CHAPTER EIGHT

Digestion of plant-cell-wall constituents by potoroine marsupials

8.1 A study of the low and variable digestion of plant-cell walls in *A. rufescens* and *P. tridactylus*

8.1.1 Introduction

THE low and variable digestibility of plant-cell-wall constituents was reported in Chapter 5. Similar results were found in the experiments described in Chapters 6 and 7, and some of these are detailed in Appendix 2. In all of these balance studies, the animals were fed grain-based diets supplemented with different levels of oat hulls.

Results from studies with domestic ruminants have shown that food particle size (Schneider and Flatt 1975; Rode *et al.* 1985), the forage:grain ratio (Schneider and Flatt 1975; Mackie *et al.* 1978; Owen and Goetsch 1986; Rode and Satter 1987; Kinser *et al.* 1988), the source and level of plant-cell walls (Hsu *et al.* 1987; Kinser *et al.* 1988) and the type of grain (Kreikemeier *et al.* 1987; Mackie *et al.* 1978) all interact to affect rumen function and, hence, the digestibility of cell-wall constituents.

In general, the digestibility of cell-walls decreases as the level of dietary concentrates is increased. This can be attributed, largely, to the rapid fermentation of the soluble carbohydrates to organic acids. The concomitant drop in pH inhibits the activity of cellulolytic bacteria and, consequently, lowers fibre digestion.

Rapid fermentation may have inhibited cellulolysis in the previous experiments with potoroine marsupials. However, this does not explain the variation between animals. Furthermore, none of the cited studies mentions variation of a similar magnitude in domestic ruminants. It may be that the variation was particularly noticeable in the previous studies of potoroines because digestibility was low. For example, there is a difference of 100% between digestibilities of 10 and 20%; the difference is only 11% if these are expressed as coefficients of indigestibility — 90 and 80% respectively.

An experiment was therefore conducted to examine whether variability in fibre digestion was within individual animals or between animals. The study examined also the effect of increasing levels of dietary fibre on food intake and fibre digestibility.

8.1.2 Materials and methods

Animals and diets

A 16-day balance study was conducted with nine *P. tridactylus* (male) and nine *A. rufescens* (eight male, one female — with a ca 50 day pouch young at the completion of the study). The animals were randomly allocated to three pelleted diets containing 180, 290 or 400 g NDF kg^{-1} (Table 8.1.1).

Table 8.1.1 Composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the experimental diets.

	Level of inclusion		
Dietary ingredient	**Low fibre**	**Medium fibre**	**High fibre**
Maize	405	300	196
Wheat	100	100	100
Oat hulls	175	350	525
Cornflour	290	220	149
Mineral mix (Table A1.6)	29	29	29
Mineral/Vitamin premix (Table A1.6)	1	1	1
Analysis			
Organic matter	957.6	952.7	944.3
Ash	42.4	47.3	55.7
Nitrogen	10.3	9.8	9.8
Acid detergent fibre	76.1	130.4	185.1
Neutral detergent fibre	179.2	290.3	397.6
Cellulose	59.6	111.6	149.5
Hemicellulose	103.1	159.9	212.5
Lignin	16.5	18.8	35.6

The general adaptation, precollection period and the 16-day collection period were as described in Chapter 4. The collection was divided into four so that the data could be treated as 4 x 4-day, 2 x 8-day, or 1 x 16-day periods. This procedure gave an indication of within-animal variation. The animals were weighed on days 1, 8 and 16 of the collection period.

Analytical

Samples were prepared for analysis and analysed using the standard procedures described in Chapter 4.

Statistical

Digestibility and balance data from the individual 4-day periods were compared by two-way analysis of variance with repeated measures, using the BMDP 2V statistical package. Values of intake, excretion, nitrogen balance and digestibility for the 16-day collection period were compared between animals, and fibre levels by two-way analysis of variance using BMDP 2V.

8.1.3 Results

All data were analysed as a split-plot in time; that is, repeated measures. The sphericity test, which measures residual effects, was non-significant ($P>0.10$) for all parameters. Thus, the data were reanalysed as a single 16-day balance study.

