted and water restricted) showed a greater initial weight loss which was followed by a gradual decline in body weight. At the end of the experiment weight loss in Groups 1 and 2 was similar. Wallabies which recieved a high nitrogen diet and were water restricted (Group 3) also showed an initial weight loss, most of which was regained at the end of the experiment. Group 4 wallabies, allowed free access to water and fed a high nitrogen diet, maintained their weight and showed weight gains after thirteen weeks.

The changes which took place in plasma urea and urea nitrogen and total nitrogen excreted in the urine have been expressed as means for each group and illustrated in Figures 16 and 17. Values for individual animals are presented in Appendix 23 (A - E). There was a large fall in urinary urea excretion after the first three weeks in both groups fed the low nitrogen diet and this remained low throughout. Total nitrogen excreted in the urine also fell gradually in these groups and, at the end of the experiment, was less in the water restricted wallabies (Group 2) than

FIGURE 16

Plasma urea (mM/l), urinary urea nitrogen ($mgN/kgW^{0.75}/day$) and total urinary nitrogen ($mgN/kgW^{0.75}/day$) of two groups of wallabies fed a low nitrogen diet and given either free (●) or restricted (○) access to water during Experiment 7, part 1.

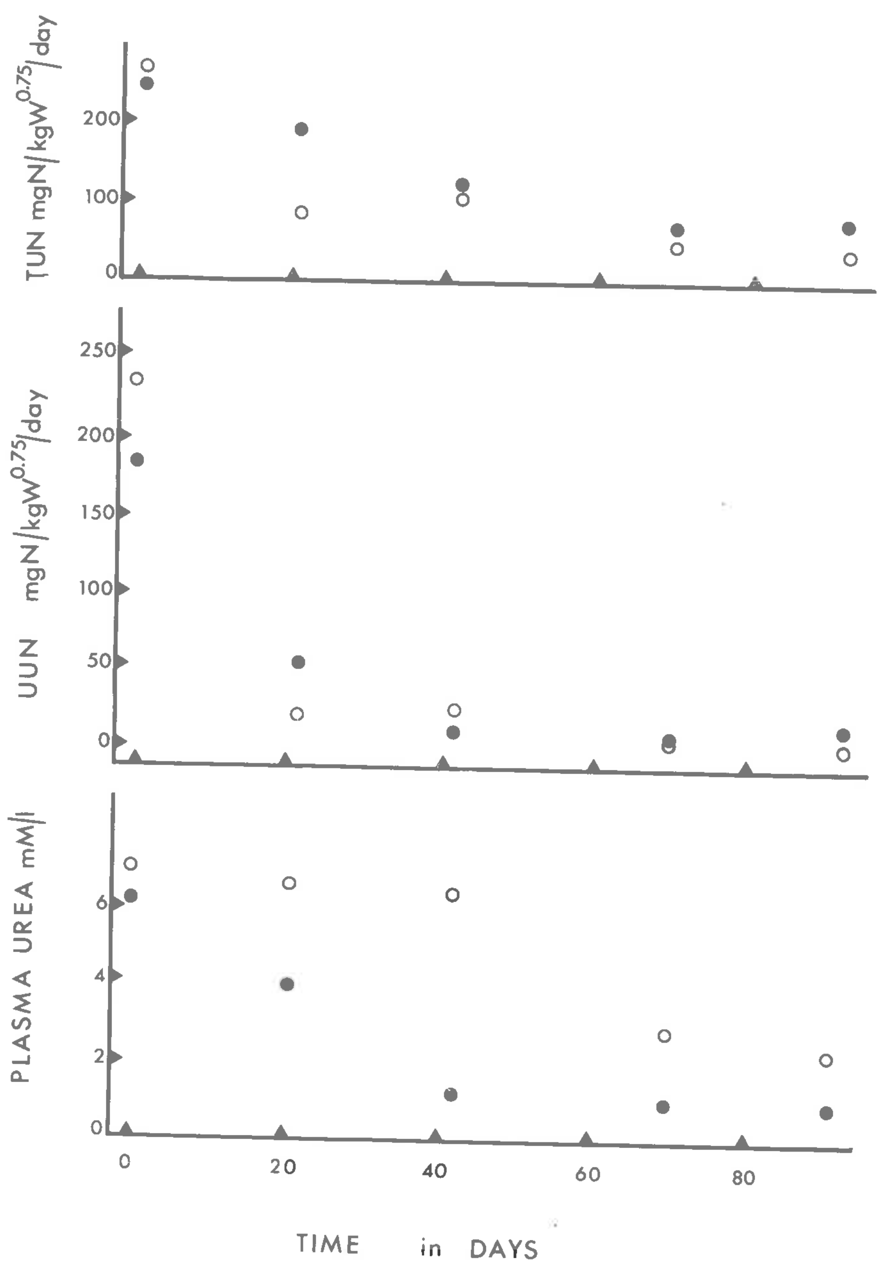
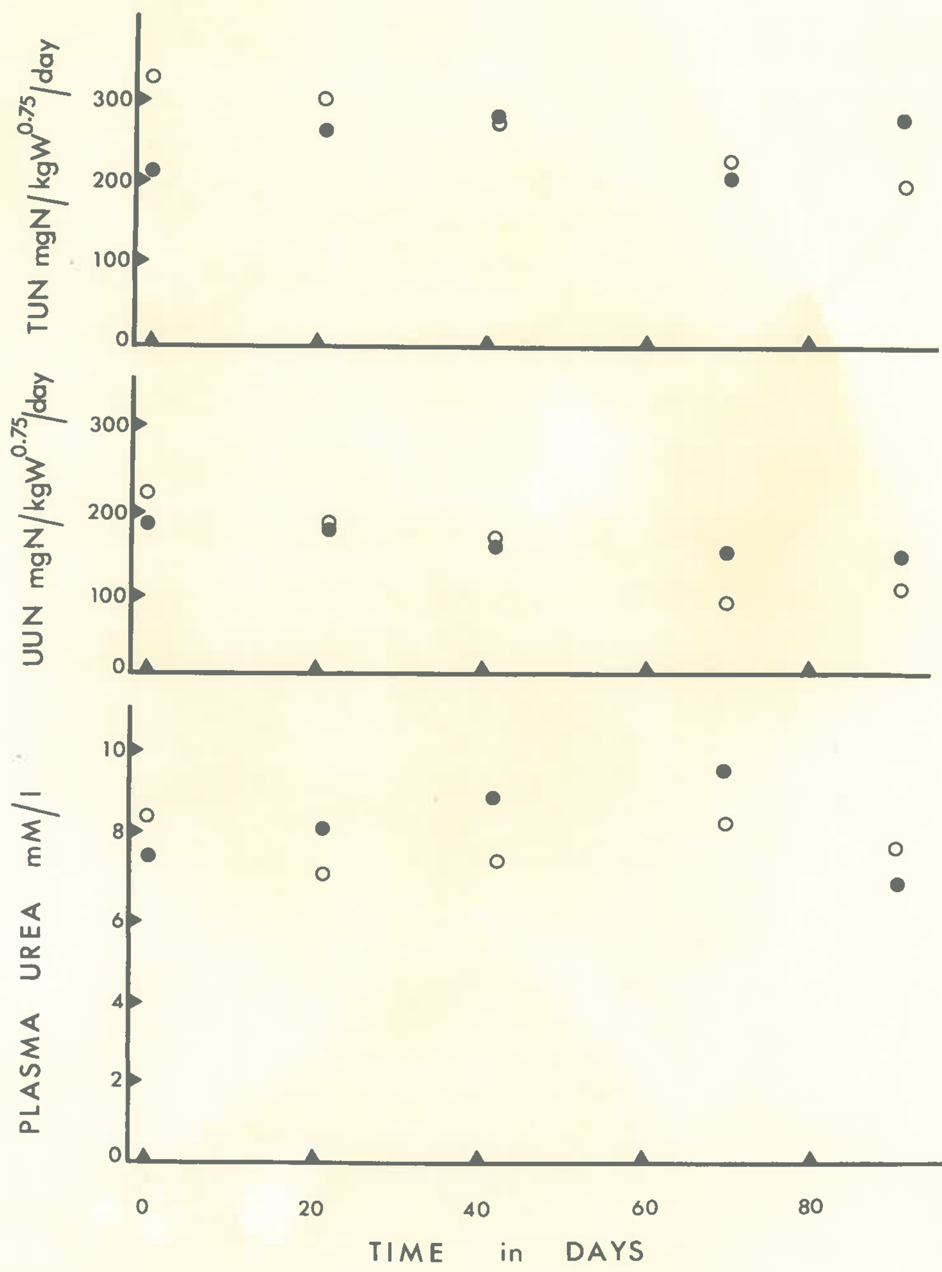

TUN mgN/kgW$^{0.75}$/day
200
100
0
UUN mgN/kgW$^{0.75}$/day
250
200
150
100
50
0
PLASMA UREA mM/l
6
4
2
0
0
20
40
60
80
TIME in DAYS

FIGURE 17

Plasma urea (mM/1), urinary urea nitrogen ($mgN/kgW^{0.75}/day$) and total urinary nitrogen ($mgN/kgW^{0.75}/day$) of two groups of wallabies fed a high nitrogen diet and given either free (●) or restricted (○) access to water during Experiment 7, part 1.



TUN mgN/kgW$^{0.75}$/day
300
200
100
0
UUN mgN/kgW$^{0.75}$/day
300
200
100
0
PLASMA UREA mM/l
10
8
6
4
2
0
0
20
40
60
80
TIME in DAYS

in those allowed water ad lib.(Group 1). Plasma urea concentrations also fell in these groups throughout the experiment but they remained higher in Group 2 animals than in Group 1. The excretion of urea in the urine of the water restricted animals was, however, less than that excreted by those allowed water ad lib. All these parameters remained fairly constant in animals fed the high nitrogen diet (Groups 3 and 4), and were significantly greater than in Groups 1 and 2 fed the low nitrogen diet. Mean U/P urea ratios for each group are presented in Table 23 and values for individual wallabies in Appendix 23 (A - E). These showed remarkable falls in Groups 1 and 2 and they remained low in Group 2 throughout the experiment and showed a slight rise in Group 1 at the end of the thirteen week experimental period. There was an overall decline in U/P urea ratios in animals receiving the high nitrogen diet (Groups 3 and 4) but at the end of the experiment these were still very much higher than in Groups 1 and 2 receiving the low nitrogen diet.

The results of the nitrogen balance trial are presented in Appendix 24 (A and B). At this stage of the experiment Group 4 wallabies had a much higher

dry matter intake than any of the animals in the other 3 groups. Group 3 wallabies had a higher dry matter intake than Groups 1 and 2 but there was no obvious difference between the low nitrogen groups. Dry faecal output of Group 4 was higher than the other groups but there was no clear difference between the other three groups. Water intake of Group 4 wallabies was higher than in the other groups which did not differ greatly except for animal 27 in Group 1 which appeared to be water addicted. There was very little difference in urine volume excreted by all animals except for animal 27 in which urine volume was high reflecting its high water intake. The excretion of water in the faeces was similar in all animals in Groups 1, 2 and 3 except again for animal 27. In these groups faecal water loss was slightly higher than urinary water loss. In Group 4 faecal water loss was of the order of 3.5 times greater than urinary water loss in this group, and it was almost double the faecal water loss in the other three groups. Nitrogen intake was very much higher in the two groups fed the high nitrogen diet than in those fed the low nitrogen diet. In Groups 1 and 2 urinary nitrogen loss was 3 to 4 times less than faecal nitrogen loss, whereas in most wallabies in

TABLE 23

Mean U/P urea ratios of all groups of wallabies throughout Experiment 7.1

Group	Treatment	Day 0	21	42	70	91
1	Low N water *ad lib.*	81	27	10	8	17
2	Low N water restricted	77	9	7	1	2
3	High N water restricted	42	40	42	26	32
4	High N water *ad lib.*	53	41	37	44	40

Groups 3 and 4 urinary and faecal nitrogen losses were of the same order, or urinary nitrogen loss was lower. Faecal nitrogen loss was lower in Groups 1 and 2 than in the other two groups. All animals in Groups 1 and 2 were in negative nitrogen balance and those in the other two groups were in positive balance except for animal 46 which was in balance. There appeared to be no difference in nitrogen balance between Groups 1 and 2. This was one of the main points of test for which the experiment was designed. Apparent digestibility of dry matter was approximately 50% in all animals except that the water restricted groups showed a slightly higher dry matter digestibility than did the water *ad lib.* groups.

(2) Experiment 7.2

The progressive changes in body weight of the two groups of wallabies are presented in Appendix 25 (A, B and C). Their body weight changes were similar to those shown by the corresponding groups receiving the same treatment in Experiment 7.1. In Group 1 there was a steady decline in body weight during the experiment whilst in Group 2 there was an initial rapid decline followed by a slower weight loss over the remainder of the experiment. The final weight

losses shown by five of the six animals in Group 2 were within the range of weight losses shown by Group 1 wallabies. The exception was animal 8, which had a 30% body weight loss at the end of the experiment. This animal also showed a different pattern of nitrogen excretion from the others when nitrogen balance was performed, in that its urinary nitrogen loss was greater than its faecal nitrogen loss. It is possible that this animal may have had a kidney infection as the percentage of urea nitrogen in the total urinary nitrogen was greater in this wallaby than in all the other wallabies. The response of animal 8 to a low nitrogen diet paralleled that of animal 39r in Experiment 5.

Plasma urea concentration, urinary urea nitrogen and total urinary nitrogen have been expressed as means for each group and the changes which took place during the experiment illustrated in Figure 18. The changes in U/P urea ratios are presented in Table 24. All these parameters had decreased dramatically from their initial values when the last collection was made. As in Groups 1 and 2 in Experiment 7.1 plasma urea concentration was significantly greater ($t_7 = 2.37^*$) in the wallabies allowed restricted access to water

FIGURE 18

Plasma urea (mM/1), urinary urea nitrogen ($mgN/kgW^{0.75}/day$) and total urinary nitrogen ($mgN/kgW^{0.75}/day$) of two groups of wallabies fed a low nitrogen diet and given either free (●) or restricted (○) access to water during Experiment 7, part 2.

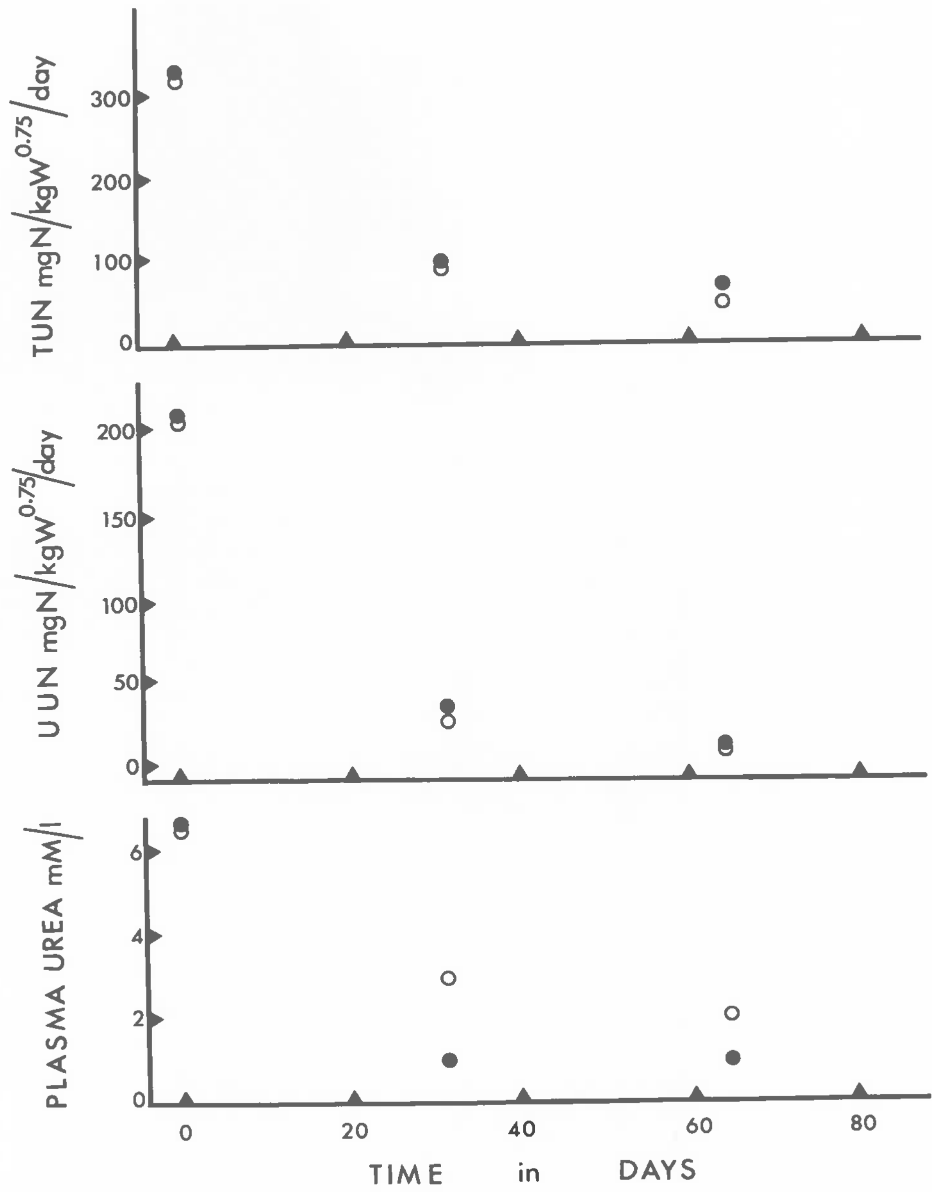

TUN mgN/kgW^0.75/day
300
200
100
0
UUN mgN/kgW^0.75/day
200
150
100
50
0
PLASMA UREA mM/l
6
4
2
0
0
20
40
60
80
TIME in DAYS

TABLE 24

Mean U/P urea ratios of both groups of wallabies throughout Experiment 7.2

Group	Treatment	Day 0	28	61
1	Low N water *ad lib.*	43	40	12
2	Low N water restricted	43	17	2

(Group 1) than in those allowed water ad lib. (Group 2). In Group 2 urinary urea excretion was significantly less ($t_7 = 3.3$*) than in Group 1 at the end of the experiment. The U/P urea ratios of both groups fell, but at the end of the experiment were lower in Group 2 than in Group 1.

To determine if haemoconcentration had caused elevated plasma urea levels in the water restricted wallabies, plasma volume was measured. Due to difficulties encountered with venous cannulation of the wallabies, values for plasma volume can only be presented for six of the experimental animals. From Table 25 it can be seen that there was a considerable variation in plasma volumes measured within the two groups. There was, however, no indication that water restriction resulted in a reduction in plasma volume.

The results of the nitrogen balance trial are summarised in Appendix 26 (A and B). During the nitrogen balance trial there was no significant difference between metabolic body weights (Kleiber, 1961), although Group 1 had a significantly higher dry matter intake than Group 2 and mean dry faecal output followed the same pattern. The water intake of Group 1 wallabies

TABLE 25

Plasma volumes of six wallabies at the end of Experiment 7.2

Group	Treatment	Wallaby	Plasma volume (ml/kgW)
1	Low Nitrogen	4	43.5
	Water *ad lib.*	7	47.6
2	Low Nitrogen	1	46.5
	Water restricted	2	44.7
		5	38.7
		10	44.3

was also significantly greater than that of Group 2 wallabies although there was little difference in urine volumes. As found in Experiment 7.1, there was a difference between the two groups in the excretion of water in the faeces; Group 1 having a significantly greater faecal water loss than Group 2. Nitrogen intake was higher in Group 1 but there was no difference between the two groups in the excretion of total nitrogen in the urine (with the exception of animal 8) although urinary urea nitrogen was significantly greater in Group 1 during the balance period than in Group 2. Faecal nitrogen excretion was higher in Group 1 (excluding 8). Overall there was no difference in nitrogen balance (excluding 8). As found in Experiment 7.1, dry matter digestibility was higher in the water restricted group.

IV. DISCUSSION

1. RUMINANT-LIKE DIGESTION AND UTILIZATION OF DIETARY NITROGEN BY THE KANGAROO ISLAND WALLABY

A. Utilization of dietary urea

Large differences in the utilization of either casein or urea nitrogen by macropods would be expected to produce changes in nitrogen excretion and retention. The results of Experiment 1 indicate that the Kangaroo Island Wallaby, like the euro (Brown, 1964, 1969), utilizes urea and casein equally well as sources of dietary nitrogen. Regardless of the form in which nitrogen was supplied, body weight fluctuated only slightly and nitrogen retention, as measured by nitrogen balance trials, was positive and did not differ significantly with the type of nitrogen supplemented.