Rather than providing a complete data set for the individual 4-day measurement periods, the results are presented in three ways:

1. Table 8.1.2 shows the means and standard errors of differences between means for all parameters measured in the 16-day balance study.

2. Table 8.1.3 shows the coefficients of variation for the digestibilities of dry matter, nitrogen, ADF and NDF.

3. Digestibilities of ADF and NDF for individual animals (five *A. rufescens* and four *P. tridactylus*) have been selected (Table 8.1.4) to illustrate specific observations.

Both *A. rufescens* and *P. tridactylus* showed variations in body mass during the 16-day balance period. However, on average, masses remained stable or slight gains were recorded. There were, however, no significant differences between species or diets.

The dry matter intakes of *P. tridactylus*, expressed on the basis of metabolic body mass, were higher ($P<0.05$) than those of *A. rufescens*. In general, as the proportion of dietary-cell-wall constituents increased, so did dry matter intake ($P<0.05$). However, *P. tridactylus* fed the high-fibre diet, ate slightly less than those fed the medium-fibre diet, but the difference was not significant.

The digestibility of fibre was not significantly different between species or dietary-fibre levels. But there was a significant interaction between species and fibre: *P. tridactylus*, fed the high-fibre diet, digested more of the NDF. The data for ADF digestibility show a similar result.

The coefficients of variation for NDF digestibility were 4-6 times those for dry matter digestibility and double those for nitrogen digestibility. The variation in ADF digestibility data was even higher — 5-16 times that for dry matter digestibility.

Table 8.1.2 Intake, digestibility and balance data from A. rufescens and P. tridactylus fed maize-oat hull diets containing 1% nitrogen and 18%, 28% or 40% NDF.

	Aepyprymnus			*Potorous*				significance		
Parameter [1]	**LF**	**MF**	**HF**	**LF**	**MF**	**HF**	**sed**	**Sp**	**Fib**	**Sp x Fib**
number	3	3	3	3	3	3				
Body mass (g)	2836	2625	2940	921	878	958				
(sem)	(62.1)	(42.0)	(80.6)	(77.9)	(16.0)	(19.8)				
change (%) [2]	0.8	0.6	5.2	1.1	3.9	1.5	1.95	ns	ns	ns
Dry matter										
intake ($g.d^{-1}$)	81	79	96	37	42	42	4.5	***	*	ns
($g.kg^{-0.75}.d^{-1}$)	37	38	43	39	46	43	2.4	*	*	ns
digestibility (%)	77	68	58	77	70	65	1.4	**	***	*
ADF digestibility (%)	14	10	8	10	10	24	4.7	ns	ns	*
NDF digestibility (%)	27	23	20	21	23	36	4.1	ns	ns	**
Nitrogen										
intake ($g.kg^{-0.75}.d^{-1}$)	0.379	0.378	0.420	0.403	0.460	0.412	0.0274	ns	ns	ns
faecal ($g.kg^{-0.75}.d^{-1}$)	0.196	0.193	0.211	0.191	0.197	0.208	0.0121	ns	ns	ns
digestibility (%)	48	49	50	52	57	50	4.7	ns	ns	ns
urinary ($g.kg^{-0.75}.d^{-1}$)	0.080	0.112	0.148	0.157	0.248	0.217	0.0497	**	ns	ns
balance ($g.kg^{-0.75}.d^{-1}$)	0.104	0.093	0.060	0.053	0.015	-0.011	0.0670	ns	ns	ns
Water intake										
($g.d^{-1}$)	116	117	123	70	90	138	21.4	ns	ns	ns
($g.kg^{-0.80}.d^{-1}$)	51	54	52	72	99	143	17.1	***	*	*
($g.100g\ DMI^{-1}$)	145	148	128	192	214	327	43.1	**	ns	ns

1 LF, MF, HF - low fibre, medium, high fibre diets
2 Body-mass change during the 16-day collection period

Table 8.1.3 Coefficients of variation for digestibility parameters

Parameter	*A. rufescens* LF	MF	HF	*P. tridactylus* LF	MF	HF
Dry matter digestibility (%)	2.1	1.5	1.5	2.1	1.7	1.3
Nitrogen digestibility (%)	5.5	4.7	4.7	4.9	4.4	3.5
NDF digestibility (%)	8.8	8.8	9.0	13.6	7.8	5.4
ADF digestibility (%)	21.4	23.2	24.4	22.9	15.9	6.9

LF, MF, HF - low-, medium-, high-fibre diets

Table 8.1.4 shows the NDF and ADF digestibilities in several *A. rufescens* and *P. tridactylus* measured over 16 days, and expressed as 4 x 4-, 2 x 8-, or 1 x 16-day collection periods.

Table 8.1.4 The digestibilities (%) of NDF in selected A. rufescens and P. tridactylus.

		Day of collection period						
Diet	**Animal**	**0-4**	**5-8**	**9-12**	**13-16**	**1-8**	**9-16**	**1-16**
LF	ArL	16	28	23	23	22	23	22
MF	ArI	24	21	9	39	23	25	24
HF	PtA	23	36	16	39	31	29	30
LF	ArG	58	20	34	21	39	28	33
MF	ArE	20	20	45	23	21	34	27
HF	PtH	26	26	45	47	26	46	37
LF	ArQ	26	21	30	28	23	29	27
MF	PtM	20	17	19	22	19	21	20
HF	PtD	36	33	36	24	34	29	32

LF, MF, HF - low-, medium-, high-fibre diets
Ar - *A. rufescens*; Pt - *P. tridactylus*

It is not possible in a 4-day balance study to measure accurately cell-wall digestibility. This is shown clearly in Table 8.1.4 by *Aepyprymnus* L and I and *Potorous* A. In these animals, NDF digestibilities were similar in the two 8-day periods, but fluctuated considerably in the 4-day measurements. In other animals, for example *Aepyprymnus* G and E and *Potorous* H, the digestibility of cell walls fluctuated in an unexplainable manner so that even the two 8-day values were markedly different. In still another group of animals, cell-wall digestibility varied little throughout the experiment. Examples are *Aepyprymnus* Q and *Potorous* M.

In terms of metabolic body mass, neither species nor diet had a significant effect on nitrogen ingestion or faecal nitrogen excretion. There were also no differences in nitrogen digestibility. The *P. tridactylus* excreted more nitrogen in their urine than did *A. rufescens* ($P<0.01$). However, this higher excretion of urinary nitrogen did not cause significant differences in nitrogen balance between species; nitrogen balance was not different between dietary-fibre levels.

Potorous and *Aepyprymnus* drank similar quantities of water. Thus, *Potorous* drank more than *A. rufescens* when expressed per kg metabolic body mass ($P<0.001$) or per 100 g dry matter intake ($P<0.01$). Dietary-fibre level was positively related to water intake ($g.kg^{-0.80}.d^{-1}$) in *P. tridactylus* but not in *A. rufescens*. These results are discussed further in Chapter 10.

8.1.4 Discussion

It is generally assumed that, among herbivores, size dictates diet. Small animals — for example, potoroines — have high mass-specific nutrient requirements and must select more concentrated diets than large herbivores (Demment and Van Soest 1985). Similarly, when fed a common diet, a small animal needs to eat more, per unit metabolic body mass, than does a larger animal. In the present study, it was observed that *P. tridactylus* ate more than the larger *A. rufescens*.

Both *A. rufescens* and *P. tridactylus* countered the nutrient diluting effect of additional increments of dietary-cell-wall constituents by eating more. Notably, the animals gained body mass — particularly when fed the high-fibre diets. Voluntary food intake is influenced by factors that depend on the animal and the diet (Journet and Remond 1976). For example, pelleted rations are highly "ingestible", because particle size is reduced. Therefore, pelleting may have allowed the potoroines to ingest the fibrous diets. But this does not prove that wild potoroines can survive on poor quality diets. However, there is circumstantial evidence that gut capacity was not a limiting factor in the present study. About 75% of the ingested cell walls remained undigested and represent "dead-space" in the gut. But despite this seemingly wasted space,

potoroines coped with fibrous diets by eating more and maximising their intake of digestible nutrients. The results of Chapter 7 suggest that the higher intakes were not facilitated by an increased rate of passage.