There was no significant correlation between nitrogen balance and nitrogen intake. This finding suggested that nitrogen retention was limited and this will be discussed more fully later. Nitrogen intake did not vary significantly with the nature of the nitrogen supplement and in addition faecal nitrogen and total urinary nitrogen were also independent of the form in which nitrogen was supplied, being positively and significantly correlated with nitrogen intake. Furthermore, blood ammonia and plasma and urinary urea, which would also be expected to reflect any difference in the utilization of casein or urea nitrogen, showed fluctuations which could not be related

to the type of nitrogen supplement. Thus, like the ruminant (see Hart et al., 1939; Wegner, Booth, Bohstedt and Hart, 1940, 1941a and b; Harris and Mitchell, 1941a and b; Johnson, Hamilton, Mitchell and Robinson, 1942; Johnson, Hamilton, Robinson and Garey, 1944; Mills, Booth, Bohstedt and Hart, 1942; Mills, Lardinois, Rupel and Hart, 1944; Reid, 1953; Williams and Tribe, 1957; Briggs, McBarron, Grainger and Franklin, 1960; Tillman and McAleese, 1963), the Kangaroo Island Wallaby can utilize urea as a source of dietary nitrogen.

In a similar experiment with sheep, Harris and Mitchell (1941a) found that utilization of casein nitrogen was significantly greater than that of urea nitrogen, when the nitrogen supplement formed the greater part of the nitrogen intake. Repp, Hale and Burroughs (1955a) have also found that replacement of more than 50% of protein with urea in diets fed to lambs does not lead to further improvement in growth rate. It has been concluded that non-protein nitrogen is utilized more efficiently by ruminants when it forms a relatively small proportion of the nitrogen intake and when readily available carbohydrates form part of the diet (Barnett and Reid, 1961; Chalupa, 1968). Contrary to these findings Tillman and McAleese (1963) report that growth in lambs was the same whether nitrogen was supplied as urea or casein. They fed purified diets containing 2.0gN/100g dry weight and

99% of the crude protein in the diet was in supplement form.

In Experiment 1, the major part of the nitrogen intake (78 - 81%) was in supplement form but utilization of urea was not significantly different from that of casein. As studies of the levels of dietary urea which can be effectively utilized by ruminants has produced conflicting results, it cannot be said whether the macropod studied here utilizes urea as a dietary nitrogen source more effectively than true ruminants. The conflicting reports on the advised level of urea intake for ruminants may arise out of differences in the composition of experimental diets, combined with variations in the general nutritional status of the experimental animals and the frequency of feeding (Chalupa, 1968; McBarron and McInnes, 1968). Brown (1964, 1969) found that urea and casein were utilized equally well as dietary nitrogen supplements by the euro. However, because nitrogen intake from the supplement formed only 18 - 36% of the total nitrogen intake, these results give no insight into the possible difference between macropod and ruminant utilization of dietary urea.

Tillman and McAleese (1963) report that, although growth rate in lambs was the same with diets supplemented with urea or casein, their growth rate was significantly greater if soyabean protein or soyabean meal formed the protein supplement. They concluded that poor growth rate in lambs, fed diets supplemented

with casein, was due to the rapid hydrolysis of casein in the rumen. These findings suggest that, although the Kangaroo Island Wallaby utilizes casein and urea equally well as sources of dietary nitrogen, protein, supplied in a form other than casein, may be utilized more efficiently than either casein or urea.

B. Production of ammonia from urea

One major problem in feeding urea in high concentrations to ruminants is the development of toxic symptoms which may result in death (Clark, Oyaert and Quin, 1951; Gallup, Pope and Whitehair, 1953; Pierce, Moule and Jackson, 1955; Repp, Hale, Cheng and Burroughs, 1955b; Nix and Anthony, 1965; McBarron and McInnes, 1968). Toxic symptoms are associated with elevated peripheral blood ammonia levels of approximately 1 - 4 mg per 100 ml (Dinning, Briggs, Gallup, Orr and Butler, 1948; Clark *et al.*, 1951; Repp *et al.*, 1955b; Davis and Roberts, 1959; Oltjen, Waller, Nelson and Tillman, 1963). The rapid increase in peripheral blood ammonia is due to a corresponding rapid hydrolysis of urea in the rumen by the action of bacterial urease (Bloomfield, Garnar and Muhrer, 1960), combined with reabsorption of ammonia directly from the rumen (Bloomfield, Komer, Wilson and Muhrer, 1966) and the inability of the liver to convert all the absorbed

ammonia to urea (Lewis, Hill and Annison, 1957).

In Experiment 1, although urea nitrogen formed the greater part of the nitrogen intake, no rise in blood ammonia levels was recorded. This suggested that either hydrolysis of urea to ammonia was not a feature of urea utilization in this wallaby, or utilization of ammonia or its conversion to urea in the liver equalled its production. The latter appeared more likely, as ammonia levels in the forestomach of the quokka fed early stage pasture, are similar to those reported in sheep fed the same diet (Moir, 1965). Nevertheless, the production of ammonia from ingested urea by the Kangaroo Island Wallaby remained to be confirmed.

The results of a preliminary experiment indicate that urea administered orally as a drench (Appendix 27) results in rapid production of ammonia and elevated blood ammonia levels in the wallaby. The only enzyme known to hydrolyse urea to ammonia is urease (Kornberg and Davies, 1955). As an endogenously produced urease has not been conclusively demonstrated in any bird of mammal studied so far (Kornberg and Davies, 1955; Levenson, Crowley, Horowitz and Malm, 1959) it must be concluded that a urease producing microbiota is present in the alimentary tract of the Kangaroo Island Wallaby. Bacterial urease has been reported in the alimentary tract of a number of birds and mammals (Visek, 1962). In the case of

the wallaby this is probably located in the forestomach where a microbial population exists (Experiment 2) as it does in the quokka (Waring, Moir and Tyndale-Biscoe, 1966) and red kangaroo (Harrop, 1965).

The results of this experiment also suggested that this macropod has a greater tolerance than true ruminants to high levels of ingested urea and the resulting high levels of blood ammonia. Toxic symptoms did not develop in wallabies drenched with urea in dosages in excess of those shown to be lethal to sheep (Gallup et al., 1953; Davis and Roberts, 1959; Oltjen et al., 1963; Nix and Anthony, 1965; McBarron and McInnes, 1968), although blood ammonia levels were similar to those found in sheep with toxic symptoms. Again, however, such a comparison between a macropod and ruminants must be qualified because the dosage of urea which results in toxicity in ruminants depends on a number of factors including the composition of the diet and the general nutritional status of the animal concerned (Repp et al., 1955b; Chalupa, 1968; McBarron and McInnes, 1968).

McBarron and McInnes (1968) found that urea administered as a drench in maximum doses of 0.26g per kg with sucrose to sheep, which had been fed an adequate diet, produced toxic symptoms but did not result in death. Furthermore, Repp et al. (1955b) report that dosages of urea double this value did not

result in the development of toxic symptoms or death in sheep fed diets of soyabean oil meal, corn and good quality hay. McBarron and McInnes (1968), however, found that sheep were more likely to develop signs of toxicity when drenched with urea, if they had been fasted.

As the wallabies drenched with urea were fed a diet rich in protein and available carbohydrate (McDonald and Hall, 1957) and had been allowed to feed prior to the experiment, this may have affected their response. Nevertheless, as the dosage of urea given to the wallabies exceeded the largest dose given by McBarron and McInnes (1968) to sheep, and no toxic symptoms developed, this macropod may have a greater tolerance to large doses of urea given orally than does the sheep. Since the cause of urea toxicity in sheep is not clearly understood (Hale and King, 1955; Moir, 1957; Lewis, 1961), a comparative study of the apparent difference in the response of sheep and the Kangaroo Island Wallaby to such treatment might help elucidate this problem.

C. The role of the forestomach microorganisms in the utilization of dietary nitrogen

The ability of ruminants to utilize urea as a dietary nitrogen source depends upon the conversion of such nitrogen into microbial protein by the rumen microorganisms (Agrawala

et al., 1953; Phillipson, Dobson and Blackburn, 1959). This process involves initial hydrolysis of urea to ammonia by bacterial urease (Bryant and Robinson, 1963). The evidence presented so far indicates that the wallaby utilizes urea or casein equally well as a dietary nitrogen source. Ingestion of urea by the wallaby results in elevated blood ammonia levels indicating the presence of a urease in the forestomach. These findings suggested that the role of the microbial population in the forestomach of the Kangaroo Island Wallaby in the digestion and utilization of urea is similar to that of the pre-gastric microorganisms in the ruminant.

The results of Experiment 2 demonstrated that utilization of dietary nitrogen by the wallaby involves its extensive incorporation into microbial nitrogen in the forestomach. The incorporation of dietary nitrogen into microbial nitrogen was extremely rapid and quantitatively similar to figures published by Weller et al. (1962) for sheep. 64 - 85% of the ingested plant nitrogen was incorporated into microbial nitrogen in the forestomach of the wallaby, a value close to that of 60 - 82% found by Weller and his colleagues for sheep.

The changes in the distribution of nitrogen in the forestomach of the wallaby, following feeding, paralleled closely those found in sheep (Blackburn and Hobson, 1960, 1961;

Weller *et al*, 1962), but the rate of attack on plant nitrogenous compounds by the forestomach microorganisms was slightly more rapid in the wallaby. In both sheep and the wallaby, however, ingestion of new plant nitrogen is followed by an initial increase in the nitrogen content of the soluble fraction of forestomach digesta and this is partly caused by an increase in ammonia nitrogen.

Sutherland, Ellis, Reid and Murray (1962) and Weller *et al*. (1962) both noted a secondary rise in ammonia in the rumen of sheep between ten and twelve hours after feeding. This was also found in the wallaby although the quantities involved were small, probably due to the feeding of a low nitrogen diet (Gray *et al*., 1958). Ammonia initially increased in the forestomach during the first three hours after feeding and after declining to a minimum some eight hours later, increased again during the remaining sixteen hours. Hungate (1966) has suggested that the cause of the secondary increase in ruminal ammonia in sheep could be caused by a decrease in ammonia utilization and its continued production from feed protein. As Hungate (1966) acknowledges, however, this is unlikely as fermentation of feed proteins by ruminants is essentially complete six to eight hours after feeding. Another possible explanation advanced by Hungate (1966) is that the increase in ammonia production is the result of the

endogenous metabolism of non-growing microbes or the digestion of one rumen microbe by another. Although this may apply to the reported study, it could be suggested that the secondary increase in the forestomach ammonia is the result of the hydrolysis of recycled urea, as urea recycling has been demonstrated in the euro. This would only be valid if the rate of urea recycling increased some eight hours after feeding but this is unknown.

The increase in bacterial nitrogen following the initial increase in soluble nitrogen, parallels the changes in the distribution of nitrogen in the rumen of sheep at different times after feeding (Blackburn and Hobson, 1960, 1961; Weller *et al.*, 1962). The initial production of ammonia, followed by an increase in bacterial nitrogen, suggests that, as in the ruminant (Boggs, 1959; Bryant and Robinson, 1963), ammonia may be intermediate in the assimilation of plant nitrogen by the forestomach microorganisms. Also as found in sheep, protozoal nitrogen, although it increased some eight or ten hours after feeding, formed only a small proportion of the total nitrogen in the forestomach at any time.

The increases noted in both bacterial and protozoal nitrogen following feeding, was probably due not only to an increase in the nitrogen content of each microorganism in the forestomach but also to an increase in the numbers of bacteria

and protozoa as it is in cows (Bryant and Robinson, 1961) and sheep (Williams, Nottle, Moir and Underwood, 1953). This could be tested by counting the numbers of forestomach microorganisms present at different times after feeding.

Although these results indicate that the incorporation of plant nitrogen, which is both protein and non-protein (Synge, 1952; Ferguson and Terry, 1954), into microbial nitrogen in this wallaby is as extensive as in sheep, they do not indicate specifically whether urea nitrogen is incorporated into microbial nitrogen. This was confirmed however by the results of Experiment 6 which demonstrated the incorporation of urea nitrogen into bacterial and protozoal nitrogen. Urea nitrogen also appeared as ammonia nitrogen in the forestomach, confirming the presence of a urease. The proportion of plant and urea nitrogen which is converted to microbial protein in the forestomach of the wallaby remains to be demonstrated. The techniques employed by Phillipson *et al.* (1959) and Land and Virtanen (1959) could be used for this purpose.

In conclusion it is clear that digestion and utilization of dietary nitrogen (including urea) by the Kangaroo Island Wallaby is similar to that found in ruminants. Ingested nitrogen is incorporated into microbial nitrogen and this is preceeded by the production of ammonia, which, in the case of

urea, indicates the presence of a urease in the forestomach. It is suggested that the utilization of dietary urea nitrogen by this macropod may be more efficient than in ruminants but this requires further research.

None of the microorganisms in the alimentary tract of macropods have been described in detail. It is evident, from the preceeding discussion, that a study of the forestomach microbiota of macropods and its role in the digestive physiology of these animals is required to provide detailed information on their role in the digestion and utilization of dietary nitrogen. In particular the synthesis of essential amino acids by the forestomach microorganisms should be investigated, as the ability of the Kangaroo Island Wallaby (Experiment 1) and euro (Brown, 1964) to utilize urea as a dietary nitrogen source indicates this occurs in macropods as it does in ruminants (Kosharov _et al._, 1967).

2. RENAL RETENTION AND UTILIZATION OF UREA IN THE KANGAROO ISLAND WALLABY

A. Urinary urea excretion and nitrogen intake, excretion and retention

Lintern and Barker (1969) demonstrated that the Kangaroo Island Wallaby shows a marked reduction in the excretion of urinary urea when nitrogen depleted. Although the site of

control of renal retention of urea was not established, kidney slice analysis suggested that this may be the same as in sheep (Schmidt-Nielsen and O'Dell, 1959). The similar response of camels (Schmidt-Nielsen et al., 1957), sheep (Schmidt-Nielsen and Osaki, 1958; Schmidt-Nielsen and O'Dell, 1959), cattle (Livingston et al., 1962; Elliott and Topps, 1963) and goats (Hill et al., 1962) to nitrogen depletion has been interpreted in terms of their digestive physiology and nitrogen metabolism. It has been suggested that renal retention of urea in these animals, as it occurs in excess of that recorded in monogastric animals, is an adaptation to a low nitrogen diet, improving their nitrogen economy by increasing the amount of urea available for recycling to the rumen (Schmidt-Nielsen, 1958).

The experimental results indicate that the Kangaroo Island Wallaby digests dietary nitrogen in a ruminant-like manner and that urea can be utilized as a dietary nitrogen source. Thus the wallaby could also utilize endogenous urea recycled to the forestomach. The following discussion outlines the extent of renal retention of urea and its relationship to the nitrogen status of the wallaby and the recycling of urea to the forestomach.

As pointed out in the introduction to Experiment 3, protein storage and hence nitrogen retention in mammals appears to be limited (see Lusk, 1928; Albanese, 1959; Swanson, 1959). It was considered that a wallaby, with complete protein stores and

receiving an adequate nitrogen diet, would not retain endogenous urea to supplement its nitrogen intake via recycling to the same extent as a wallaby in negative nitrogen balance and with depleted protein stores, if the extent of urea retention and recycling is related to the nitrogen economy of the wallaby. Therefore in order to place any interpretation on the significance of renal retention of urea and its utilization via recycling, it was essential to be able to judge nitrogen status of the experimental animals and preferably to study urea excretion and utilization under conditions of maximum and minimum nitrogen retention.

Experiment 3 of the reported study was designed to determine whether nitrogen retention is limited in the Kangaroo Island Wallaby and to diagnose whether its extent could be judged from easily measurable parameters. It was found, by combining the results of the reported experiment with those of Barker (1968) for the same animals, that nitrogen retention, as measured by nitrogen balance trials, increased linearly with nitrogen intake up to intakes of 400mgN/kg$W^{0.75}$/day. However, at nitrogen intakes in excess of this value, nitrogen retention remained almost constant. Ingested nitrogen not retained was excreted mainly as urea nitrogen in the urine, since both total nitrogen and urinary urea nitrogen showed an increased rate of excretion per unit nitrogen intake at intakes

greater the 400mgN/kgW$^{0.75}$/day. There was, however, a small and constant increase in the excretion of urinary non-urea nitrogen over the entire range of nitrogen intakes recorded in the reported experiment and that of Barker (1968). Contrary to the pattern of nitrogen excretion in the urine, faecal nitrogen did not increase at nitrogen intakes greater than 400-500mgN/kgW$^{0.75}$/day and even decreased slightly at nitrogen intakes in excess of 800mgN/kgW$^{0.75}$/day.

Data for nitrogen excretion and retention from Experiment 3 and that of Barker (1968) showed a certain degree of overlap. There was, however, no overlap in the nitrogen contents of the diets presented in the two experiments. The overlap in parameters measured was due, no doubt, to the depressed dry matter intake and nitrogen intake noted in all animals during period I of Experiment 3. This may have been due to the extreme depression of appetite resulting from the high temperatures recorded during the prefeeding period prior to period 1.

One of the aims of Experiment 3 was to determine whether nitrogen intake and retention could be judged from easily measurable parameters. The percentage of urea nitrogen excreted in the total urinary nitrogen was positively and significantly correlated with nitrogen intake over the entire range of nitrogen intakes measured during Experiment 3 and the

experiment of Barker (1968). It was considered that this parameter could be used as an index of nitrogen intake and hence nitrogen retention in wallabies subjected to the same experimental procedure. To a lesser extent U/P urea ratios can be used to the same end, as this parameter was also positively and significantly correlated with nitrogen intake during Experiment 3. However, as plasma urea was not measured by Barker (1968), the relationship between U/P urea ratios and nitrogen intake and retention at low nitrogen intakes could not be established. Nevertheless, Lintern and Barker (1969) found that U/P urea ratios declined to low values in male wallabies fed a low nitrogen diet and in negative nitrogen balance, similar to that obtained by Barker (1968) for female wallabies with a similar nitrogen intake. Thus the use of U/P urea ratios as an index of nitrogen intake and nitrogen retention may be valid.

It is difficult to explain the cause of a limitation in nitrogen retention in wallabies with high nitrogen intakes from the data available and any explanation at this stage is of a speculative nature. It would be expected that animals fed a high protein diet which is adequate in every respect, would initially exhibit an increase in nitrogen retention which would reach a maximum value, and once the limited protein stores had been laid down, nitrogen balance would return to equilibrium

(Albanese, 1959). This, however, did not occur in the reported experiment. It is clear that in data of this kind, the time factor involved in the measurement of nitrogen retention by nitrogen balance trials, must be considered. Values for nitrogen retention represent a mean value over a ten day collection period. However, even if this time factor is considered, animals with the maximum nitrogen intake recorded, would be expected to fill their protein reserves more rapidly than those receiving a somewhat lower nitrogen intake and hence mean nitrogen retention for a ten day period would be lower in the former than in the latter. Therefore, the upper limit to nitrogen retention measured in Experiment 3 cannot be explained in terms of protein storage.

An explanation for this pattern of nitrogen retention with increasing nitrogen intake can be offered by comparing what is known of digestion and utilization of dietary nitrogen in this species with that of true ruminants. There is ample evidence in the literature that protein degredation in the rumen results in ammonia production (Annison, Chalmers, Marshall and Synge, 1954; Annison, 1956; Pearson and Smith, 1943; Warner, 1956; Lewis and McDonald, 1958; Moore and King, 1958; Blackburn and Hobson, 1960, 1961; Williams, Gutierrez and Doetsch, 1960; Preston, Whitelaw and MacLeod, 1963) and these workers have shown that the more soluble proteins (of which

casein is one) give rise to higher concentrations of ammonia in the rumen. Ammonia is absorbed from the rumen (McDonald, 1948), converted to urea in the liver and excreted in the urine. Neither urea nor ammonia nitrogen can be utilized by the host animals for essential amino acid synthesis unless it is subsequently recycled to the rumen (Kosharov et al., 1967). Therefore excessive production and movement of ammonia from the rumen into the blood stream may form a considerable loss of dietary nitrogen to the ruminant (Blackburn, 1965).

It has been demonstrated that digestion of dietary nitrogen in the Kangaroo Island Wallaby is ruminant-like and involves extensive incorporation of dietary nitrogen into microbial nitrogen in the forestomach, with the initial production of ammonia (Experiment 2). It is clear that urea, administered as a drench, is hydrolysed to ammonia as extensively as in ruminants. Therefore the factor limiting nitrogen retention in wallabies fed high levels of casein in the diet may have been the rate of ammonia production exceeding its conversion to microbial nitrogen. This suggestion is supported by the relationship found between faecal nitrogen and nitrogen intake when nitrogen retention was maximal. These parameters, although positively and significantly correlated at low nitrogen intakes (Barker, 1968) were not correlated at nitrogen intakes which resulted in limited nitrogen retention in Experiment 3.