The *P. tridactylus*, fed the high-fibre diet, digested 36% of the dietary NDF. This is between 9 and 16 percentage units greater than the NDF digestibility of any of the other diets by either species. Although inconclusive, the increase in fibre digestion and concomitant, albeit non-significant, decrease in food intake suggests that fermentation products contributed a greater proportion of the nutrient requirements of the *P. tridactylus* fed the high-fibre diet than of either species fed on the other diets.

The higher digestion of cell-walls in the *P. tridactylus* fed the high-fibre diet suggests a relationship between soluble carbohydrate levels and cellulolytic activity. A similar relationship has been recognized in ruminants for a long time (Burroughs *et al.* 1949). However, some recent studies (for example Kerley *et al.* 1985; Kinser *et al.* 1988) which describe the feeding of fibrous by-products to ruminants, provide the most interesting data for comparison with the present results. Kinser *et al.*'s sheep digested between 25 and 30% of the NDF in pelleted diets containing 60% maize and 22% ground corncobs or ground cottonseed hulls. This is very similar to the cell-wall digestion by *A. rufescens* and *P. tridactylus* fed the low- and medium-fibre diets. Kinser *et al.* (1988) reported also higher NDF digestibilities when the maize was reduced to 42% and the roughage component of the diet was increased to 39%.

In a similar study to that of Kinser *et al.* (1988), Kerley *et al.* (1985) fed diets containing 15, 35, 38 and 50% ground maize, and detected no significant differences in NDF digestibilities. They did, however, report a drop in ADF digestibility from 48% to 34% when the level of corn in the ration was increased from 15 to 50%. Of interest are their standard errors of the means. In agreement with the present study, these were much higher for cell-wall digestibility than for either nitrogen or dry matter digestibilities.

Three other findings by Kerley et al. (1985) are relevant to the present study. First, ruminal ammonia-nitrogen levels in animals fed 50% corn were half those of animals fed 15, 35 or 38% corn. Secondly, although all diets produced similar levels of ruminal short-chain fatty-acids, more propionic acid was produced in animals fed 50% ground corn. Finally, more nitrogen escaped ruminal degradation in animals fed the high level of corn.

These cited studies point to similarities between the foregut environments of potoroines and domestic ruminants. How do grains influence the fermentation environment? The basic mechanism seems well understood. The soluble carbohydrate is fermented rapidly, causing a sudden drop in pH. These conditions favour species

capable of rapid metabolism and a tolerance to some downward shift in pH. Hiltner and Dehority (1983) showed that cellulose digestion occurs simultaneously with the utilisation of the soluble carbohydrate. However, when the pH drops below 6.3, cellulose hydrolysis is impeded because the enzyme — cellulase — is acid sensitive (Stewart 1977). Of particular importance to overall cell-wall digestion is the length of time that the pH remains below 6.0 (Mackie and Gilchrist 1979).

As discussed in Chapter 2, the foregut environment is regulated by the rate of flow and the composition of saliva, and the rate of removal of fermentation products. The rate of production of saliva depends on diet, the amount of chewing and the animal species; salivary composition depends on species alone. In ruminants, saliva flow is highest during rumination (Kaufman 1976). A reduction in particle size depresses rumination and hence rumen buffering via saliva. Potoroines do not ruminate. Thus the rate of their saliva flow is presumably linked to how intensely and for how long they chew.

The pattern and level of feeding can affect foregut pH. The pH in the foregut is lowered when the level of feeding doubled from 1% to 2% of body mass. This was true for both roughage and concentrate diets (Rumsey *et al.* 1970). Kaufman (1976) reported that a higher ruminal pH is maintained by more frequent feeding. In all my experiments with potoroines, animals were fed to appetite rather than a set amount per unit metabolic mass. Furthermore, although potoroines housed in metabolism cages usually started feeding when the lights went out, food consumption rates differed noticeably among animals. Thus, food intake and feeding patterns differed between animals. These factors may, in turn, have given rise to different patterns of foregut pH.