It has been concluded (Blaxter, 1964) that the bulk of the nitrogen excreted in the faeces of cows and sheep (Blaxter and Martin, 1962; Campling, Freer and Balch, 1962) which cannot be attributed to the classical metabolic fraction (Mitchell and Bert, 1954), also is not attributable to undigested food nitrogen. Rather, any increase in faecal nitrogen in cows and sheep, fed an adequate nitrogen diet supplemented with urea or casein, is due to ingested nitrogen which is converted to microbial nitrogen in the rumen and subsequently excreted, undigested, in the faeces. This was supported by the finding of Martin and Blaxter (1963, unpublished data; cited Blaxter, 1964), that if additional casein and urea are introduced directly into the abomasum, no increase in faecal nitrogen occurs. If this is true for the wallaby, then any limit in the incorporation of dietary nitrogen into microbial nitrogen would result in an upper limit in nitrogen excretion in the faeces. This assumes that metabolic faecal nitrogen (MFN) is independent of the diet fed. This assumption does not appear to be true, as MFN excretion in ruminants may vary with the amount of food eaten (Mukherjee and Kehar, 1949) and the ratio of carbohydrate/protein in the diet (Blaxter and Martin, 1962).

Therefore, if dietary nitrogen, which is not incorporated into microbial nitrogen, is absorbed from the forestomach in

the form of ammonia, this, after conversion to urea in the liver, would be excreted in the urine. Such a system explains the upper limit to nitrogen retention, rapid increase in urinary urea nitrogen and hence total urinary nitrogen and the upper limit to faecal nitrogen excretion at high nitrogen intakes in the reported experiment. However, the decline in faecal nitrogen at nitrogen intakes in excess of 800mgN/kgW$^{0.75}$/day can only be explained if, at these high nitrogen intakes, incorporation of dietary nitrogen into microbial nitrogen declines, or MFN declines.

Whether inadequate energy or a deficiency such as sulphur in the experimental diets limited the utilization of casein nitrogen by the forestomach microbiota, as it may in the ruminant (Starks, Hale, Garrigus, Forbes and James, 1954; Warner, 1956; Lewis and McDonald, 1958; Reis and Reid, 1959), cannot be said. As sulphates in the diet were plentiful and available carbohydrates formed 39% of the diet and the bulk of the remainder was in the form of oaten chaff (McDonald and Hall, 1957), this seems unlikely. However, this could be tested by feeding high nitrogen diets of variable carbohydrate or sulphate content to wallabies and measuring nitrogen retention.

In Experiment 1, when utilization of dietary urea and casein by the wallaby were compared, nitrogen intakes of 550-750mgN/kgW$^{0.75}$/day were recorded. At these levels of

nitrogen intake, nitrogen intake and nitrogen retention were not significantly correlated. In addition the slope of the line of best fit calculated for these parameters was not significantly different from zero (t_6 = 1.64 NS). This finding suggests that nitrogen retention was limited in the wallabies during Experiment 1 as it was during Experiment 3.

Contrary to the results of Experiment 3, a positive and significant correlation was found between faecal nitrogen and nitrogen intake during the balance periods of Experiment 1. However, as the values obtained for faecal nitrogen were similar to those obtained in Experiment 3 over the same range of nitrogen intakes, this correlation cannot be considered representative of the pattern of faecal nitrogen excretion at nitrogen intakes below the range recorded in Experiment 1.

This conclusion is supported by the value obtained from the data of Experiment 1 for metabolic faecal nitrogen (MFN). MFN is the endogenous fraction of faecal nitrogen and comprises the nitrogenous residues of digestive juices and epithelial cells sloughed off from the lining of the alimentary tract. It is usually determined as the faecal nitrogen excretion from a low protein or nitrogen free diet (Schneider, 1935; Mitchell and Bert, 1954; Armstrong and Mitchell, 1955). However, Mitchell and Bert (1954), demonstrated a linear relationship between faecal nitrogen output per unit dry matter intake and

the percentage nitrogen content of the food eaten over a range of nitrogen intakes for rabbits, dogs, swine and sheep. They found that extrapolation of the regression line to give faecal nitrogen output at zero nitrogen intake gave values which were in close agreement with those obtained when MFN was determined directly by feeding a low protein diet. As the nitrogen content of faeces and food eaten (expressed per unit dry matter intake) were positively and significantly correlated in Experiment 1 ($r = +0.82^{*}$), MFN can be calculated (Mitchell and Bert, 1954). The regression equation relating these parameters is

$$Y = 0.29X + 36.8$$

where Y = faecal nitrogen ($mgN/kgW^{0.75}/day$)

X = nitrogen intake ($mgN/kgW^{0.75}/day$).

Therefore, if it is assumed that the indirect method of Mitchell and Bert (1954) for calculating MFN is valid for the Kangaroo Island Wallaby, 0.037gN/100g dry weight is the theoretical excretion of faecal nitrogen by a wallaby fed a nitrogen free diet. Clearly this value is meaningless. Not only is it lower than the values reported for macropods including the Kangaroo Island Wallaby (0.27 - 0.41gN/100g dry matter intake) (Brown, 1964; Brown and Main, 1967; Barker, 1968) and ruminants (0.41 - 0.48gN/100g dry matter intake) (Hutchinson and Morris, 1936; Harris and Mitchell, 1941a;

Harris, Work and Henke, 1943; Mukherjee and Kehar, 1949; Blaxter and Wood, 1951), it is also significantly lower than values obtained for monogastric mammals (0.10- 0.24gN/100g dry matter intake) (Allison and Anderson, 1945; Armstrong and Mitchell, 1955).

Values obtained in Experiment 1 for U/P urea ratios and the percentage of urea nitrogen excreted in the total urinary nitrogen were also similar to those obtained for the same range of nitrogen intakes in Experiment 3. Therefore, it is concluded that nitrogen retention was limited in the wallabies of Experiment 1. Accordingly the same arguments can be advanced to explain this limitation in nitrogen retention as were advanced for Experiment 3.

The highly speculative nature of the above discussion cannot be emphasised too strongly. The necessity to draw so extensively on data obtained for true ruminants typifies how little is known of the most elementary features of macropod digestion and utilization of dietary nitrogen. Clearly a detailed study of the fermentative and synthetic activities of the forestomach microorganisms and their nutritional requirements is needed.

B. The site of control and extent of renal retention of urea

It was demonstrated in Experiment 3 that nitrogen retention, under the experimental conditions imposed, is limited in wallabies with nitrogen intakes in excess of those required to maintain nitrogen equilibrium. However, maximum nitrogen retention, in terms of protein storage (see Lusk, 1928; Swanson, 1959; Mitchell, 1962) was not achieved in these wallabies. Nevertheless it was considered that if a mechanism, related to the nitrogen status of the wallaby, controls the extent of renal retention and utilization of urea, then it would be detected in a study which compared these processes in wallabies in maximum positive (Experiment 3) and minimum negative (Barker, 1968) nitrogen balance. In the following experiments these levels of nitrogen retention were diagnosed, using the criteria set down in Experiment 3, by measuring U/P urea ratios and the percentage of total urinary nitrogen excreted as urea nitrogen in wallabies fed low or high nitrogen diets.

Experiment 4 was designed to investigate the site of control and extent of renal retention of urea in wallabies with maximum and minimum nitrogen retention. The aim of the experiment was to establish whether renal retention of urea is as extensive as in ruminants and occurs through renal tubular reabsorption (Schmidt-Nielsen *et al.*, 1957, 1958).

(i) ETC clearance as a measure of GFR.

It was found necessary to establish a method for measuring GFR in the wallaby, as no report has been published on this aspect of macropod renal physiology. As the wallaby is difficult to handle and responds adversely to caging and handling, it was not practicable to infuse an exogenous substance, the clearance of which could be used as a measure of GFR over twenty four hours. It was necessary therefore to find an endogenous constituent of the plasma and urine which could be used for this purpose. ETC clearance was an obvious choice as it has been widely used as an index of GFR in mammals. However, as pointed out in the introduction to Experiment 4, ETC clearance is only a valid measure of GFR in some mammals and opinion is divided as to its use for this purpose.

Rheburg (1926 a and b) first used total creatinine chromogen as an index of GFR in man on the grounds that it was concentrated in the urine to a greater extent than any other identified constituent. It was already known that total creatinine chromogen is concentrated in the urine of man in remarkably constant amounts daily and varies little with diet or urine flow rate (Folin, 1905; Schaffer, 1908). However, it was

Popper and Mandel (1937, cited Mandel et al., 1953) who first employed ETC clearance as a clinical measure of GFR. Since then a controversy has raged (Wesson, 1957; O'Connor, 1962; Pitts, 1969) as to whether ETC fulfils the criteria outlined by Smith (1956) for a substance whose clearance is an index of GFR.

These criteria are:

(1) It must be amenable to precise and quantitative determination in plasma and urine.

(2) It must be completely filterable at the glomerulus.

(3) It must be physiologically inert.

(4) It must not be reabsorbed or secreted by the tubules.

ETC appears to satisfy the first three requirements set down by Smith (1956). The adsorption-elution technique, using Lloyd's reagent, of Hare (1950) which was employed during Experiment 4, has enabled ETC to be accurately estimated in both plasma and urine. The creatinine molecule is small enough to be completely filterable at the glomerulus (Smith, 1956) in mammals studied so far and Bloch and Schoenheimer (1939) have demonstrated that the conversion of creatine phosphate and creatine to creatinine in mammalian muscle to be

biologically irreversible, rendering creatinine physiologically inert. However, as pointed out by O'Connor (1962), the evidence for or against tubular excretion or reabsorption of ETC is indirect and appears to be the main objection to its use as an index of GFR. The only way of assessing whether all the ETC filtered by the kidney is actually excreted in the urine is by comparing its clearance with a reference substance known to fulfil the criteria of Smith (1956) and also by demonstrating that the clearance of ETC is unaffected by a wide range of urine flow rates and plasma concentrations.

Inulin, a polysaccharide, has been widely used as a standard reference substance since it was first suggested quite independently by Richards (1938) and Smith (1943), and it was employed for this purpose during Experiment 4. It fulfils the criteria of Smith (1956) being amenable to precise and accurate determination in plasma and urine (Davidson and Sackner, 1963), it is completely filterable at the glomerulus (Smith, 1956) and furthermore is physiologically inert; it is metabolised at a rate too low to affect clearance estimations (Finkenstaedt, O'Meara and Merrill, 1953). It is neither secreted nor reabsorbed by the kidney tubules, although again this evidence is indirect, relying on the finding that

its clearance is unaffected over a wide range of plasma concentrations. Although this last property of inulin has been challenged for man (Ferguson, Olbrich, Robson and Stewart, 1950) dogs and cats (Eggleton and Habib, 1951), it has been widely used as an index of GFR and a standard reference substance in a number of species (Mandel et al., 1953; Schmidt-Nielsen et al., 1957, 1958; Ramsay and Coxon, 1967; Campbell and White, 1967). Thurau, Valtin and Schnermann (1968), in their recent review, have concluded that in mammals studied so far, inulin is a valid measure of GFR and the assumption was made that this was also true for the wallaby.

The results of Experiment 4 indicate that, under conditions of high urine flow rate, ETC and inulin clearance are identical. O'Connor (1962) objected to the assumption that ETC clearance and inulin clearance remain identical during periods of high and low urine flow rates, as reports for or against this assumption are conflicting (Shannon, 1936b; Chelsey, 1938; Ladd et al., 1956). However, in the reported study, ETC clearance was independent of urine flow rate (Table 26). It appears therefore that ETC clearance may be a valid measure of GFR in the Kangaroo Island Wallaby.

<u>TABLE 26</u>

ETC clearance (ml/min) and urine flow rate (ml/min) (in parenthesis) of four wallabies fed high, maintenance or low nitrogen diets and with and without water loading during Experiment 4

Diet	Hydration	Animal			
		392r	394r	10	56
Maintenance	Water	5.1(1.2)	7.0(2.0)	10.6(2.6)	9.7(2.6)
High-N	Loaded	5.2(1.3)	7.0(2.2)	10.6(2.6)	9.6(2.5)
Low-N		5.2(1.0)	7.0(1.9)	10.6(2.4)	9.6(2.4)
High-N	Not water	5.2(.04)	7.0(.05)	10.6(.06)	9.6(.04)
Low-N	Loaded	5.1(.05)	6.9(.04)	10.5(.08)	9.6(.04)

Nevertheless, it remains to be demonstrated that ETC clearance is independent of variations in plasma ETC concentrations. Slight variations in plasma ETC concentrations occurred during Experiment 4. They were lower when the wallabies were water loaded than when they were not water loaded. This was due no doubt to an increase in plasma volume resulting from reabsorption of water introduced into the forestomach. Nevertheless, regardless of these small fluctuations in plasma ETC concentrations, ETC clearance remained unchanged (Table 26). In the light of these findings ETC clearance was accepted as a measure of GFR in the Kangaroo Island Wallaby.

(ii) Urea excretion and GFR

As found in sheep and camels (Schmidt-Nielsen *et al.*, 1957, 1958), renal regulation of urea excretion in the Kangaroo Island Wallaby is brought about by renal tubular reabsorption of urea. GFR was independent of nitrogen intake whilst urea clearance was dependent upon nitrogen intake. In addition, the fraction of filtered urea excreted in the urine of the wallabies receiving the low nitrogen diet was between 1 - 2%, which lies within the range of values reported for sheep and camels similarly nitrogen depleted

(Schmidt-Nielsen *et al.*, 1957, 1958). U/P urea ratios were also within the range recorded for sheep and camels receiving a low nitrogen diet (Table 27). The high values obtained by Cocimano and Leng (1967) for sheep cannot be explained. Thus renal retention of urea in the nitrogen depleted wallaby is as efficient as in nitrogen depleted sheep and camels. In the wallabies receiving the high nitrogen diet and diagnosed to be in positive nitrogen balance (Experiment 3), the fraction of filtered urea excreted ranged from 76% to 87%, being somewhat greater than values obtained for nitrogen sufficient sheep (42 - 60%) and camels (30 - 50%) (Schmidt-Nielsen *et al.*, 1957, 1958).

It was found that wallabies 394r and 56, when depleted of nitrogen, retained considerably more urea per day through renal tubular reabsorption, than when they received an adequate nitrogen diet. In wallabies 392r and 10, however, the amount of urea retained daily by the kidney was only slightly greater during periods of nitrogen depletion. The significance of this finding is not clear. A number of workers have demonstrated that there is more than one site of urea reabsorption in the tubules of the mammalian kidney and the relative importance of these sites of

TABLE 27

U/P Urea ratios at low urine flow rates of mammals fed a low nitrogen diet

Animal	U/P Urea	Reference
Rat	4-6	Lassiter, Mylle and Gottschalk (1966)
	4-39	Truniger and Schmidt-Nielsen (1964)
Man	25-40	Murdaugh and Schmidt-Nielsen (1957)
Dog	50-60	Schmidt-Nielsen (1958)
Camel	4	Schmidt-Nielsen *et al.* (1957)
Sheep	4-12	Schmidt-Nielsen and O'Dell (1959)
	5-9	Schmidt-Nielsen *et al.* (1958)
	34	Cocimano and Leng (1967)
Kangaroo Island Wallaby	11-12	Lintern and Barker (1969)
	2-3	This thesis

reabsorption appears to depend upon the nitrogen status and degree of hydration of the animal concerned (Schmidt-Nielsen and O'Dell, 1959; Bray, 1960; Bray and Preston, 1961; Lassiter, Gottschalk and Mylle, 1961; Schmidt-Nielsen, O'Dell and Osaki, 1961; Rabinowitz and Kellogg, 1963; Ullrich *et al.*, 1967). The measurement made of the amount of urea retained per day by the kidney of the wallaby provides no information on the relative contribution of the sites of urea reabsorption in the renal tubule or the amount of retained urea which subsequently becomes available to the wallaby for use. Nevertheless, the increase recorded in the total amount of urea retained by the kidney of the nitrogen depleted wallaby may be a reflection of enhanced renal retention of urea. Such extensive renal retention of urea may be of survival value during periods of nitrogen stress, as urea recycling to the forestomach may also be enhanced. This possibility was investigated further in Experiments 5 and 6.

C. Renal retention of injected urea

The results of Experiment 5 indicate a further parallel between the renal handling of urea by the Kangaroo Island Wallaby and the ruminant. Like camels (Schmidt-Nielsen *et al.*, 1957) the wallaby, when depleted of nitrogen, retains nearly all urea injected intravenously. This has also been reported in the nitrogen depleted euro (Brown, 1964). Renal retention of injected urea also occurs in sheep (Schmidt-Nielsen and Osaki, 1958; Houpt, 1959; Somers, 1961d) and goats (Houpt, 1959; Hill *et al.*, 1962) receiving a low nitrogen diet, although to a lesser extent than reported in camels, euros and Kangaroo Island Wallabies. Whether this is merely a reflection of a difference in the nitrogen status of the experimental animals used or a true difference in the ability of the kidney to retain injected urea is unknown. Nitrogen sufficient wallabies also responded to injected urea in a manner similar to camels and sheep receiving an adequate nitrogen diet (Schmidt-Nielsen *et al.*, 1957; Somers, 1961d) as some 60% of the injected urea was recovered in the urine within twenty four hours after the administration of urea.

It was found that GFR (as measured by ETC clearance) was not affected in either group of wallabies by the increased filtered urea load arising from the injection of urea. It was concluded, therefore, that renal retention of injected urea

is brought about by renal tubular reabsorption. It appears from this finding that reabsorption of urea by the renal tubules of wallabies depleted of nitrogen is enhanced when urea is injected, even though the filtered urea load during the post-injection period may be more than double that recorded prior to the injection of urea. This mechanism does not appear to operate as extensively in nitrogen sufficient wallabies.

U/P urea ratios of less than unity were recorded on two occasions in the nitrogen depleted wallabies during the post-injection period and, as pointed out, the estimated U/P urea ratios were too high for the nitrogen depleted group during the post-injection period. Schmidt-Nielsen (1958) has stated that active reabsorption of urea or any other filterable solute in the renal tubule is only demonstrated when its concentration in the plasma exceeds that in the urine. It is suggested therefore that reabsorption of injected urea in the renal tubule of the nitrogen depleted wallabies involves an active transport system.

The results of Lintern and Barker (1969) support this suggestion. They demonstrated that the concentration of endogenous urea in the tissue of the inner stripe of the outer zone of the renal medulla of nitrogen depleted wallabies may exceed that in the urine. Based on similar evidence, active renal tubular reabsorption of urea has been suggested

for a number of mammals including sheep (Schmidt-Nielsen, and O'Dell, 1959), rats (Bray and Preston, 1961) and dogs (Goldberg, Wojtczak and Ramirez, 1967). Ullrich et al. (1967) have recently provided more direct evidence for active reabsorption of urea in the kidney of a nitrogen depleted mammal. They found that uphill transport of urea occurs in the kidney of the nitrogen depleted rat, but that movement of urea was passive in the kidney of rats fed an adequate nitrogen diet. The site of urea reabsorption was located as the medullary region of the collecting duct.

It appears, therefore, from the results of Experiments 4 and 5, together with those of Lintern and Barker (1969), that renal retention of urea by nitrogen depleted wallabies and sheep may involve active transport of urea out of the renal tubule, that the region of the renal tubule concerned is located in the outerzone of the renal medulla and that the extent of reabsorption of urea may be enhanced when the filtered urea load is temporarily increased by injecting urea into these animals. It is concluded that, if the biochemical mechanism by which injected urea is retained in nitrogen depleted wallabies is induced, then the stimulus for induction is not the circulating plasma urea levels. Some other effect of a lowered nitrogen intake may induce this mechanism.

Schmidt-Nielsen and Osaki (1958) suggested that renal retention of injected urea in nitrogen depleted sheep is a

response to changes in the balance of circulating hormonal levels brough about by a lowered nitrogen intake. They found, however, that ACTH had no clear cut or immediate effect on renal retention of urea in sheep. It is possible, nevertheless, that renal retention of injected urea in both nitrogen depleted ruminants and macropods is induced either by a hormone or some other factor in the blood stream, the level of which is controlled by the levels of nitrogen intake. Further research on this problem is required. It could be investigated in wallabies by perfusing the kidney of a nitrogen sufficient wallaby with arterial blood from a nitrogen depleted wallaby and measuring renal retention of urea in the treated kidney. The untreated kidney could be used as a control. The same could be done for nitrogen depleted wallabies by perfusing one kidney in each with arterial blood from nitrogen sufficient wallabies.

As pointed out, the ability of nitrogen depleted ruminants and wallabies to retain exogenous and endogenous urea has been interpreted in terms of utilization of urea by these animals via the urea recycling mechanism.