Microbiological aspects of the potoroine gut are unknown. Even so, microbial species are known to modify ruminal pH. Protozoa, whose numbers generally increase with dietary concentrates, engulf starch grains and ferment them more slowly than do bacteria. Protozoa engulf bacteria also, further reducing the rate of fermentation. Mackie *et al.* (1978) studied the microbial and chemical changes in the rumen during the stepwise adaptation of sheep to concentrate diets. Their research suggests that two factors are critical in determining forestomach pH. First, if the amount of grain exceeds the capacity of protozoa to remove a large portion of it, the bacterial fermentation may proceed uncontrollably. Secondly, if there is not a substantial increase in acid-tolerant lactate users, lactic acid accumulates and pH drops rapidly. Plant-cell walls from different sources are digested at different rates, and react differently to the addition of soluble carbohydrate. For example, Burroughs *et al.* (1949) reported that 40% starch decreased the digestion of corncobs by 22 percentage units, but alfalfa digestion was decreased by only 2-5 percentage units. The explanation for their findings was unclear.

Oat hulls were selected for the present work because they are low in nitrogen, easily pelleted and contain much NDF (ca 70%). Rowe and Crosbie (1988) showed that the digestibility of oat hulls and oat grain is inversely related to the hull lignin content. Oat hulls can be well digested. For example, Hsu et al. (1987) fed a diet containing 80% oat hulls and 14% soybean meal to lambs and reported NDF digestibilities of 40%. This diet contained about 2% nitrogen, which is double that of the potoroine diets. However, a nitrogen deficiency does not seem to be the reason for the minimal fibre digestion because the fibre in the basal ration (Appendix 1, Table A1.9) also is poorly digested. Instead, the generally low digestion of NDF and ADF in the present experiment implies that forestomach pH often fell below 6.0. Differences in the actual extent of fibre digestion between individual animals suggests that there was both between- and within-animal variation in the extent and duration of pH depression. This was probably related to differences in feeding patterns among animals, and also from day to day within the same animal.

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8.2 The digestibility of a lucerne-based diet by three species of potoroine marsupials

8.2.1 Introduction

IN the balance studies in which potoroines have been fed grain-oat hull diets, the digestibilities of cell-wall components have been low and variable. This is not surprising if we consider conventional theories relating body size to diet quality. These theories predict that small herbivores, such as potoroines, must select nutrient-rich diets because they lack the large gut capacity needed to process plant-cell walls. However, the relatively slow passage of grain-oat hull diets through the gastrointestinal tracts of potoroines (Chapter 7) refutes any suggestion that limited digesta retention restricts the breakdown of structural carbohydrates. Instead, it appears that some other factor(s) is inhibiting cellulolysis. This latter hypothesis was raised in Section 8.1, in which it was suggested that the activity of cellulolytic microbes may have been depressed by low pH conditions resulting from fermentation of soluble carbohydrates.

It is clear that much could be gained by the alternative approach of avoiding diets rich in oat hulls and soluble carbohydrates. Because similar digestive studies were being conducted in this laboratory with *Macropus robustus robustus* and *M. r. erubescens* fed pelleted lucerne diets (Freudenberger pers. comm.), it seemed logical, for comparative purposes, to feed similar diets to potoroines. Not only might this approach answer questions about oat hulls and soluble carbohydrates, but it would provide also an opportunity to compare digestive efficiencies in macropodoid species of markedly different body size.

8.2.2 Materials and methods

Animals and diets

Four *A. rufescens*, six *P. tridactylus* and three *B. penicillata*, all adult males housed in outdoor enclosures, were offered a pelleted diet containing 972 g.kg^{-1} lucerne and 28 g.kg^{-1} mineral mix. Because many animals were housed in each outdoor enclosure food consumption was monitored indirectly — by weighing the animals. When all animals lost 5-10% of their body mass it was clear that the diet was unacceptable. Therefore, the animals were allowed to regain condition and were then offered a diet containing 720 g.kg^{-1} lucerne, 250 g.kg^{-1} maize and 30 g.kg^{-1} mineral/vitamin mix. The chemical composition of this diet is shown in Table 8.2.1. Although animals initially lost mass on the new diet they did consume it. The animals were moved from the outdoor enclosures to individual metabolism cages in a partially controlled environment

described in Chapter 4. They were fed the experimental diet for a further 14 days, prior to the start of a 7-day balance study. One representative from each species failed to maintain a steady feed intake and was discarded from the experiment.