The only evidence to suggest that extensive renal retention of injected urea is restricted to animals with a ruminant or ruminant-like digestion is that provided by Schmidt-Nielsen _et al._ (1957). They found that the donkey,

a non-ruminant herbivore, when nitrogen depleted, excreted injected urea almost quantitatively in the urine. Since it has been assumed that post-gastric fermentation in non-ruminant herbivores prohibits these animals from benefiting from microbial protein synthesis, Schmidt-Nielsen *et al.* (1957) concluded that renal retention of urea would not contribute to the nitrogen economy of these animals and therefore it would not be expected to occur. This was supported by their results. However, this finding must be treated with caution. The results were based on one donkey which was fed a diet of hay alone, which may have been low in available energy as well nitrogen. As camels (Schmidt-Nielsen *et al.*, 1957) sheep, goats, (Houpt, 1959), euros (Brown, 1964) and Kangaroo Island Wallabies were fed diets low in nitrogen but rich in available carbohydrates, comparisons between these results and those obtained for the donkey may not be valid, particularly as Houpt (1959) has demonstrated that retention of injected urea by nitrogen depleted sheep is reduced from 52% to 22% when available carbohydrates are withdrawn.

Although there is little direct evidence in the literature to suggest that extensive renal retention of injected urea is peculiar to nitrogen depleted animals with a ruminant or ruminant-like digestion, there is considerable indirect evidence, if it can be assumed that feeding a single high

protein or urea meal to a nitrogen depleted animal is analogous to injecting urea. This is a reasonable assumption as such treatment also gives rise to elevated plasma urea levels (Schmidt-Nielsen, 1958; Schmidt-Nielsen and Osaki, 1958). Schmidt-Nielsen and Osaki (1958) found that U/P urea ratios in nitrogen depleted lambs fell from 25 to 5 when these animals were fed a high protein meal and that urea clearance fell to 1-2% of the GFR. However, in man and dogs receiving a low nitrogen diet (see Schmidt-Nielsen, 1958 for review) a high protein or urea meal is followed by an increase in the excretion of urea in the urine. Thus, in those animals known to recycle and utilize endogenous urea, renal retention of exogenous urea is pronounced during periods of nitrogen depletion. However in those animals (man and dogs) where the urea recycling mechanism does not appear to operate, renal retention of exogenous urea does not occur.

Some retention of exogenous urea (administered parenterally) has been reported in rats and mice (Leifer, Roth and Hemplemann, 1948; Dintzis and Hastings, 1953). Houpt (1963) has also shown that injected urea is retained by nitrogen depleted rabbits. However, as these animals are known to practice coprophagy (Moir, 1965), they presumably digest hindgut microbiota. Thus it is conceivable that urea recycling may occur to the hindgut and protein synthesised

from this non-protein source by the microbiota would ultimately contribute to the dietary amino acid pool of the animal. This would provide an interesting field of research, as nitrogen depleted rats exhibit U/P urea ratios similar to those recorded in nitrogen depleted ruminants (Table 27).

Houpt (1959), working with sheep and goats, found that injected urea which disappears from the total body water available for urea dilution, and which is not recovered in the urine, enters another compartment of the body, namely the digestive tract. As injected urea gradually disappeared from the bloodstream (and therefore the urea dilution volume) of the wallaby during Experiment 5, and this could not be entirely accounted for by urinary urea excretion in either the nitrogen sufficient or depleted wallabies, some of this urea probably entered the digestive tract. Furthermore, as more injected urea was retained by the nitrogen depleted wallabies it was postulated that more injected urea entered the digestive tract in these animals and was utilized. This possibility was investigated in Experiment 6.

D. Recycling of injected urea to the alimentary tract

The results of Experiment 6 are in agreement with those of Experiment 5 and confirm that extensive renal retention of injected urea occurs in the nitrogen depleted Kangaroo Island

Wallaby, paralleling closely the response of nitrogen depleted camels, sheep and goats to injected urea (Schmidt-Nielsen et al., 1957; Schmidt-Nielsen and Osaki, 1958; Houpt, 1959). At the termination of the experiment (which occurred three hours after the injection of urea) 45% of the injected urea had been excreted by the nitrogen sufficient wallabies and only 0.3% by the wallabies depleted of nitrogen.

Using the indirect method of Houpt (1959), injected urea was found to enter the digestive tract of both the nitrogen depleted and sufficient wallabies. However, more injected urea was present in the digestive tract of the nitrogen depleted wallabies than in the nitrogen sufficient wallabies (14% and 5% respectively) at the end of the experimental period.

Houpt (1959) also measured the amount of endogenous urea which moves directly from the blood stream into the rumen of sheep and goats using animals with saline filled rumen (Simmonet et al., 1957). Although this technique enables the amount of urea which actually enters the rumen to be measured, it does not account for any effect of the rumen microbiota on the extent of recycling and utilization of urea. As any improvement in the nitrogen economy of the animal from recycled urea depends upon the conversion of urea to microbial protein, a study of the importance of microbial utilization of

recycled urea should be included in any study of urea recycling to rumen. The technique used by Houpt (1959) is further limited, as any interpretations on the control of the movement of urea into the rumen, based on data from this technique, depends upon the measurement of concentration gradients from the plasma to rumen or rumen to plasma. In the presence of an active microbiota, under normal physiological conditions, such gradients may be different due to conversion of urea to ammonia and microbial protein (Houpt and Houpt, 1968).

The measurements made of the amount of salivary urea entering the rumen by cannulation of salivary glands also limits (Somers 1961a,b,c,d) any conclusion on the significance of this urea to the animal, as its utilization by the microbiota in the rumen is not assessed. However, this technique allows the affect of plasma urea concentration on the accumulation of urea in the saliva to be studied (Somers, 1961d).

A further method employed for detecting the recycling of urea to the rumen is by injecting urea intravenously and measuring changes in ruminal ammonia concentrations (Weston and Hogan, 1967; Varady et al., 1967). This technique enables some interpretation to be made on the relationship between the movement of urea into the rumen and the concentration of urea in the plasma. It does not, however, enable the utilization of recycled urea by the microorganisms to be established and

provides no information on the extent of urea recycling in the absence of injected urea. The former limitation can be remedied by using urea labelled with C^{14} or N^{15} (Hill et al., 1962).

In Experiment 6 the technique employed was injection of urea and N^{15} labelled urea was used, as in a study of nitrogen metabolism, it is more meaningful to follow the movement and utilization of the nitrogenous moiety of the urea molecule.

This technique was chosen in the light of the results of Experiment 5. It was considered that the difference observed in the extent of renal retention of injected urea in nitrogen depleted and sufficient wallabies might be a reflection of an adaptive response of the kidney aimed at improving nitrogen economy by increasing the amount of urea available for recycling (Schmidt-Nielsen et al., 1957, 1958; Schmidt-Nielsen and Osaki, 1958; Houpt, 1959). It was thought that these differences might be reflected as differences in the amount of urea recycled to and utilized by the forestomach microbiota. Weston and Hogan (1967) and Somers (1961d) have suggested that when circulating plasma urea levels are high, movement of urea into the rumen of sheep is limited.

When N^{15} labelled urea was injected intravenously into nitrogen sufficient and depleted wallabies, excess N^{15} was detected in the forestomach digesta from both groups. As the

excess N^{15} could only have arisen from injected urea N^{15}, the ability of the Kangaroo Island Wallaby, like the ruminant (Phillipson, 1964) and euro (Brown, 1964), to recycle urea to the forestomach was confirmed. It was found that the amount of injected urea present in the forestomach digesta of the nitrogen depleted wallabies was significantly greater than that in the forestomach digesta of the nitrogen sufficient wallabies. This trend was the same as that observed when the indirect method of Houpt (1959) was used to estimate the total amount of injected urea present in the digestive tract.

Part of the N^{15} arising from injected urea was incorporated into microbial nitrogen in the forestomach of both groups of wallabies. This finding confirmed the suggestion, based upon the results of Experiments 1 and 2, that digestion of urea nitrogen involves its incorporation into microbial nitrogen in the forestomach. A difference was found between the two groups of wallabies in the extent of incorporation of recycled urea nitrogen into microbial nitrogen. At the termination of the experiment, 40% to 58% of the recycled urea nitrogen was in the form of microbial nitrogen in the forestomach of wallabies in negative nitrogen balance, whereas only 19% was detected in the form of microbial nitrogen in the nitrogen sufficient wallabies. In the latter group the remaining 81% was in the form of soluble nitrogen, and of this some 50% was

ammonia nitrogen. Ammonia nitrogen arising from recycled urea formed only 25% of the fraction of soluble nitrogen arising from recycled urea in the forestomach digesta of the wallabies receiving the low nitrogen diet.

Injected urea nitrogen was also detected in the caeca of both groups of wallabies. As found for the forestomach, the amount of injected urea present in the caeca of the nitrogen depleted wallabies was greater than that found in the caeca of wallabies fed the high nitrogen diet. It is unlikely that injected urea nitrogen detected in the caecum arose from onward passage of forestomach digesta, as no excess N^{15} was detected in digesta from the distal end of the duodenum. Therefore, N^{15} labelled urea probably entered the caecum directly from the blood stream, but the quantities involved were considered too small to be important in the nitrogen economy of the wallaby. Nevertheless, as Williams (1965) has shown that the hindgut of ruminants is an active site of fermentation, the movement of urea into the caeca of wallabies together with the observation of a microbial population in this region of the hindgut suggests that the hindgut may play a part in the nitrogen metabolism of this species.

The values obtained using the indirect method of Houpt (1959) agreed well with those obtained using the direct method of N^{15} labelled urea. The differences between the two methods

may reflect the amount of injected urea present in other regions of the alimentary tract. It is clear, however, that recycling of injected urea in the wallaby occurs mainly to the forestomach, only a small proportion entering the hindgut.

As pointed out, urea may enter the rumen of the ruminant both with the saliva (Somers, 1961a,b,c and d) and directly from the blood stream across the rumen wall (Houpt, 1959). In Experiment 6 the relative contribution of these two pathways was not determined but could be estimated using the standard techniques of cannulation of the salivary glands and filling the forestomach with saline.

Brown (1964) using these techniques in the euro, demonstrated that urea enters the forestomach by both these pathways and, like the ruminant (Somers, 1961a; Houpt, 1959), more urea enters the forestomach directly from the blood stream than enters with the saliva. Brown (1964) also found that the amount of urea recycled to the forestomach of the euro is quantitatively similar to that recycled to the rumen of sheep (Somers, 1961a; Houpt, 1959). Whether this is true for the wallaby cannot be determined from the results of Experiment 6 and the accumulation of urea in the forestomach must be estimated in the absence of injected urea in order to make a quantitative comparison of the extent of urea recycling in the wallaby, euro (Brown, 1964) and sheep (Somers, 1961a; Houpt, 1959).

Data gathered during Experiment 6 does, however, give information on the relative utilization of recycled urea by nitrogen sufficient and depleted wallabies. The results indicate that differences exist not only in the amount of injected urea retained by the kidney but also in the amount of urea which, after recycling to the forestomach, is utilized by the microorganisms of nitrogen depleted and sufficient wallabies. It appears that utilization of recycled urea is greater in the nitrogen depleted wallabies than it is in wallabies receiving an adequate nitrogen diet. Gray *et al.* (1958) found that the amount of nitrogen leaving the rumen of sheep which had been fed a diet low in nitrogen, exceeded their dietary nitrogen intake. In nitrogen sufficient sheep the situation was reversed. The results indicate that this may also be true of the wallaby during periods of nitrogen depletion and sufficiency.

Conflicting conclusions have resulted from investigations of the control of the transfer of blood urea, either directly from the blood stream or with the saliva, into the rumen. Somers (1961d), suggests that the amount of urea transferred via the saliva may be limited at high plasma urea concentrations. Gartner, Decker and Hill (1961), using C^{14} labelled urea, concluded that transfer of urea from the blood stream into the rumen is also limited when plasma urea concentrations are high.

They suggested that a carrier system, possibly an active transport system, exists in the rumen epithelium for urea transfer. Similar findings were reported by Weston and Hogan (1967). However, Engelhardt and Nickel (1965) and Cocimano and Leng (1967) found that transfer of urea from the blood into the rumen of sheep to be linearly related to the urea concentration difference between the plasma and rumen. No urea transfer occurred at zero gradient.

Houpt and Houpt (1968), working with sheep and goats, have advanced another hypothesis to explain the transfer of urea from the blood to the rumen when bacterial urease in the ruminal epithelium has not been destroyed. They proposed that, under normal ruminal conditions, urea is hydrolysed to ammonia in the ruminal epithelium and that, according to the nitrogen status of the animal, this may result in either facilitated or limited transfer of urea into the rumen. The inner cornified layer of the rumen wall may be traversed with greater ease by the small, lipid soluble ammonia molecule than by the relatively larger urea molecule. The ammonia may move in the direction of its concentration gradient and hence more urea nitrogen would enter the forestomach of the nitrogen depleted ruminant because ruminal ammonia concentrations are low (Weller _et al._, 1962) and less in nitrogen sufficient animals in which ruminal ammonia concentrations are high. This system would enable

urea recycling to be enhanced during periods of nitrogen depletion and this would be enhanced further if plasma urea concentrations were elevated through renal retention of urea. This last point is also true of the simple diffusion theory of Engelhardt and Nickel (1965) and Cocimano and Leng (1967), however under the system proposed by these workers, urea transfer into the rumen need not necessarily be limited when plasma urea concentrations are high.

It is not known whether the difference detected in the amount of injected urea nitrogen in the forestomach of the two groups of wallabies was due to an absolute difference in the total amount of injected urea which entered the forestomach, or merely a difference in the extent of utilization of urea by the microorganisms and its rate of removal in the form of ammonia. Until this is known, no hypothesis can be advanced to explain the presence of more injected urea in the forestomach of the nitrogen depleted wallabies than was detected in the forestomach of the nitrogen sufficient wallabies. Clearly the nitrogen depleted wallabies would enjoy improved nitrogen economy if transfer of urea into the forestomach of this group is facilitated in some way. Application of the technique used by Houpt and Houpt (1968) for sheep and goats to the wallaby may help elucidate this problem.

3. THE EFFECT OF CONCURRENT WATER AND NITROGEN DEPLETION ON RENAL RETENTION OF UREA AND NITROGEN RETENTION IN THE KANGAROO ISLAND WALLABY

Experiments 7.1 and 7.2 were designed to establish whether this wallaby, like cattle (Livingston *et al.*, 1962, 1964; Payne, 1964, 1965, 1966) shows improved nitrogen retention and decreased urinary urea excretion when both water and nitrogen depleted. In addition, the suggestion of Moir (1965) that renal retention of urea might improve water balance, under certain conditions, was tested. As the results of Experiment 7.1 were inconclusive and provided information which lead to the more detailed study described in Experiment 7.2, only the results of Experiment 7.2 will be discussed.

In ruminants, water and dry matter intake have been found to be positively and significantly correlated and a decline in dry matter intake is a consistent feature of water restriction (Leitch and Thomson, 1944; Clark and Quin, 1949; Winchester and Morris, 1956; Phillips, 1960; Thornton and Yates, 1968). Water and dry matter intake have also been shown to be positively and significantly correlated in the Kangaroo Island Wallaby (Barker, 1968; Experiments 1 and 3). In Experiment 7.2, dry matter intake was significantly less in the water restricted group of wallabies than in those wallabies allowed water *ad lib.*, agreeing with the findings for true ruminants.

Phillips (1960) found that the ratio water intake/ dry matter intake was significantly reduced in cattle which were given food ad lib. but allowed only restricted access to water. This ratio was also significantly reduced in the water restricted wallabies in Experiment 7.2 ($t_7 = 13.7^{***}$). Contrary to these findings, Winchester and Morris (1956) and Thornton and Yates (1968) report that this ratio was not significantly affected in cattle allowed limited access to water. It is necessary to establish the regression equations relating dry matter intake and water intake in wallabies with either a restricted or unrestricted water intake before the true significance of the difference in the ratio water intake/ dry matter intake is established. The design of the Experiments7.1 and 7.2 did not allow this to be calculated.

Barker (1968), reported that nitrogen intake and dry matter intake were positively and significantly correlated in female Kangaroo Island Wallabies. Therefore, water restriction, which results in a decline in dry matter intake, would also be expected to lead to a lower nitrogen intake. In Experiment 7.2 the water depleted wallabies had a significantly lower nitrogen intake than those wallabies allowed water ad lib. This could only be attributed to the lower dry matter intake of this group, as the nitrogen content of the food eaten by the two groups was not significantly different.

Payne (1964) provides only scanty data on dry matter and nitrogen intakes in his experiment. Nevertheless, it is clear that water restriction lead to a decline in dry matter intake in both *Bos taurus* and *Bos indicus*. However, although nitrogen intake was also reduced in *B. indicus*, this was not found in *B. taurus*. This anomally was not discussed.

A decline in the excretion of urea nitrogen in the urine during periods of nitrogen depletion is well documented in ruminants (Schmidt-Nielsen *et al.*, 1957, 1958; Hill *et al.*, 1962; Livingston *et al.*, 1962; Elliott and Topps, 1963) and has also been shown to occur in the Kangaroo Island Wallaby (Lintern and Barker, 1969; this thesis). This was confirmed in Experiments 7.1 and 7.2 and it was also demonstrated that wallabies both water and nitrogen restricted show a further reduction in the excretion of urea nitrogen in the urine. At the end of Experiment 7.2 the wallabies with a restricted water intake excreted significantly less urea nitrogen in the urine than those allowed free access to water. This difference may have been due to the lower nitrogen intake recorded in the water restricted group. Thus, as found in cattle (Livingston *et al.*, 1962) and rabbits (Houpt, 1963), water restriction leads to decline in the excretion of urea nitrogen in the urine. Whether the reduction in urinary urea nitrogen in nitrogen depleted rabbits and cattle receiving a restricted water intake was

attributable to a decline in nitrogen intake cannot be established from the data of Livingston et al. (1962) and Houpt (1963).

Contrary to the findings of Livingston et al. (1962, 1964) no significant difference was detected in total urinary nitrogen excretion in the two groups of wallabies. This anomally cannot be explained. Total urinary nitrogen excretion and nitrogen intake have been found to be positively and significantly correlated in this wallaby (Barker, 1968; Experiment 1, this thesis). Therefore, as nitrogen intake was reduced in the water restricted wallabies, their excretion of nitrogen in the urine would also be expected to be less than that of the wallabies allowed water ad lib. As the excretion of urinary urea nitrogen was significantly less in the water restricted group, presumably water restriction resulted in an increase in the excretion of some other nitrogenous compound in the urine. This should be investigated.

Livingston et al., (1964) found that water restriction resulted in an improvement in nitrogen balance in water restricted cattle, in two cases from negative to positive. Under the conditions of the reported experiment no significant difference was detected in nitrogen retention (as measured by nitrogen balance trials) between the two groups of wallabies. Again, as nitrogen intake and nitrogen retention have been

found to be positively and significantly correlated in this wallaby (Barker, 1968), the nitrogen balance of the water restricted wallabies would be expected to be lower than that of the wallabies allowed water *ad lib.*, since their nitrogen intake was lower.

The excretion of nitrogen in the faeces of the water restricted wallabies was significantly less than that excreted by those wallabies allowed water *ad lib.* Although this may have been due to the lower nitrogen intake of this group, it may also have been a reflection of their greater dry matter digestibility. However this is doubtful, as apparent nitrogen digestibility was similar in both the water restricted and unrestricted wallabies. Johnson *et al.* (1966) report an increase in nitrogen digestibility in cattle allowed a restricted water intake but this was not found by French (1956). It is possible that apparent nitrogen digestibility may not reflect true nitrogen digestibility when dry matter intake is reduced in response to restricted access to water, as Kehar and Mukherjee (1949) have found that metabolic faecal nitrogen increases in cattle as absolute food intake decreases.

As pointed out, dry matter digestibility was significantly greater in the water restricted wallabies than in the control wallabies. This is a well documented feature of ruminants with a restricted water intake (Larsen, Hungerford and Bailey,

1917; Balch, Balch, Johnson and Turner, 1953; French, 1956; Phillips, 1961; Thornton and Yates, 1968) and in these animals may be due to decreased dry matter intake associated with a decreased rate of passage (Blaxter, Graham, Wainman, 1956; Raymond, Minson and Harris, 1959). Brown (1966), in his review of this subject, concluded that as the level of dry matter intake in ruminants increases, dry matter digestibility decreases.

At the termination of Experiment 7.2, plasma urea concentration was significantly greater in the water restricted wallabies than in those allowed water *ad lib.* (t_7 = 2.37*). As plasma urea concentrations have been shown to decrease in the wallaby when nitrogen intake is lowered (Lintern and Barker, 1969; this thesis), the water restricted wallabies would be expected to have lower plasma urea concentrations than those allowed water *ad lib.* as their nitrogen intake was lower. There was no indication that the elevated plasma urea concentrations in the water restricted wallabies resulted from a reduction in plasma volume. Schmidt-Nielsen *et al.* (1957) found that plasma urea concentration was elevated in dehydrated camels. However, although there was a reduction in plasma volume in these animals, this was insufficient to account for the rise in plasma urea concentration (Schmidt-Nielsen, 1964). In the reported study, increased renal retention of

urea may have led to the elevated plasma urea levels in the water restricted wallabies.

It is clear from this study that water restriction does not lead to a significant improvement in nitrogen balance in nitrogen depleted Kangaroo Island Wallabies. Although the wallabies which were concurrently water and nitrogen restricted excreted significantly less urea nitrogen in the urine than those wallabies allowed water ad lib., the quantities were considered too small to be significant to the animal's nitrogen economy. However, the differences in plasma urea concentration and dry matter digestibility between the experimental and control wallabies remains unexplained and requires further investigation. It is possible that increased plasma urea concentrations may result in increased recycling of urea to the forestomach in the water restricted wallabies. This in turn may result in increased dry matter digestibility as Egan (1965) has demonstrated a decrease in the weight of cotton thread in the rumen of sheep when urea recycling is increased. This, however, remains to be tested.

No significant difference was detected in urine volume between the two groups of wallabies during the reported study. Therefore, under the conditions of this experiment, additional renal retention of urea did not lead to an improvement in water economy through decreased urine volume in the wallabies

both water and nitrogen restricted. Thus the suggestion of Moir (1965) was not confirmed in the reported experiment for the Kangaroo Island Wallaby.

Thornton and Yates (1968) report that faecal water excretion was quantitatively more important in reducing water excretion in water restricted cattle than any reduction in urine volume. The reported study indicates that this is also true of the Kangaroo Island Wallaby, as faecal water output was significantly lower in the water restricted wallabies than in those allowed water _ad lib._ These findings agree with those of Weeth, Sawhney and Lesperance (1967) but are contrary to those of Balch _et al._ (1953). Thornton and Yates (1968) also additionally noted that the ratio urine volume/ water intake increased in the water restricted cattle and this was also reported by Phillips (1961). However, contrary to the results of Phillips (1961), Thornton and Yates (1968) reported a significant reduction in the proportion of the water intake excreted as faecal water by water restricted cattle; Phillips (1961) found this ratio to be constant.

In Experiment 7.2 the ratio urine volume/ water intake was greater in the water restricted wallabies than in those allowed water _ad lib._, whilst the ratio faecal water/ water intake was significantly less in the former than the latter. These results agree well with those of Thornton and Yates (1968).

However, the decline in faecal water excretion in the water restricted wallabies may merely be a reflection of reduced water intake rather than any increase in reabsorption of water in the hindgut as a specific response to water restriction.

It is concluded from the results of these experiments that wallabies receiving a low nitrogen diet and with a restricted water intake show no improvement in nitrogen retention or water balance through additional renal retention of urea. However this requires further research. It appears from work with both dogs (Konishi and McCay, 1960; Bressani and Braham, 1964) and cattle (Johnson *et al.*, 1964) receiving adequate nitrogen diets, that an improvement in nitrogen retention during periods of water restriction is closely related to a reduction in urine volume. As pointed out previously, renal retention of urea may result in enhancement of the renal concentrating ability of some mammals (Bray, 1960; Lassiter *et al.*, 1961; Schmidt-Nielsen *et al.*, 1961; Rabinowitz and Kellogg, 1963). Such additional renal retention of urea during periods of partial dehydration may account for improvements in nitrogen retention noted in water restricted dogs and cattle. In dogs this may involve the action of antidiuretic hormone (ADH) (Jaenike, 1960). It is possible that the Kangaroo Island Wallaby may also exhibit improved nitrogen balance if water restriction is sufficient to produce a reduction in urine

volume. The role of urea in the water economy of the wallaby requires further research as the relative renal medullary thickness of the wallaby indicates that this species has the capacity to produce an extremely concentrated urine (Appendix 22).

4. COMPARISON OF THE DIGESTIBLE NITROGEN REQUIREMENT, ENDOGENOUS URINARY NITROGEN EXCRETION, AVERAGE DRY MATTER INTAKE AND CREATININE COEFFICIENT OF THE KANGAROO ISLAND WALLABY WITH THAT OF OTHER MACROPODS AND EUTHERIANS

Brown (1964) reported that the euro has a lower digestible nitrogen requirement, average dry matter intake and minimum urinary nitrogen excretion than eutherians of similar body weight. He interpreted these differences in terms of the standard energy metabolism of the euro, which he predicted, based on measurements of heart rate, to be lower than that of eutherians. It was subsequently found by Fraser and Kinnear (1969) that the creatinine coefficient of the euro was also significantly lower than that of eutherians and they attributed this difference to the lower standard energy metabolism of this species.

Brown (1964) suggested that the low standard energy metabolism and digestible nitrogen requirement of the euro, might be of survival value during the summer months when nitrogen in the diet is low, water scarce and temperatures high

(Ealey, 1962), allowing the euro to persist on pastures unsuitable for sheep.

The Kangaroo Island Wallaby is also thought to experience a shortage of nitrogen and water towards the end of summer on Kangaroo Island, although this probably is not as extreme as that experienced by the euro (Ealey, 1962). Therefore it was of interest to consolidate available data from this thesis and work of others on the digestible nitrogen requirement, average dry matter intake, minimum urinary nitrogen excretion, creatinine coefficient and standard energy metabolism of the Kangaroo Island Wallaby, as values obtained for these parameters may provide further information on the wallaby's ability to survive when nitrogen intake is low, temperatures high and water scarce on Kangaroo Island towards the end of summer.

A. Standard Energy Metabolism

Dawson and Hulbert (1969) found that the standard energy metabolism of the Kangaroo Island Wallaby and a number of other marsupials is significantly lower than that of eutherians of similar body weight agreeing with the findings for the euro (Brown, 1964).

B. Digestible nitrogen requirement

Absorbed nitrogen or digestible nitrogen requirement is

calculated for making comparisons of the nitrogen requirements of animals fed different types of diets. It is the amount of ingested nitrogen actually absorbed from the digestive tract and is calculated from the difference between daily nitrogen intake and the residual food nitrogen excreted in the faeces. To calculate residual food nitrogen in the faeces, an estimate of MFN has to be made.

No estimate of MFN could be made from the data of Experiment 3, as the nitrogen content of the faeces and food eaten expressed per unit dry matter intake were not significantly correlated ($r = +0.07$ NS). This was due to the relationship found between faecal nitrogen and nitrogen intake at nitrogen intakes in excess of 400mgN/kgW$^{0.75}$/day. However, Barker (1968) found a significant correlation between these parameters at lower nitrogen intakes and estimated the digestible nitrogen requirement of the Kangaroo Island Wallaby to be 248mgN/kgW$^{0.75}$/day. It can be seen from Table 28 that this is within the range of values obtained for the quokka and euro but considerably less than that obtained for sheep.

C. Minimum urinary nitrogen excretion

A relationship between basal energy metabolism and endogenous urinary nitrogen excretion was demonstrated by Smuts (1935), who showed that 2.0 mg of nitrogen were excreted

TABLE 28

The digestible nitrogen requirement for several mammals

Species	Digestible Nitrogen Requirement ($mgN/kgW^{0.73}/day$)	Reference
Marsupials		
Euro (*Macropus robustus*)	160	(Brown, 1964)
Quokka (*Setonix brachyurus*)	126-245	(Brown, 1964)
Tammar (*Protemnodon eugenii*)	$248 mgN/kgW^{0.75}/day$	(Barker, 1968)
Ruminants		
Sheep	360	(Harris and Mitchell, 1941a)
	495	(Moir and Williams, 1950)

in the urine per Calorie of basal metabolic energy. This relationship was confirmed by Brody (1945), who found that endogenous urinary nitrogen excretion varied with the power 0.7 of the body weight giving an average value for this relationship in a wide range of eutherian mammals of 146mgN/kgW$^{0.73}$.

Since endogenous urinary nitrogen excretion cannot be determined directly, it has been defined by Mitchell (1962) as the lowest level of urinary nitrogen attained after a certain definite period, differing with animal size, on a near nitrogen free but otherwise complete diet.

Brown (1964) measured minimum urinary nitrogen excretion in euros with low nitrogen intakes to gain an indication of the level of endogenous urinary nitrogen excretion in this species. He obtained a value of 34mgN/kgW$^{0.73}$/day. This value is considerably lower than those obtained for eutherian species (Table 29) and about one fifth of the eutherian average (Brody, 1945). Values for minimum urinary nitrogen excretion for male and female Kangaroo Island Wallabies fed low nitrogen diets have been calculated from the data of Barker (1968) and that obtained in Experiment 4. Although Kleiber (1961) has shown that body weight raised to the power 0.75, for comparative purposes, is a more accurate estimate of the metabolic body weight of mammals, all values in Table 29 have been expressed

in terms of body weight raised to the power 0.73, as they were expressed in this manner in the reports cited. It can be seen that with the exception of camels, the values calculated for minimum urinary nitrogen excretion in this wallaby, like the euro, are considerably lower than values obtained for eutherians. There also appears to be a sex difference, values obtained for male wallabies being considerably lower than those obtained for female wallabies. As a number of factors may affect the levels of minimum urinary nitrogen excretion in mammals such a comparison between macropods and eutherians must be qualified.

Animals deprived of nitrogen for extended periods have been shown to excrete levels of nitrogen in the urine which are less than endogenous levels. This phenomenon reflects a loss of nitrogen from a reduced cytoplasmic mass (Fisher, 1954). Furthermore, Ashworth (1935) has demonstrated that the time required for minimum nitrogen excretion to be achieved depends on the level of nitrogen intake prior to the determination of endogenous output. This may reflect the status of the labile nitrogen reserves in the body (Allison, 1959). In addition Brody (1945) and Mitchell (1962) have suggested that, in general, endogenous urinary nitrogen excretion is higher for growing animals than adult animals when related to metabolic weight or surface area. However, contrary to these findings, Smuts and Marais (1938) and Armstrong and Mitchell (1955) working with

TABLE 29

Minimum or endogenous urinary nitrogen excretion of some eutherian and macropod species

Species of Animal	Body Weight (kg)	Urinary Nitrogen Excretion*	Urinary Nitrogen Excretion+	Reference
Mice	0.02- 0.03	15.4	224(215-248)	Brody (1945)
Rats	0.17- 0.23	41.9	137(111-157)	"
Guinea Pigs	0.33- 0.50	75.4	140(129-154)	"
Rabbits	1.16- 2.78	268	150(116-175)	"
Goats	24.2 -62.0	1860	124(112-137)	Hutchinson and Morris (1936)
Sheep	33.0 -42.0	1245	91 (78-104)	Smuts and Marais (1938)
	31.8 -41.4	1367	96 (89-100)	Moir and Williams (1950)
	31.9 -42.0	1781	127 (79-182)	Brody (1945)
Pigs	24.0 -79.0	2031	131 109-141)	Du Toit and Smuts (1941)
Camel	250	3750	67	Schmidt-Nielsen _et al._ (1957)
Eutherian (Av)		-	146	Brody (1945)
Euro	8.5 -19.7	210	34 (21- 48)	Brown and Main (1967)
Kangaroo Island Wallaby	♀ 4.2 - 5.3	206	66 (57- 84)	This thesis
	♂ 4.0 - 6.3	112	33 (31- 38)	" "

* Mean values (mgN/day)   + Mean and range (mgN/kgW$^{0.73}$/day)

sheep and pigs respectively report no significant difference in endogenous urinary nitrogen excretion of growing or adult animals. These factors may have influenced the levels of minimum urinary nitrogen excretion in the Kangaroo Island Wallaby and euro but as pointed out by Brown and Main (1967) they cannot explain the difference observed between macropods and eutherian species. Since there appears to be some difference between the minimum urinary nitrogen excretion of ruminants and non-ruminants (Table 29), this may explain in part the difference observed between the eutherian average for minimum urinary nitrogen excretion and the values obtained for macropods, as macropods have a ruminant-like digestion.

D. Average Dry Matter Intake

The range of dry matter intakes in macropods so far studied, also appears to be lower than that recorded for eutherian herbivores. Mean dry matter intakes have been calculated from the data of Experiment 3 for female Kangaroo Island Wallabies and for males of this species from data in Experiment 1. It is clear from Table 30 that the range of dry matter intakes for the wallaby falls within the range of values obtained for the quokka and euro, being lower than that reported for eutherian herbivores. Mean dry matter intake has been related to body weight raised to the power 0.73. Although

this is not correct (Kleiber, 1961), it is the only way a comparison can be made with earlier work. Again there appears to be a difference between mean values for male and female wallabies.

Conclusions from comparative data of this kind must be treated with caution as in most cases diets differed in composition. Barker (1968) demonstrated a significant negative correlation between dry matter intake and mean daily temperature during the prefeeding period outdoors and in addition also found that nitrogen intake and dry matter intake were positively and significantly correlated. Although the former correlation was found in Experiment 3 and also by Brown and Main (1967) in the euro and Kelsall (1965) in the tammar, these workers did not find a correlation between nitrogen intake and dry matter intake agreeing with the results of Experiment 3. Again, as factors controlling food intake in macropods have not been studied, this can only be explained in terms of what is known from studies of true ruminants.

Weston (1967) reports that, in sheep fed a wheaten hay diet, the primary factor limiting dry matter intake was a deficiency of nitrogen in the diet and that, once this had been remedied, the next limiting factor was the resistance of the diet to removal from the rumen. This may explain the lack of correlation between nitrogen and dry matter intake in Experiment 3.

TABLE 30

Average dry matter intake of macropods and some eutherian herbivores (g/kgW$^{0.73}$/day)

Species	Average dry matter intake (g/kgW$^{0.73}$/day)	Diet	Reference
Kangaroo Island Wallaby	♀ 25(10-37) ♂ 37(29-44)	Oaten chaff and concentrates	Exp. 3. This thesis Exp. 1. This thesis
Euro	42(38-44)	Oaten chaff and concentrates	Brown (1964)
Quokka	39(17-55)	Sheep cubes, oaten and lucern chaff	Calaby (1958)
Deer	66	Alfalfa & grain	Bissel, Harris, Strong and James (1955)
Goats	74	-	Majumdar (1960)
Sheep	80	Forage	Crampton, Donefer and Lloyd (1960)
Sheep	51-90	Long fodders	Blaxter, Wainman and Wilson (1961)
Cattle	69	Lucern Hay and grass	Ashton (1962)
Rabbits	53	Oaten chaff and concentrates	Brown (1964)

However, in Experiment 1, these parameters were positively and significantly correlated although nitrogen intakes were within the range recorded during Experiment 3. This anomally cannot be explained.

If the difference found in mean dry matter intake between macropods and eutherian herbivores is a true difference, it may be associated with the lower standard energy metabolism of the former.

E. Creatinine Coefficient

The concept of a relationship between total creatinine chromogen excreted daily in the urine (which is nearly all ETC (Pitts, 1969)) and body weight in man was first advanced by Schaffer (1908). He found that daily excretion of total creatinine chromogen, on an individual basis, was remarkably constant and related to body weight. This relationship was further investigated by Brody (1945) who found that an exponential relationship existed between the excretion of ETC and live weight (Creatinine N(mg) = $12.7W^{0.896}$) which was true in mammals ranging in size from mice to cattle.

The mean creatinine coefficients for wallabies fed high and low nitrogen diets during Experiment 4 were 21.55 and 21.73 respectively. These values are not significantly different (t_6 = 0.58 NS) and agree closely with the values of 21.80 and

21.46 found for the Western Australian race of this species fed a high and low nitrogen diet respectively (Fraser and Kinnear, 1969).

As pointed out by Fraser and Kinnear (1969) the creatinine coefficient for the euro is significantly less than that of any mammal studied. Furthermore the creatinine coefficients of the Kangaroo Island Wallaby and tammar (the Western Australian race of P. eugenii) are also slightly lower than those reported for eutherian species (with the exception of the camel and pig) (Table 31). Since the nitrogen requirements (Brown and Main, 1967; Barker, 1968), endogenous urinary nitrogen excretion (Brown and Main, 1967; this thesis) and standard energy metabolism of macropods (Dawson and Hulbert, 1969) are lower than those of eutherians it seems that the explanation for a slightly lower creatinine coefficient in macropods may lie in the lowered nitrogen metabolism of these animals. It has been found in the rat that ETC is excreted at a rate amounting to 2% of the total creatine in the body (Bloch, Schoenheimer and Rittenberg, 1941) and in a man a rate of 1.64% has been calculated (Hoberman, Sims and Peters, 1948). Creatine, which is largely found in the muscle in relatively constant amounts in eutherians (Spector, 1956) is therefore converted to ETC at a relatively constant rate. Thus in macropods either creatine in the tissues is lower or the rate of breakdown of creatine

is slower. The latter is more likely as the standard energy metabolism of *P. eugenii* is lower than that of eutherians (Dawson and Hulbert, 1969). In addition Fraser and Kinnear (1969) have speculated the low ETC excretion by macropods may be a reflection of retention of ETC by recycling to the forestomach. However, although this may be true, from the results of the reported study it is clear that there is no renal retention of ETC occurring in a manner analogous to renal retention of urea, as ETC excretion by wallabies fed the low nitrogen diet was not significantly different from those fed the high nitrogen diet. In addition ETC clearance was not significantly different from inulin clearance (Experiment 4).

It appears that the Kangaroo Island Wallaby, like the euro, (Brown, 1964; Fraser and Kinnear, 1969), has a lower digestible nitrogen requirement (Barker, 1968), minimum urinary nitrogen excretion, average dry matter intake, and creatinine coefficient (with the exception of the pig and camel, Table 31) than eutherian mammals of similar body weight. Low values obtained for these parameters probably arise from the depressed standard energy metabolism of the wallaby (Dawson and Hulbert, 1969). As pointed out, this may contribute to the survival of the wallaby on Kangaroo Island when water and nitrogen may be in short supply and temperatures high towards the end of summer.

TABLE 31

Values of creatinine coefficients for various mammals

Species	Weight (kg)	Creatinine* Coefficient	Reference
Rat	0.068	57.0	Mitchell, Beadles and Kruger (1926, cited Mitchell, 1962)
Dog	-	28.1	Bressani and Braham (1964)
Rabbit	2.072	46.0	Brody (1945)
Pig	67.6	19.5	Smuts (1935)
Man (male)	59.9	23.4	Bleiler and Schedl (1962)
Camel	515.0	17.91	Brody (1945)
Cattle	322.0	26.05(19.17-32.28)	Brody (1945)
Cattle	317-340	30.1	Dinning, Gallup and Briggs (1949)
Sheep	50.4	23.43	Moir (unpublished data; cited Fraser and Kinnear, 1969)
Non-eutherian species			
Quokka (*Setonix brachyurus*)	3.25	25.40	Ramsay (1966)
Tammar (*Protemnodon eugenii*)	3.79	21.65	Fraser and Kinnear (1969)
Euro (*Macropus robustus*)	14.63	12.28	Fraser and Kinnear (1969)
Kangaroo Island Wallabyq (*Protemnodon eugenii*)	5.867	21.64	This thesis

* ETC (mg) excreted in the urine per kg body weight per day

V. CONCLUSION

The results of the study reported herein indicate that the Kangaroo Island Wallaby may improve its nitrogen economy during periods of nitrogen depletion in a manner analogous to ruminants. The wallaby, through the synthetic activities of the pre-gastric microbiota can utilize urea as a dietary nitrogen source and endogenous urea arising from the bloodstream may contribute to the nitrogen entering the forestomach. It is clear that the extent of renal retention of urea in the nitrogen depleted wallaby is quantitatively similar to that reported in nitrogen depleted ruminants (Schmidt-Nielsen _et al._, 1957, 1958) and is also brought about by the reabsorption of urea in the renal tubule.. Whether the extent of urea recycling to the forestomach of the wallaby is also quantitatively similar to that reported in the ruminant (Houpt, 1959; Somers, 1961a and b) remains to be demonstrated. However, the results indicate that the incorporation of recycled urea nitrogen into microbial nitrogen in the forestomach is more extensive in nitrogen depleted wallabies than in wallabies receiving an adequate nitrogen diet. Similar findings with sheep prompted Weller _et al._ (1962) to suggest that net synthesis of microbial protein may only occur when there is a critical shortage of nitrogen in the diet.

The extent to which the nitrogen status of the wallaby controls the amount of urea retained by the kidney and recycled to the forestomach is still unknown. From the small amount

of data available on the control of renal retention of urea in nitrogen depleted ruminants, it appears that this may be facilitated through an active transport system located in the region of the renal tubule situated in the medullary region of the kidney (Schmidt-Nielsen and O'Dell, 1959). The results of the reported investigation together with those of Lintern and Barker (1969) suggest that renal retention of urea in the nitrogen depleted wallaby may be facilitated in a similar manner. In the preceeding discussion suggestions have been made outlining further research which may aid in the investigation of the control of renal retention of urea in the wallaby.

Whether urea recycling to the forestomach of the wallaby is controlled by the level of nitrogen intake also remains to be demonstrated. It appears from the conflicting hypotheses which have been advanced to explain this process in the ruminants (Gartner et al., 1961; Engelhardt and Nickel, 1965; Weston and Hogan, 1967; Houpt and Houpt, 1968), that a considerable amount of research is required before the relationship between the quantity of urea recycled and nitrogen status is elucidated.

The importance of renal retention and utilization of endogenous urea in the survival of the wallaby on Kangaroo Island when there is a critical shortage of nitrogen in the diet is unknown. Phillipson (1964) has pointed out that recycled urea may have a favourable effect on the nutrition of the

ruminant in two ways. It may increase the supply of amino nitrogen passing into the abomasum through the protein synthetic activities of the ruminal microorganisms. It may also assist in maintaining an adequate concentration of bacteria and protozoa in the rumen. This in turn may enhance the digestion of cellulose and fibre in the diet by the nitrogen depleted ruminant. Recycled urea may have similar effects on the utilization of herbage by the Kangaroo Island Wallaby, thereby contributing to both the energy and nitrogen balance of the wallaby.

The additional renal retention of urea which occurs in nitrogen and water depleted wallabies does not appear to significantly improve either nitrogen retention or water balance. However, as pointed out, this facet of macropod nitrogen metabolism and water turnover requires further research, in particular the role of urea in the renal concentrating mechanism.

It is suggested that the lower digestible nitrogen requirement of the wallaby may also be of survival value by enabling these animals to persist on pastures which would not support sheep (Moir and Williams, 1950). Corresponding survival value may be gained during periods of heat and dehydration from the lower standard energy metabolism of this species (Dawson and Hulbert, 1969).

It is evident that more information is required on both

the nitrogen metabolism, water turnover and ecology of the Kangaroo Island Wallaby before the significance of a ruminant-like digestion and nitrogen metabolism in the survival of this species on Kangaroo Island can be assessed.

VI. APPENDICES

APPENDIX 1

Body weight (kg), plasma urea (mM/l), blood ammonia (mg/100ml), total urinary nitrogen (mgN/kgW$^{0.75}$/day) and urinary urea nitrogen (mgN/kgW$^{0.75}$/day), U/P urea ratios and the percentage of urea nitrogen excreted in the total urinary nitrogen of four male wallabies fed diets supplemented with urea (U) or casein (C) during Experiment 1.

A. Body Weight (kg)

Animal	Period	Diet	Week 0	Week 2	Week 3	Week 4
44	I	U	7.376	7.274	7.233	7.282
	II	C	7.045	6.984	7.093	7.060
6	I	C	5.689	5.605	5.604	5.612
	II	U	5.560	5.562	5.482	5.540
89	I	C	6.085	6.090	5.978	6.148
	II	U	5.904	5.824	5.868	5.804
3	I	U	6.080	6.050	6.045	6.000
	II	C	6.205	6.136	6.150	6.137

APPENDIX I (cont.)

B. Plasma Urea (mM/l)

Animal	Period	Diet	Week			
			0	2	3	4
44	I	U	5.7	5.8	5.8	7.7
	II	C	10.3	10.3	10.0	9.0
6	I	C	7.8	6.0	7.7	6.8
	II	U	8.8	9.3	8.0	9.0
89	I	C	7.0	7.2	7.7	6.8
	II	U	9.3	7.8	7.3	8.7
3	I	U	7.2	6.3	7.7	7.7
	II	C	8.0	7.3	7.5	7.7

APPENDIX I (cont.)

C. Blood Ammonia (mg/100ml)

Animal	Period	Diet	Week 0	2	3	4
44	I	U	0.21	0.35	0.35	0.35
	II	C	0.24	0.24	0.31	0.28
6	I	C	0.24	0.17	0.22	0.31
	II	U	0.24	0.21	0.31	0.24
89	I	C	c.17	0.28	0.38	0.21
	II	U	0.10	0.21	0.35	0.21
3	I	U	0.35	0.28	0.35	0.35
	II	C	0.41	0.24	0.10	0.24

APPENDIX I (cont.)

D. Total Urinary Nitrogen (mgN/kgW$^{0.75}$/day)

Animal	Period	Diet	Week			
			0	2	3	4
44	I	U	424	416	431	398
	II	C	372	502	321	349
6	I	C	496	298	370	343
	II	U	375	438	433	433
89	I	C	533	340	395	395
	II	U	286	377	302	311
3	I	U	286	419	391	397
	II	C	202	256	265	433

APPENDIX I (cont.)

E. Urinary Urea Nitrogen (mgN/kgW$^{0.75}$/day)

Animal	Period	Diet	Week			
			0	2	3	4
44	I	U	352	327	371	343
	II	C	304	257	257	301
6	I	C	406	223	292	296
	II	U	305	255	352	358
89	I	C	393	275	286	322
	II	U	225	274	197	247
3	I	U	170	313	290	358
	II	C	153	175	187	369

APPENDIX I (cont.)

F. U/P Urea Ratios

Animal	Period	Diet	Week 0	Week 2	Week 3	Week 4
44	I	U	78	73	80	77
	II	C	63	65	71	75
6	I	C	65	80	78	78
	II	U	73	75	79	78
89	I	C	83	81	79	84
	II	U	83	91	82	83
3	I	U	86	92	85	88
	II	C	79	101	98	91

APPENDIX I (cont.)

G. Percentage of Urea Nitrogen excreted in the Total Urinary Nitrogen

Animal	Period	Diet	Week			
			0	2	3	4
44	I	U	83	79	86	88
	II	C	82	51	80	86
6	I	C	82	75	79	87
	II	U	81	58	81	83
89	I	C	74	81	72	82
	II	U	79	73	65	80
3	I	U	59	75	74	90
	II	C	76	68	71	85

APPENDIX 2A

Daily dry matter excretion and water intake and excretion (expressed as a function of metabolic weight) of four wallabies fed diets supplemented with urea (U) and casein (C) during two nitrogen balance trials performed during Experiment 1

Period	I				II			
Treatment	U	C	C	U	C	U	U	C
Animal	44	6	89	3	44	6	89	3
Water Intake*	77.5	67.1	76.0	70.0	75.7	72.0	68.3	83.1
Urine Volume*	15.0	21.8	13.2	14.4	17.6	13.4	13.6	11.6
Faecal Water*	51.1	38.1	50.0	47.0	50.7	49.4	45.3	57.5
Dry Faecal Output°	20.8	14.2	16.0	18.1	20.3	18.4	19.7	22.6
Body Weight (mean $kgW^{0.8}$)	4.892	3.972	4.229	4.207	4.784	3.917	4.102	4.274
Body Weight (mean $kgW^{0.75}$)	4.421	3.644	3.868	3.846	4.339	3.597	3.756	3.902

* $ml/kgW^{0.8}/day$ ° $g/kgW^{0.75}/day$

APPENDIX 2B

Variations in parameters measured during the nitrogen balance trials of Periods I and II of Experiment 1 with period and treatment

Parameter	Period		Treatment	
	t_6	Sig.	t_6	Sig.
Urine Volume (ml/kgW$^{0.8}$/day)	0.87	N.S.	0.86	N.S.
Water Intake (" " ")	2.12	N.S.	0.91	N.S.
Faecal Water (" " ")	1.05	N.S.	0.21	N.S.
Dry Matter Intake (g/kgW$^{0.75}$/day)	0.38	N.S.	0.97	N.S.
Wet Faecal Output (" " ")	0.87	N.S.	0.28	N.S.
Dry Faecal Output (" " ")	1.80	N.S.	0.49	N.S.
Nitrogen Intake (mg/kgW$^{0.75}$/day)	0.65	N.S.	1.36	N.S.
Faecal Nitrogen (" " ")	0.25	N.S.	0.97	N.S.
Total Urinary Nitrogen (" ")	0.90	N.S.	1.30	N.S.
Nitrogen Balance (" " ")	0.16	N.S.	1.47	N.S.
Urinary Urea Nitrogen (" ")	1.15	N.S.	0.55	N.S.
Apparent Nitrogen Digestibility (%)	2.45	*	0.00	N.S.
Apparent Dry Matter Digestiblity (%)	1.06	N.S.	2.24	N.S.

APPENDIX 3

Nitrogen content of feed supplied to and consumed (mg/100g dry weight) by each wallaby during the nitrogen balance trials of Experiment 1. Diet C was supplemented with casein and diet U with urea.

	Nitrogen content of feed supplied (mg/100g dry weight)		Nitrogen content of feed consumed* (mg/100g dry weight)	
Animal	Period I	Period II	Period I	Period II
44	1.81 (U)	1.80 (C)	1.83	1.79
6	1.80 (C)	1.81 (U)	2.01	1.92
89	1.80 (C)	1.81 (U)	1.98	1.77
3	1.81 (U)	1.80 (C)	1.93	1.82

* Calculated from the difference between the nitrogen content of the feed presented and the nitrogen content of feed refused.

APPENDIX 4

Distribution of nitrogen (mgN/100g of stomach contents) in the forestomach of the wallaby at different times after feeding and the weight of forestomach digesta (g) at the conclusion of Experiment 2

Time killed after commencement of feeding	Animal	Plant Nitrogen	Bacterial Nitrogen	Protozoal Nitrogen	Soluble Nitrogen	Ammonia Nitrogen	Weight of forestomach digesta
3	54	26	63	4	13	4	272
5	0	16	92	6	8	3	252
8	25	14	85	15	3	2	232
12	32	11	82	14	7	3	196
24	23	9	53	7	11	4	147

APPENDIX 5A

Body weight and daily dry matter and water intake and excretion measured during the nitrogen balance trials of Experiment 3 for four female wallabies

Period	Treatment	Animal	Mean Body Weight ø	Dry Matter Intake +	Dry Faeces +	Water Intake o	Urine Volume o	Faecal Water o	Apparent Dry Matter Digestibility*
I	A	2	4.184	28.9	13.1	74.8	10.9	35.5	55
	B	16	5.266	39.5	17.9	112.0	13.8	45.8	55
	C	1	5.861	88.5	32.7	171.0	38.0	78.4	63
	D	18	4.939	60.6	26.5	142.8	21.8	76.6	56
II	A	18	4.806	90.7	47.3	223.0	25.1	144.9	48
	B	2	4.775	65.3	28.2	132.0	23.8	74.1	57
	C	16	5.086	63.2	28.1	145.0	29.5	68.8	56
	D	1	5.625	86.2	35.6	186.5	51.2	67.7	59
III	A	16	4.880	82.2	42.9	217.0	30.7	113.3	48
	B	1	5.552	103.1	42.3	205.5	39.7	93.6	59
	C	18	4.715	114.0	50.5	244.5	44.9	155.9	56
	D	2	4.147	70.0	17.1	117.5	31.2	48.3	76
IV	A	1	5.465	103.7	50.3	221.0	58.8	116.8	51
	B	18	4.613	104.5	56.5	285.0	41.7	181.3	46
	C	2	4.140	75.4	34.7	180.5	33.7	97.6	54
	D	16	4.767	87.9	38.7	281.5	131.9	95.8	56

ø kg; + g/day; o ml/day; * %

APPENDIX 5B

Daily nitrogen intake and excretion measured during the nitrogen balance trials of Experiment 3 for four female wallabies

Period	Treatment	Animal	Nitrogen Intake°	Urinary Nitrogen°	Faecal Nitrogen°	Nitrogen Balance°	Apparent Nitrogen Digestibility+	Urinary Urea Nitrogen°
I	A	2	471	308	219	- 56	54	18
	B	16	1120	566	302	+251	73	125
	C	1	2513	1465	827	+230	67	727
	D	18	2396	1296	746	+354	69	762
II	A	18	1077	514	472	+ 91	56	195
	B	2	1045	505	470	+ 70	55	200
	C	16	1722	804	768	+149	55	362
	D	1	2812	1774	830	+208	70	1136
III	A	16	1423	494	740	+189	48	174
	B	1	1756	817	780	+160	56	253
	C	18	2902	2074	568	+260	80	1430
	D	2	2693	2055	432	+206	84	1439
IV	A	1	1198	614	530	+ 54	56	265
	B	18	2184	1316	677	+192	69	790
	C	2	2257	1374	644	+239	71	891
	D	16	3253	2378	608	+268	81	1855

° mgN/day; + %

APPENDIX 5C

Plasma urea and U/P urea ratios measured during the nitrogen balance trials of Experiment 3 for four female wallabies

Period	Treatment	Animal	Plasma Urea+	U/P Urea
I	A	2	3.8	12
	B	16	6.8	47
	C	1	8.7	78
	D	18	9.5	131
II	A	18	6.0	46
	B	2	8.5	35
	C	16	8.7	51
	D	1	9.7	81
III	A	16	6.2	33
	B	1	7.5	30
	C	18	8.0	141
	D	2	8.5	192
IV	A	1	5.7	29
	B	18	7.7	88
	C	2	8.3	112
	D	16	9.7	215

\+ mM/l

APPENDIX 6

Daily dry matter excretion and water turnover measured during the nitrogen balance trials of Experiment 3 for four female wallabies expressed as a function of their metabolic weights

Period	Treatment	Animal	Dry Faeces°	Water Intake+	Urine Volume+	Faecal Water+
I	A	2	4.5	23.8	3.5	11.2
	B	16	5.1	29.7	3.7	12.1
	C	1	8.7	41.6	9.2	19.1
	D	18	8.0	39.8	6.1	21.3
II	A	18	14.6	63.5	7.1	41.3
	B	2	8.7	37.8	6.8	21.2
	C	16	8.3	39.5	8.0	18.7
	D	1	9.7	46.8	12.9	17.0
III	A	16	13.1	61.1	8.6	31.9
	B	1	11.7	52.2	10.1	23.8
	C	18	15.9	70.7	13.0	45.1
	D	2	5.9	37.7	10.0	15.5
IV	A	1	14.1	56.8	15.1	30.0
	B	18	18.0	83.9	12.3	53.4
	C	2	12.0	57.9	10.8	31.3
	D	16	11.0	80.7	37.8	27.5

° $g/kgW^{0.75}/day$ + $ml/kgW^{0.8}/day$

APPENDIX 7

Summary of analysis of variance of parameters measured during the nitrogen balance trials of Experiment 3 and presented in Appendix 6. Mean measurements are also presented.

Parameter	Treatment A	B	C	D	F	Statistical Significance
Dry faeces⁺	11.5	10.9	11.2	8.9	2.74	NS
Water Intake°	51.3	50.9	52.4	51.2	0.02	NS
Urine Volume°	8.6	8.2	10.3	16.7	1.49	NS
Faecal Water°	28.6	27.6	28.5	20.3	3.45	*
	Period I	**II**	**III**	**IV**		
Dry faeces⁺	6.6	10.3	11.6	14.0	18.85	**
Water Intake°	33.7	46.9	55.4	69.8	10.08	**
Urine Volume°	5.6	8.7	10.4	19.0	3.17	NS
Faecal Water°	15.9	24.6	29.1	35.5	14.72	**
	Animal 1	**2**	**16**	**18**		
Dry faeces⁺	11.0	7.8	9.6	14.1	18.85	**
Water Intake°	42.6	39.3	52.7	64.5	10.08	**
Urine Volume°	11.8	7.8	14.5	9.6	0.82	NS
Faecal Water°	22.5	19.8	22.5	40.3	19.24	**

⁺ $g/kgW^{0.75}/day$ ° $ml/kgW^{0.8}/day$

APPENDIX 8

Correlation coefficients of regressions between paired measurements made on four female Kangaroo Island Wallabies during the nitrogen balance trials of Experiment 3.

	Dry Faeces	Water Intake	Urine Volume	Faecal Water	Nitrogen Intake	Urinary Nitrogen	Faecal Nitrogen	Nitrogen Balance
Dry Matter Intake	0.84 ***	0.80 ***	0.43 NS	0.77 ***	0.47 NS	0.42 NS	0.45 NS	0.25 NS
Dry Faeces		0.86 ***	0.34 NS	0.89 ***	0.21 NS	0.14 NS	0.39 NS	0.12 NS
Water Intake			0.61 *	0.82 ***	0.44 NS	0.39 NS	0.40 NS	0.29 NS
Urine Volume				0.22 NS	0.56 *	0.58 *	0.22 NS	0.27 NS
Faecal Water					0.21 NS	0.17 NS	0.28 NS	0.16 NS
Nitrogen Intake						0.91 ***	0.49 NS	0.73 **
Urinary Nitrogen							0.30 NS	0.62 *
Faecal Nitrogen								0.49 NS

APPENDIX 9

Regression equations describing the relationships between significantly correlated parameters (Appendix 8) measured during Experiment 3

1. Dry matter intake and dry faecal output

$$Y = 0.5X - 1.3$$

Y = dry faecal output ($g/kgW^{0.75}/day$)
X = dry matter intake ($g/kgW^{0.75}/day$)

2. Dry matter intake and water intake

$$Y = 2.2X + 0.1$$

Y = water intake ($ml/kgW^{0.8}/day$)
X = dry matter intake ($g/kgW^{0.75}/day$)

3. Dry matter intake and faecal water output

$$Y = 1.4X - 7.7$$

Y = faecal water output ($ml/kgW^{0.8}/day$)
X = dry matter intake ($g/kgW^{0.75}/day$)

4. Dry faecal output and water intake

$$Y = 4.1X + 7.9$$

Y = water intake ($ml/kgW^{0.8}/day$)
X = dry faecal output ($g/kgW^{0.75}/day$)

5. Dry faecal output and faecal water output

$$Y = 2.9X - 4.8$$

Y = faecal water output ($ml/kgW^{0.8}/day$)
X = dry faecal output ($g/kgW^{0.75}/day$)

6. Water intake and urine volume

$$Y = 0.3X - 4.1$$

Y = urine volume ($ml/kgW^{0.8}/day$)
X = water intake ($ml/kgW^{0.8}/day$)

7. Water intake and faecal water output

$$Y = 0.6X - 4.6$$

Y = faecal water output ($ml/kgW^{0.8}/day$)
X = water intake ($ml/kgW^{0.8}/day$)

8. Urine volume and nitrogen intake

$$Y = 2.1X + 378.1$$

Y = nitrogen intake ($mgN/kgW^{0.75}/day$)
X = urine volume ($ml/kgW^{0.8}/day$)

9. Urine volume and total urinary nitrogen

$$Y = 16.8X + 167.8$$

Y = total urinary nitrogen ($mgN/kgW^{0.75}/day$)
X = urine volume ($ml/kgW^{0.8}/day$)

10. Nitrogen intake and total urinary nitrogen

$$Y = 0.8X + 118.7$$

Y = total urinary nitrogen ($mgN/kgW^{0.75}/day$)
X = nitrogen intake ($mgN/kgW^{0.75}/day$)

11. Nitrogen intake and nitrogen balance

$$Y = 0.09X - 1.2$$

Y = nitrogen balance ($mgN/kgW^{0.75}/day$)
X = nitrogen intake ($mgN/kgW^{0.75}/day$)

12. Total urinary nitrogen and nitrogen balance

$$Y = 0.1X + 18.7$$

Y = nitrogen balance (mgN/kgW$^{0.75}$/day)
X = total urinary nitrogen (mgN/kgW$^{0.75}$ / day)

APPENDIX 10

Urinary urea nitrogen estimated from the urine samples collected during periods 2-5 by Barker (1968) in a series of nitrogen balance trials performed on the four female wallabies used in Experiment 3.

Period	Treatment*	Animal	Urinary urea Nitrogen+
2	A	2	3
	B	18	5
	D	16	18
	E	1	59
3	A	16	3
	C	18	10
	D	1	24
	E	2	75
4	B	1	11
	C	2	16
	D	18	39
	E	16	94
5	A	18	2
	B	16	9
	C	1	17
	D	2	45

+ $mgN/kgW^{0.75}/day$

* Nitrogen content of diets
A = 0.30%; B = 0.53%; C = 0.65%; D = 0.83%; E = 1.33%

APPENDIX 11

A comparison of Endogenous True Creatinine (ETC) and Inulin clearance during two thirty minute clearance periods in four anaesthetised, water-loaded Kangaroo Island wallabies which had been fed a maintenance diet (1.5gN/100g dry weight) during Experiment 4.

Animal	Clearance Period	Plasma Inulin (mg/100ml)	Plasma ETC (mg/100ml)	Urinary Inulin (mg/100ml)	Urinary ETC (mg/100ml)	Inulin U/P	ETC U/P	ETC U/P / Inulin U/P
392r	1	10.1	0.81	42.58	3.41	4.22	4.22	1.000
	2	10.1	0.81	42.58	3.41	4.22	4.22	1.000
394r	1	9.7	1.19	34.40	4.24	3.55	3.57	1.006
	2	9.9	1.19	32.10	4.02	3.24	3.38	1.043
10	1	9.6	0.78	39.30	3.18	4.09	4.06	0.993
	2	9.2	0.81	37.68	3.33	4.10	4.12	1.005
56	1	8.5	0.90	32.75	3.41	3.85	3.78	0.982
	2	8.3	0.81	31.48	2.97	3.79	3.68	0.971

$\overline{X} = 1.00$

APPENDIX 12A

Six hourly ETC (mg/100ml) and urea (mM/l) concentrations in the plasma of four male wallabies measured over a thirty six hour period during Experiment 4. The wallabies were allowed food (1.5gN/100g dry weight) and water *ad lib.* during the sampling period

Animal	392r		394r		10		56	
	ETC	Urea	ETC	Urea	ETC	Urea	ETC	Urea
Time								
11 a.m.	1.24	8.2	1.43	9.0	1.05	8.8	1.14	6.2
5 p.m.	1.24	7.9	1.36	8.6	1.05	8.1	1.14	5.9
11 p.m.	1.24	8.3	1.33	9.1	1.09	8.9	1.16	6.1
5 a.m.	1.24	8.0	1.36	8.7	1.05	8.0	1.16	6.0
11 a.m.	1.43	8.3	1.33	9.1	1.05	8.3	1.24	6.5
5 p.m.	1.24	7.8	1.38	8.6	1.05	7.9	1.24	5.8
11 p.m.	1.24	8.2	1.33	9.3	1.05	8.6	1.16	6.5

APPENDIX 12B

Six hourly ETC (mg/100ml) and urea (mM/1) concentrations in the plasma of four male wallabies measured over a thirty six hour period during Experiment 4. The wallabies were deprived of food and water during, and eight hours prior to, the collection period.

Animal	392r		394r		10		56	
	ETC	Urea	ETC	Urea	ETC	Urea	ETC	Urea
Time								
11 a.m.	1.22	7.9	1.37	8.7	1.08	8.4	1.12	5.8
5 p.m.	1.22	7.8	1.37	8.7	1.08	8.4	1.12	5.8
11 p.m.	1.22	7.9	1.37	8.7	1.11	8.4	1.12	5.8
5 a.m.	1.22	7.9	1.37	8.7	1.09	8.3	1.12	5.8
11 p.m.	1.22	7.9	1.37	8.7	1.09	8.3	1.12	5.8
5 p.m.	1.22	7.9	1.37	8.7	1.09	8.3	1.08	5.8
11 p.m.	1.22	7.9	1.37	8.7	1.09	8.3	1.09	5.8

APPENDIX 13A

Daily values for parameters measured on three alternate days during period I of Experiment 4 for the two male wallabies fed a low nitrogen diet.

Animal	392r			394r		
Day	1	2	3	1	2	3
Body Weight+	4.012	4.014	4.013	6.060	6.063	6.061
Urine Volume°	80	65	72	59	59	60
Plasma Urea*	2.1	2.0	2.0	1.9	1.8	1.8
Plasma ETCø	1.2	1.2	1.2	1.3	1.3	1.3
Urinary Urea≠	14	14	13	18	18	17
Urinary ETC≠	84.8	90.6	91.6	126.9	128.7	130.9
U/P Urea	1	2	2	3	3	3
Urinary Nitrogen≠	86	87	86	101	101	100
GFR°	7126	7306	7697	9837	9977	10147
Renal Retention of Urea≠	884	863	910	1104	1090	1079
% Filtered Urea Excreted	1.6	1.6	1.5	1.6	1.6	1.5
Urea Nitrogen % Urinary Nitrogen	8	7	8	8	8	8
Creatinine Coefficient●	21.14	22.57	22.83	20.94	21.23	21.60

+ kg; ° ml/day; * mM/l; ø mg/100ml; ≠ mg/day; ● ETC excreted in the urine per kg body weight per day

APPENDIX 13B

Daily values for parameters measured on three alternate days during Period I of Experiment 4 for two male wallabies fed a high nitrogen diet.

Animal	10			56		
Day	1	2	3	1	2	3
Body Weight[+]	7.159	7.158	7.157	6.456	6.455	6.457
Urine Volume[o]	76	91	99	64	58	68
Plasma Urea[*]	8.7	8.2	8.4	7.0	7.1	7.3
Plasma ETC[ø]	1.0	1.0	1.0	1.0	1.0	1.0
Urinary Urea[≠]	6 350	6197	6534	4992	5046	5304
Urinary ETC[≠]	152.0	154.4	153.0	135.7	140.6	140.1
U/P Urea	160	138	131	186	204	178
Urinary Nitrogen[≠]	3352	3293	3423	2648	2854	3189
GFR[o]	15200	15440	15300	13570	13921	14010
Renal Retention of Urea[≠]	1584	1400	1101	765	884	872
% Filtered Urea Excreted	80.0	81.6	88.6	86.7	85.1	85.9
Urea Nitrogen % Urinary Nitrogen	89	88	90	89	83	78
Creatinine Coefficient[⊕]	21.23	21.57	21.38	21.02	21.78	21.70

[+] kg; [o] ml/day; [*] mM/l; [ø] mg/100ml; [≠] mg/day; [⊕] ETC excreted in the urine per kg body weight per day

APPENDIX 13C

Daily values for parameters measured on three alternate days during Period II of Experiment 4 for two male wallabies fed a high nitrogen diet

Animal	392r			394r		
Day	1	2	3	1	2	3
Body Weight[+]	4.724	4.726	4.722	6.602	6.601	6.603
Urine Volume[o]	51	78	60	60	67	99
Plasma Urea*	7.5	7.5	7.6	9.2	9.0	9.3
Plasma ETC[ø]	1.4	1.4	1.4	1.4	1.4	1.4
Urinary Urea[≠]	2616	2504	2610	4788	4623	4871
Urinary ETC[≠]	102.0	108.7	103.6	141.8	142.1	138.0
U/P Urea	114	71	95	145	128	88
Urinary Nitrogen[≠]	1653	1594	1667	2940	2781	2834
GFR[o]	7338	7709	7507	10057	10078	10073
Renal Retention of Urea[≠]	686	965	813	764	819	750
% Filtered Urea Excreted	79.2	72.2	76.2	86.2	84.9	86.7
Urea Nitrogen % Urinary Nitrogen	74	74	74	77	78	81
Creatinine Coefficient[⊕]	21.59	23.00	21.94	21.48	21.53	20.90

[+] kg; [o] ml/day; * mM/l; [ø] mg/100ml; [≠] mg/day; [⊕] ETC excreted in the urine per kg body weight per day

APPENDIX 13D

Daily values for parameters measured on three alternate days during Period II of Experiment 4 for two male wallabies fed a low nitrogen diet

Animal	10			56		
Day	1	2	3	1	2	3
Body Weight^{+}	6.308	6.308	6.308	5.610	5.611	5.609
Urine Volume^{o}	129	120	100	50	70	40
Plasma Urea*	1.5	1.6	1.0	1.7	1.7	1.8
Plasma ETC$^{\emptyset}$	0.9	0.9	0.9	0.9	0.9	0.9
Urinary Urea$^{\neq}$	27	29	27	18	17	15
Urinary ETC$^{\neq}$	136.8	138.7	133.2	122.7	123.0	123.8
U/P Urea	2	3	3	4	3	4
Urinary Nitrogen$^{\neq}$	142	145	147	122	119	113
GFR^{o}	15371	15242	14966	13787	13820	13910
Renal Retention of Urea$^{\neq}$	1356	1434	1320	1389	1393	1487
% Filtered Urea Excreted	2.0	2.0	2.0	1.3	1.2	1.0
Urea Nitrogen % Urinary Nitrogen	9	9	9	7	7	6
Creatinine Coefficient$^{\bullet}$	21.69	21.99	21.12	21.87	21.92	22.07

$^{+}$ kg; ^{o} ml/day; * mM/l; $^{\emptyset}$ mg/100ml; $^{\neq}$ mg/day; $^{\bullet}$ ETC excreted in the urine per kg body weight per day

APPENDIX 14A

A comparison of ETC and Inulin clearance during two thirty minute clearance periods in four anaesthetised water-loaded Kangaroo Island wallabies during Experiment 4. Animals 392r and 394r were fed a high nitrogen diet and animals 10 and 56 a low nitrogen diet. Clearance was measured immediately after Period II.

Animal	Diet	Clearance Period	Plasma Inulin (mg/100ml)	Plasma ETC (mg/100ml)	Urinary Inulin (mg/100ml)	Urinary ETC (mg/100ml)	Inulin U/P	ETC U/P	ETC U/P / Inulin U/P
392r	High N	1	10.9	0.99	45.03	4.14	4.13	4.18	1.012
		2	10.9	0.81	44.25	3.26	4.06	4.02	0.990
394r		1	10.1	1.01	31.93	3.13	3.16	3.10	0.981
		2	10.1	1.00	31.93	3.15	3.16	3.15	0.997
10	Low N	1	9.7	0.69	42.58	3.06	4.39	4.43	1.009
		2	9.8	0.71	44.23	3.15	4.51	4.44	0.984
56		1	8.3	0.70	33.58	2.82	4.05	4.03	0.995
		2	8.3	0.70	34.40	2.89	4.14	4.13	0.998

$\bar{X} = 0.996$

$t_6 = 0.35$ NS

APPENDIX 14B

A comparison of ETC and Inulin clearance during two thirty minute clearance periods in four anaesthetised water-loaded Kangaroo Island wallabies during Experiment 4. Animals 392r and 394r were fed a low nitrogen diet and animals 10 and 56 a high nitrogen diet. Clearance was measured immediately after Period I.

Animal	Diet	Clearance Period	Plasma Inulin (mg/100ml)	Plasma ETC (mg/100ml)	Urinary Inulin (mg/100ml)	Urinary ETC (mg/100ml)	Inulin U/P	ETC U/P	ETC U/P / Inulin U/P
392r	Low N	1	9.9	1.01	52.40	5.25	5.29	5.25	0.993
		2	9.9	1.00	49.95	5.03	5.05	5.03	0.996
394r		1	10.1	1.10	36.85	3.64	3.65	3.64	0.997
		2	10.1	1.10	36.03	3.58	3.57	3.58	1.003
10	High N	1	9.2	0.82	39.30	4.21	4.27	4.21	0.986
		2	9.2	0.73	39.30	4.07	4.27	4.07	0.953
56		1	8.6	0.82	32.75	3.70	3.81	3.70	0.971
		2	8.6	0.82	33.58	3.88	3.90	3.88	0.995

$\bar{X} = 0.987$

$t_6 = 0.94$ NS

APPENDIX 15

Body weight (kg) during the prefeeding period and the experimental period of Experiment 5 when an intravenous injection of urea was given to eight wallabies fed a low or high nitrogen diet. On day 23 the wallabies were removed to metabolism cages and on day 31 they were injected with urea.

Diet	High Nitrogen				Low Nitrogen			
	39	7	1	4	39r	392r	89	394r
Day								
1	5.578	6.535	7.549	5.979	6.908	4.899	7.334	7.237
14	5.441	6.510	7.414	5.864	6.428	4.724	6.822	7.003
23	5.462	6.544	7.544	5.905	6.145	4.529	6.544	6.857
28	5.430	6.367	7.280	5.711	5.984	4.393	6.398	6.664
29	5.485	6.293	7.268	5.648	6.003	4.482	6.208	6.530
31	Urea Injection (700mg/animal)							
33	5.457	6.214	7.116	5.591	5.798	4.334	6.149	6.397
36	5.484	6.329	7.004	5.570	5.683	4.228	6.003	6.312

APPENDIX 16

Urinary urea (mg/day) during the prefeeding period and the experimental period of Experiment 5 when an intravenous injection of urea was given to eight wallabies fed a high or low nitrogen diet. The wallabies were removed to metabolism cages on day 23 and on day 31 they were injected with urea.

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
1	2520	2528	3843	2574	2179	3084	2873	1455
23	3352	3912	5166	3557	554	78	86	31
29	3300	3461	4559	3398	296	31	29	11
30	3366	3440	4577	3379	263	25	29	18
31	3341	3469	4583	3393	222	27	26	14
	Urea Injection (700mg/animal)							
32	3797	3904	4983	3808	368	69	14	26
33	3263	3354	4410	3312	342	11	15	15
34	3226	3472	4309	3180	166	9	21	13
35	3150	3306	4366	3216	159	8	15	11
36	3296	2976	4231	3203	155	10	15	12

APPENDIX 17

Total urinary nitrogen (mg/day) during the prefeeding period and the experimental period of Experiment 5 when an intravenous injection of urea was given to eight wallabies fed a high or low nitrogen diet. On day 23 the wallabies were removed to metabolism cages and on day 31 they were injected with urea

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
1	1932	1825	2528	1925	2052	2096	1870	1262
23	1968	2263	2881	2139	499	153	151	235
29	2184	2054	2647	1850	255	151	176	233
30	1987	2044	2691	1952	491	167	153	168
31	2007	2058	2633	2020	347	178	189	165
	Urea Injection (700mg/animal)							
32	2215	2145	2832	2213	425	192	168	175
33	1953	2071	2614	2027	394	86	176	174
34	2070	2094	2560	1974	252	109	220	170
35	2030	2054	2596	1960	260	103	180	218
36	2038	1702	2577	1958	254	116	182	219

APPENDIX 18

Plasma urea (mM/l) measured during the prefeeding period and experimental period of Experiment 5 when an intravenous injection of urea was given to eight wallabies fed a low or high nitrogen diet. On day 23 the wallabies were removed to metabolism cages and on day 31 they were injected with urea.

Diet	High Nitrogen				Low Nitrogen			
Animal	39	7	1	4	39r	392r	89	394r
Day								
1	5.7	7.4	7.4	6.5	7.6	5.9	8.0	8.5
23	7.3	8.2	8.0	7.3	7.6	3.6	1.9	2.3
28	6.5	7.6	7.7	7.1	6.4	1.3	1.4	1.3
29	6.4	7.2	8.0	7.0	5.8	1.4	1.5	1.4
30	6.5	7.3	7.5	7.0	6.3	1.3	1.4	1.3
31	6.4	7.6	7.3	6.4	6.3	2.5	1.5	1.5
Urea Injection (700mg/animal)								
31+10min	10.4	10.8	10.7	10.4	12.0	7.0	7.5	8.1
31+30min	9.2	9.4	9.5	9.5	10.9	5.6	5.8	6.4
31+270min	7.2	8.3	8.0	7.4	10.2	5.7	5.2	5.4
32	6.6	7.1	7.7	6.2	8.6	4.4	5.3	5.2
33	6.2	7.1	7.5	6.6	8.4	3.7	4.1	4.6
34	6.2	7.1	7.5	6.6	8.4	3.5	3.8	4.4
35	6.5	7.1	7.6	6.4	6.5	3.6	3.2	3.1
36	6.5	7.2	7.7	6.5	6.6	3.4	2.6	2.5

APPENDIX 19

A.

Body Weight (kg) during the prefeeding and experimental periods of Experiment 6 for five wallabies, two fed a high nitrogen diet and three a low nitrogen diet.

Animal	Diet	Day 0	12	18	32	37	42
39	Low Nitrogen	5.965	5.454	5.367	5.259	5.197	5.148
1	Low Nitrogen	7.866	7.381	7.366	6.941	6.792	6.711
7	Low Nitrogen	6.316	5.911	5.723	5.402	5.316	5.232
392r	High Nitrogen	5.159	5.139	5.013	5.114	5.186	5.233
4	High Nitrogen	6.053	6.033	5.979	5.889	5.861	5.908

APPENDIX 19 (cont.)

B. Urinary urea (mg/day) during the prefeeding period of Experiment 6 for five wallabies, two fed a high nitrogen diet and three a low nitrogen diet.

Animal	Diet	Day			
		0	18	32	37
39	Low Nitrogen	3672	103	45	37
7	Low Nitrogen	2510	83	41	35
1	Low Nitrogen	2194	47	23	20
392r	High Nitrogen	1536	2518	3071	3192
4	High Nitrogen	2087	2400	2646	2785

C. Plasma urea (mM/l) during the prefeeding period of Experiment 6 for five wallabies, two fed a high nitrogen diet and three a low nitrogen diet.

Animal	Diet	Day			
		0	18	32	37
39	Low Nitrogen	5.9	2.5	1.2	0.6
7	Low Nitrogen	5.6	2.3	1.5	0.9
1	Low Nitrogen	6.2	2.2	1.9	2.3
392r	High Nitrogen	5.1	6.1	6.5	6.6
4	High Nitrogen	5.4	6.3	6.7	6.3

APPENDIX 19 (cont.)

D. Total Urinary Nitrogen (mg/day) during the prefeeding period of Experiment 6 for five wallabies, two fed a high nitrogen diet and three a low nitrogen diet.

Animal	Diet	Day			
		0	18	32	37
39	Low Nitrogen	2152	213	110	143
7	Low Nitrogen	1573	193	105	96
1	Low Nitrogen	1430	129	157	124
392r	High Nitrogen	1098	1671	1807	1831
4	High Nitrogen	1404	1504	1676	1656

APPENDIX 20

Estimation of injected urea excreted in the urine for five wallabies after an intravenous injection of N^{15} labelled urea (14.643 atom % excess N^{15}) during the three hour experimental period of Experiment 6

Animal	Diet	Urinary Urea Nitrogen (mg/3 hours)	Atom % excess N^{15}	N^{15} in urinary urea nitrogen (mg/3 hours)	Injected Urea nitrogen excreted in the urine (mg/3 hours)	Injected urea excreted in the urine* (mg/3 hours)
39	Low Nitrogen	3.2	3.438	0.111	0.75	1.6
7		2.7	5.360	0.145	0.99	2.1
1		2.7	5.111	0.138	0.94	2.0
392r	High Nitrogen	302.3	5.124	15.490	105.8	213.5
4		335.0	5.058	16.887	115.2	245.0

$$\text{* Injected urea excreted in the urine (mg/3 hours)} = \frac{\text{Urinary urea nitrogen (mg/3 hours)} \times \text{atom \% excess } N^{15}}{0.14643 \times 0.47}$$

APPENDIX 21

Total nitrogen (mg), atom % excess N^{15} and total N^{15} (mg) in the forestomach and associated bacterial protozoal and soluble fractions of five wallabies, two of which were fed a high nitrogen diet and three a low nitrogen diet during Experiment 6

Animal	Diet	Forestomach Digesta			Bacterial Fraction			Protozoal Fraction			Soluble Fraction		
		TN*	%°	N^{15+}	TN*	%°	N^{15+}	TN*	%°	N^{15+}	TN*	%°	N^{15+}
39	Low Nitrogen	710	0.600	4.260	346	0.701	2.429	39	0.101	0.039	75	2.403	1.802
7	Low Nitrogen	819	0.524	4.292	365	0.528	1.929	60	0.147	0.088	98	2.322	2.276
1	Low Nitrogen	867	0.465	4.032	375	0.409	1.535	92	0.100	0.092	132	1.827	2.412
392r	High Nitrogen	1223	0.090	1.101	583	0.024	0.129	183	0.015	0.028	169	0.557	0.941
4	High Nitrogen	1944	0.066	1.283	1075	0.020	0.215	213	0.035	0.025	210	0.472	0.991

* Total nitrogen (mg) in particular fraction of digesta

° Atom percentage excess N^{15} in nitrogen of particular fraction of digesta

\+ N^{15} (mg) in particular fraction of digesta

APPENDIX 22

Sperber (1944) made a comprehensive study of the mammalian kidney (both marsupial and eutherian) and concluded that the kidneys of mammals found in arid habitats generally have thicker renal medullas than those of moist environments. Schmidt-Nielsen and O'Dell (1961) subsequently demonstrated in a number of mammals from different habitats a positive correlation between renal medullary thickness and the ability to concentrate electrolytes in the urine .

In order to gain an indication of the renal concentrating ability in the Kangaroo Island Wallaby kidney size and renal medullary thickness was measured in 12 male and 5 female wallabies. The data was collected during the years 1966-1969. Whenever a healthy animal was killed for experimental purposes measurements were made on fresh kidneys using vernier callipers. The kidney size was determined as the cube root of the product of the dimensions of the kidney in millimetres. The relative thickness of the cortex and the medulla is defined as the ratio cortex or medullary thickness (mm) x 10/ kidney size after Sperber (1944). Values for these parameters are presented in Table A.

APPENDIX 22

TABLE A. The relative dimensions of kidneys of four species of macropod

Species		Kidney Size+ (mm)	Thickness of		Relative thickness of≠		References
			cortex (mm)	medulla (mm)	medulla	cortex + medulla	
Euro		29(27-32)	5.6(5.0-6.8)	21.1(19.0-24.0)	7.2(7.0-7.5)	9.1(8.7-9.6)	Brown (1964)
Red Kangaroo		43(42-43)	7.6	27.9(27.4-28.4)	6.6(6.5-6.6)	8.4(8.3-8.4)	Brown (1964)
Tammar		22(21-22)	3.4(2.8-4.0)	15.3(15.0-15.5)	7.1(7.0-7.1)	8.7(8.5-8.9)	Brown (1964)
G.I.* Tammar		-	-	-	5.9(5.0-6.8)	-	Kinnear, Purohit & Main (1968)
A° Tammar		-	-	-	6.8(6.4-7.9)	-	
Kangaroo Island Wallabyø	♀	23(22-24)	1.8(1.7-2.1)	18.7(18.5-14.1)	8.2(7.8-8.5)	9.0(8.6-9.3)	This Thesis & Lintern (1966)
	♂	23(22-25)	2.3(1.5-4.7)	18.7(15.4-21.6)	8.1(7.3-8.8)	8.9(8.2-9.6)	
Quokka		26	4.7	14.1(13.3-15.0)	5.5(5.1-5.8)	7.3(6.9-7.6)	Brown (1964)

* Tammar wallabies (*Protemnodon eugenii*) obtained from Garden Island, Western Australia

° Tammar wallabies (*Protemnodon eugenii*) obtained from Abrolhos Islands, Western Australia

ø South Australian race of the Western Australian tammar wallaby *Protemnodon eugenii*

\+ Kidney size = cube root of the product of the dimensions

≠ Relative thickness of medulla = medulla (mm) x 10/ kidney size

Sperber (1944) points out that relative medullary thickness varies with kidney size, smaller kidneys having a relatively thicker renal medulla. From Table A it is clear that kidney size in the macropods listed ranged from 21 to 43 mm. Sperber (1944) found that the maximum relative medullary thickness for species from arid habitats and with kidneys within this size range was 6.0. Thus the greater relative medullary thickness of the kidneys of the euro, tammar, red kangaroo and Kangaroo Island Wallaby suggests that these macropods may be adapted to a certain degree of dehydration.

It has been demonstrated that the relative medullary thickness of the kidneys of quokkas, euros and tammars correlates well with observed maximum urine electrolyte concentrations (Bentley, 1955; Ealey, 1962; Kinnear, Purohit and Main, 1968). Although the ability of the Kangaroo Island Wallaby to concentrate electrolytes and urea in the urine has not been studied, the large values obtained for the relative thickness of the renal medulla of this species suggests this is within the range of euros and tammars. Kinnear, Purohit and Main (1968) have shown that the tammar can concentrate electrolytes in the urine to a greater extent than the Kangaroo Rat <u>Dipodomys merriami</u> (Schmidt-Nielsen and Schmidt-Nielsen, 1950; Schmidt-Nielsen, 1964; Carpenter, 1966) which is noted for its renal concentrating ability. From Table B it is clear that the

relative renal medullary thickness of macropods tends to be greater than eutherians with similar kidney size. The data of Bentley (1955), Ealey (1962) and Kinnear *et al.* (1968) suggests that this is a reflection of a greater ability to concentrate electrolytes in the urine. Thus as the Kangaroo Island Wallaby has the greatest relative renal medullary thickness of all the macropods studied so far, this wallaby may also be able to produce urine as concentrated as *Dipodomys merriami.* This would be of considerable advantage to this species in the field when water may not be readily available during the summer months.

APPENDIX 22

TABLE B. Kidney size and relative renal medullary thickness in a number of eutherians and marsupials

Animal	Kidney Size (mm)	Relative renal medullary thickness	Reference
Eutherian			
Beaver	36	1.3	Schmidt-Nielsen and O'Dell (1961)
Pig	66	1.6	
Man	64	3.0	"
Dog	40	4.3	"
Cat	24	4.8	"
Rat	14	5.8	"
Kangaroo rat	5.9	8.5	"
Jerboa	4.5	9.3	"
Psammomys	13	10.7	"
Marsupial			
Quokka	26	5.5	Brown (1964)
Red Kangaroo	43	6.6	"
Tammar	22	7.1	"
Garden Island Tammar	-	5.9	Kinnear *et al.* (1968)
Abrolhos Island Tammar	-	6.8	"
Kangaroo Island Wallaby	23	8.2	This thesis
Euro	29	7.2	Brown (1964)

APPENDIX 23A

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratio, and body weight for each wallaby during collection 1 of Experiment 7 part 1.

Treatment	High Nitrogen; Water *ad lib.*									
Animal	27	3	42	4	28	46	47	39	33	44
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	302	194	229	252	358	290	351	218	166	257
Urinary urea nitrogen ($mg/kgW^{0.75}/day$)	229	136	157	193	361	215	242	244	122	212
Plasma urea (mM/l)	7.0	5.5	6.0	8.1	7.2	7.0	10.7	7.3	7.1	8.2
U/P urea ratio	75	86	80	72	78	39	45	63	39	57
Body Weight (kg)	7.243	6.376	5.212	6.625	5.341	5.541	5.269	4.186	6.652	7.593

APPENDIX 23B

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratio and body weight for each wallaby during collection 2 of Experiment 7 part 1.

Treatment	Low Nitrogen Water *ad lib.*		Low Nitrogen Water Restricted			High Nitrogen Water Restricted		High Nitrogen Water *ad lib.*		
Group	1		2			3		4		
Animal	27	3	42	4	28	46	47	39	33	44
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	206	188	106	87	94	261	354	176	296	319
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	21	93	29	15	22	157	210	56	290	200
Plasma urea (mM/l)	3.4	4.7	6.5	7.8	5.9	7.9	6.3	9.5	9.0	6.1
U/P urea ratio	5	49	12	5	11	45	35	12	44	68
Body Weight (kg)	6.657	5.876	4.493	6.040	4.719	5.298	4.660	4.138	6.524	7.642

APPENDIX 23C

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 3 of Experiment 7 part 1.

Treatment	Low Nitrogen Water *ad lib.*		Low Nitrogen Water Restricted			High Nitrogen Water Restricted		High Nitrogen Water *ad lib.*		
Group	1		2			3		4		
Animal	27	3	42	4	28	46	47	39	33	44
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	152	119	86	75	150	222	304	277	295	253
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	5	11	9	9	64	129	199	110	196	161
Plasma urea (mM/l)	1.5	1.0	3.1	7.0	9.6	6.1	8.7	10.8	8.5	7.3
U/P urea ratio	4	17	6	3	11	43	40	18	37	56
Body Weight (kg)	6.598	5.781	4.323	5.870	4.614	5.411	4.554	4.271	6.593	7.772

APPENDIX 23D

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 4 of Experiment 7 part 1

Treatment	Low Nitrogen Water *ad lib.*		Low Nitrogen Water Restricted			High Nitrogen Water Restricted		High Nitrogen Water *ad lib.*		
Group	1		2			3		4		
Animal	27	3	42	4	28	46	47	39	33	44
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	90	58	50	36	40	177	274	169	193	274
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	7	6	2	2	2	89	94	103	133	205
Plasma urea (mM/l)	1.2	0.8	3.2	2.3	2.9	9.0	10.3	8.2	8.1	9.0
U/P urea ratio	5	11	1	2	1	29	22	33	41	57
Body Weight (kg)	6.054	5.274	3.963	5.675	4.323	5.265	4.450	4.220	6.683	7.775

APPENDIX 23E

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 5 of Experiment 7 part 1

Treatment	Low Nitrogen Water *ad lib.*		Low Nitrogen Water Restricted			High Nitrogen Water Restricted		High Nitrogen Water *ad lib.*		
Group	1		2			3		4		
Animal	27	3	42	4	28	46	47	39	33	44
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	121	40	37	37	30	188	206	194	328	288
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	27	9	2	2	2	119	88	64	160	200
Plasma urea (mM/l)	1.2	0.6	3.6	2.5	1.1	7.2	8.3	7.9	6.9	5.7
U/P urea ratio	10	24	1	1	3	40	24	19	34	68
Body Weight (kg)	5.571	5.007	4.170	5.652	4.228	5.474	4.680	4.240	6.733	7.907

APPENDIX 24A

Daily food and water intake and excretion and apparent dry matter digestibility of four groups of wallabies related to their metabolic weight measured over a ten day period during Experiment 7 part 1.

Animal	Treatment	Body Weight ($kgW^{0.75}$)	Dry Matter Intake^{o}	Dry Faeces^{o}	Apparent Dry Matter Digestibility*	Urine Volume^{+}	Faecal Water^{+}	Water Intake^{+}
27	Low Nitrogen	3.847	31.4	16.5	47	47.1	36.1	95.3
3	Water _ad_ _lib._	3.516	27.7	13.1	53	18.6	24.5	51.4
42	Low Nitrogen	2.831	34.8	16.3	53	17.4	25.6	52.7
4	Water Restricted	3.711	28.5	12.7	55	18.1	18.0	39.5
28		3.007	31.0	13.7	56	17.7	21.2	49.4
46	High Nitrogen	3.493	36.1	16.0	57	11.5	26.6	42.1
47	Water Restricted	3.054	38.6	16.1	58	14.4	27.3	48.6
39	High Nitrogen	2.948	46.5	23.0	50	13.6	56.8	82.8
33	Water _ad_ _lib._	4.148	46.3	23.3	50	15.1	57.6	85.4
44		4.656	42.0	19.3	54	13.9	46.9	70.1

^{o} $g/kgW^{0.75}/day$; $^{+}$ $ml/kgW^{0.8}/day$; * %

APPENDIX 24B

Daily nitrogen intake and excretion of four groups of wallabies related to their metabolic weight measured over a ten day period during Experiment 7 part 1

Animal	Treatment	Nitrogen Intake+	Urinary Nitrogen+	Faecal Nitrogen+	Nitrogen Balance+
27	Low Nitrogen	70	61	155	-146
3	Water *ad lib.*	54	39	140	-125
42	Low Nitrogen	74	45	174	-143
4	Water Restricted	65	38	123	- 96
28		84	33	164	-113
46	High Nitrogen	405	183	222	0
47	Water Restricted	534	264	197	+ 73
39	High Nitrogen	411	161	212	+ 38
33	Water *ad lib.*	601	266	275	+ 60
44		560	277	268	+ 15

+ $mgN/kgW^{0.75}/day$

APPENDIX 25A

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 1 of Experiment 7 part 2.

Treatment	High Nitrogen; water *ad lib.*									
Animal	3	4	7	11	1	2	5	8	9	10
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	312	229	308	371	254	296	385	371	251	411
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	224	208	207	218	133	149	212	173	239	331
Plasma urea (mM/l)	6.1	7.1	6.7	6.4	6.5	5.5	7.5	7.6	6.3	6.2
U/P urea ratio	31	58	30	52	30	32	35	25	68	52
Body Weight (kg)	5.699	5.782	6.303	7.260	6.818	6.096	5.647	6.662	6.053	6.072

APPENDIX 25B

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 2 of Experiment 7 part 2.

Treatment	Low Nitrogen Water *ad lib.*				Low Nitrogen Water Restricted					
Group	1				2					
Animal	3	4	7	11	1	2	5	8	9	10
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	101	163	61	47	96	140	71	114	68	78
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	22	46	42	14	22	37	32	30	25	16
Plasma urea (mM/l)	0.7	0.7	1.1	0.9	4.2	2.0	1.8	3.3	3.8	2.5
U/P urea ratio	36	36	59	28	8	25	26	13	14	11
Body Weight (kg)	5.237	5.362	5.811	6.871	6.016	5.447	4.847	5.603	5.341	5.479

APPENDIX 25C

Total urinary nitrogen, urinary urea nitrogen, plasma urea, U/P urea ratios and body weight for each wallaby during collection 3 of Experiment 7 part 2

Treatment	Low Nitrogen Water *ad lib.*				Low Nitrogen Water Restricted					
Group	1				2					
Animal	3	4	7	11	1	2	5	8	9	10
Total urinary nitrogen ($mgN/kgW^{0.75}/day$)	80	50	50	61	49	42	47	154	53	54
Urinary urea nitrogen ($mgN/kgW^{0.75}/day$)	7	7	7	5	3	2	2	103	3	3
Plasma urea (mM/l)	0.9	0.7	1.1	0.7	3.7	1.7	1.7	6.1	1.4	1.5
U/P urea ratio	10	13	10	13	1	2	3	26	3	3
Body Weight (kg)	4.666	4.848	5.245	6.263	5.636	5.101	4.607	4.828	5.054	5.043

APPENDIX 26A

Daily food and water intake and excretion and apparent dry matter digestibility of two groups of wallabies related to their metabolic weight measured over a ten day period during Experiment 7 part 2

Animal		Body Weight ($kgW^{0.75}$)	Dry Matter Intake°	Dry Faeces°	Apparent Dry Matter Digestibility	Water Intake+	Urine Volume+	Faecal Water+
3	Low Nitrogen Water *ad lib.*	3.241	38.7	22.1	43	77.4	22.9	47.8
4		3.309	34.4	18.3	47	55.4	23.3	25.9
7		3.519	36.1	19.4	46	64.9	20.0	37.1
44		4.017	35.1	18.3	48	65.3	15.9	36.4
	$\bar{X}$	3.521	36.1	19.5	46	65.7	20.5	36.8
1	High Nitrogen Water Restricted	3.718	31.5	14.6	54	41.9	18.3	20.5
2		3.428	29.2	14.9	49	45.7	16.9	21.7
5		3.152	32.2	15.8	51	50.0	19.3	19.2
8ø		3.333	34.0	16.4	52	47.1	17.5	22.0
9		3.388	33.2	16.2	51	46.2	16.6	22.1
10		3.387	32.7	15.1	54	46.3	14.7	25.8
	$\bar{X}$	3.415	31.8	15.3	52	46.0	17.2	21.9
	t_7 =	0.57 NS	3.76**	4.84**	4.13**	4.68**	1.89NS	3.62**

° $g/kgW^{0.75}$/day; + $ml/kgW^{0.8}$/day. ø data from animal 8 have not been included in means or statistical analyses

APPENDIX 26B

Daily nitrogen intake and excretion, partitioned urinary nitrogen excretion and apparent nitrogen digestibility of two groups of wallabies related to their metabolic weight measured over a ten day period during Experiment 7 part 2

Animal	Treatment		Nitrogen Intake+	Urinary Nitrogen+	Faecal Nitrogen+	Nitrogen Balance+	Apparent Nitrogen Digesibility¢	Urinary Urea Nitrogen+
3	Low Nitrogen Water *ad lib.*		101	57	163	-119	-61	5
4			96	35	171	-110	-78	6
7			96	36	161	-101	-68	6
44			94	44	143	- 93	-65	7
		$\overline{X}$	97	43	159	-106	-65	6
1	High Nitrogen Water Restricted		85	47	135	- 97	-59	2
2			77	38	147	-108	-91	1
5			87	35	144	- 92	-66	2
8ø			95	194	182	-281	-92	108
9			94	43	145	- 94	-54	2
10			89	45	147	-103	-65	2
		$\overline{X}$	86	42	144	- 99	-67	2
		t_7 =	3.21*	0.19 NS	2.60*	1.18 NS	0.23 NS	14.58***

+ $mgN/kgW^{0.75}/day$. ø data from animal 8 have not been included in means or statistical analyses. ¢ %.

APPENDIX 27

HYDROLYSIS OF UREA TO AMMONIA AND UREA TOXICITY IN THE KANGAROO ISLAND WALLABY

1. Introduction

The results of Experiment 1 demonstrate that the Kangaroo Island Wallaby utilizes urea and casein equally well as sources of dietary nitrogen. Harris and Mitchell (1941a) have shown in sheep that dietary urea is utilized less efficiently than dietary casein when the supplement forms the major part of the nitrogen intake. In addition Barnett and Reid (1961) report that non-protein nitrogen is utilized more efficiently by ruminants when it forms a relatively small proportion of the total nitrogen intake.

The major problem in feeding urea in high concentrations to ruminants is the development of toxic symptoms which may result in death (Moir, 1957). Urea toxicity has usually been demonstrated in animals which have been drenched with urea and it has generally been agreed that the onset of toxic symptoms is associated with elevated blood ammonia levels (Clark *et al.*, 1951; Gallup et al., 1953; Pierce *et al.*, 1955; Repp *et al.*, 1955b; Davis and Roberts, 1959; Coombe, Tribe and Morrison, 1960; Briggs *et al.*, 1960; Oltjen *et al.*, 1963; Nix and Anthony, 1965; McBarron and McInnes, 1968). The rise in blood ammonia

in animals drenched with urea is due to rapid hydrolysis of urea to ammonia by the action of bacterial urease in the rumen (Bloomfield *et al.*, 1966) combined with the inability of the liver to convert the additional ammonia to urea (Lewis *et al.*, 1957).

In Experiment 1, blood ammonia levels showed no increase in those wallabies fed the urea supplemented diet although urea nitrogen formed the major portion of the total nitrogen intake. This finding could mean that hydrolysis of urea to ammonia is not a feature of urea utilization in this macropod, or else production of ammonia from urea was equal to its utilization by the microbial population in the forestomach and its conversion to urea in the liver. The former suggestion is unlikely as ammonia concentrations in the forestomach of the quokka fed new growth pasture are similar to those in ruminants fed a similar diet (Moir, 1965). The failure, however, to produce ammonia from ingested urea by the Kangaroo Island Wallaby and hence the absence of a bacterial urease could not be disregarded.

In the following experiment wallabies which had been fed a maintenance diet which was rich in available carbohydrates (McDonald and Hall, 1957) were drenched with urea. The aim of the experiment was to establish whether ingestion of urea results in elevated blood ammonia levels. This would indicate the presence of a urease and in addition would show whether this

macropod develops those symptoms characteristic of urea toxicity in sheep. In the experiment reported herein a urea dosage of 0.79 - 0.81 g of urea per kg body weight was given. The lethal dose of orally administered urea in sheep has been found to range from 0.4 to 0.6 g of urea per kg body weight (Gallup et al., 1953; Davis and Roberts, 1959; Oltjen et al., 1963; Nix and Anthony, 1965).

2. Experimental Procedure

Eight adult female Kangaroo Island Wallabies were fed a maintenance diet containing 1.5gN/100g dry weight for three weeks.

On the day of the experiment the animals were weighed and a blood sample taken from the lateral tail vein. The animals were then divided randomly into two groups of four. Animals 70, 25, 29 and 32 were drenched by stomach tube with a known amount of an aqueous solution of urea (0 5g/ml). Animals 1, 2, 3 and 4 were drenched with a similar volume of water (Table).

Blood samples were taken at 0, 10, 20, 30, 40, 60 and 120 minutes from the lateral tail vein and assayed for ammonia.

APPENDIX 27

TABLE A. Urea dosage for each animal (g/kg) introduced into the stomach by drenching

Animal	Body Weight (kg)	Mls of Solution	Dosage of urea (g/kg)
70	5.143	8.0	0.79
25	5.014	8.0	0.79
29	5.337	8.8	0.81
32	5.198	8.4	0.81
1	4.620	7.6	0
2	4.788	7.6	0
3	4.750	7.6	0
4	5.092	8.0	0

APPENDIX 27

TABLE B. Blood ammonia levels (mg/100ml) before and after the urea drench for animals 70, 25, 29 and 32 and before and after an equivalent water drench for animals 1, 2, 3 and 4

Treatment	Urea				Water			
Animal	70	25	29	32	1	2	3	4
Time (min)								
0	0.17	0.10	0.17	0.07	0.10	0.14	0.07	0.14
10	0.21	0.28	0.24	0.35	0.10	0.14	0.07	0.17
20	1.00	1.00	1.10	1.17	0.10	0.21	0.07	0.21
30	1.72	1.72	1.86	1.86	0.10	0.20	0.10	0.17
40	2.55	1.31	1.38	1.45	0.09	0.14	0.07	0.17
60	0.76	0.76	0.83	0.69	0.10	0.07	0.04	0.17
120	0.48	0.27	0.34	0.55	0.10	0.14	0.07	0.14

3. Results

Blood ammonia concentrations (mg/100ml) for each wallaby before and after drenching with either urea or water are presented in Table B. It can be seen that blood ammonia levels rose rapidly in those wallabies drenched with urea, reaching a maximum of 1.7 - 2.6 mg/100ml between 30 - 40 minutes after drenching. In the control wallabies drenched with water, blood ammonia levels remained almost unchanged.

Symptoms associated with urea toxicity in sheep include respiratory difficulties, excessive salivation, muscular tremors incoordination, and bloat. If the dosage is lethal, death usually occurs 1½ - 2½ hours after the initial appearance of symptoms (Dinning *et al.*, 1948; Clark *et al.*, 1951; Repp *et al.*, 1955b; Lewis *et al.*, 1957; Davis and Roberts, 1959; Oltjen *et al.*, 1963). None of these symptoms were detected in the wallabies drenched with urea and the wallabies survived without apparent ill effect.

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