Table 8.2.1 Composition ($g.kg^{-1}$ ADM) and chemical analysis ($g.kg^{-1}$ ODM) of the experimental diet.

Dietary ingredient	Level of inclusion
Lucerne chaff	720
Maize	250
Mineral mix (Table A1.6)	29
Mineral/Vitamin premix (Table A1.6)	1
Analysis	
Organic matter	904.2
Ash	95.8
Nitrogen	25.7
Acid detergent fibre	298.9
Neutral detergent fibre	505.2
Cellulose	222.2
Hemicellulose	206.3
Lignin	76.7

Experimental

Much more of the pelleted lucerne diet was spilled than of any diet fed previously to the potoroines. Moreover, spilled feed was quickly contaminated by the copious urine produced on this diet, making accurate measurements of food intake difficult. To resolve this problem, food was replenished and faeces collected several times during the peak feeding period (1800-2400h). As well as minimising the contamination of spilled feed, this procedure allowed estimates of faecal water output. The animals were weighed at the start and end of the collection period. Food and water intake, food refusals and faecal and urinary output were recorded daily and complete samples were frozen. Urine was collected into Polythene bottles containing 5 ml glacial acetic acid.

Analytical

The analytical procedures were those described in Chapter 4. The spilled food was analysed to enable precise determinations of urine output and the consumption of individual dietary constituents.

Statistical

Intake, excretion and digestibility coefficients were compared among species by one-way analysis of variance using the BMDP 1V statistical package. When the species main effect was significant, species means were compared using the least significant difference procedure (Steel and Torrie 1960). Because of the unequal replication it is not possible to present a single value for the standard error of the difference between means. Instead, the error mean square is presented.

8.2.3 Results

The mean data for intake, output and digestibility measurements are shown in Table 8.2.2. Whereas the *Bettongia* and *Aepyprymnus* maintained or gained mass over the 7-day balance study, all *Potorous* suffered losses that ranged from 0.5 to 8.0% of initial body mass ($P<0.01$). This occurred despite the fact that, when expressed as a function of metabolic body size, there were no interspecific differences in either the intake or output of any dietary constituent. Also, the three species digested similar proportions of dry matter, organic matter, ADF and NDF. They differed in their abilities to digest crude protein, with *Aepyprymnus* digesting more than *Potorous* ($P<0.05$) which, in turn, digested more than *Bettongia* ($P<0.05$). Although non-significant, there was a trend for *B. penicillata* to excrete more faecal nitrogen per unit metabolic body mass than did the other species. This alone seemed the major contributor to the depressed ($P<0.05$) nitrogen digestibility.

When expressed as a function of metabolic body mass, there were no significant species differences in the component parameters of nitrogen balance — viz, nitrogen intake, and urinary and faecal nitrogen losses. Hence, nitrogen retention was similar in all species.

The data for water flux were extremely variable among individuals and this masked any possible species differences. All aspects of water metabolism in this experiment are discussed further in Chapter 10. Nevertheless, *P. tridactylus* appeared to consume more water and excrete more urine than did the other species, but further experimentation with greater replication would be necessary to verify this trend.

P. tridactylus excreted more ($P<0.05$) respiratory water than did *A. rufescens*. The method of calculating respiratory water by difference is open to criticism because it summates all errors. However, it is important to note that animals defaecated early in the dark period and rarely defaecated later in the night. Thus it was possible to collect faeces soon after their appearance and thus to measure accurately faecal water output. There was also a peak in urine excretion soon after dark.

Table 8.2.2 Intake, digestibility and balance data from A. rufescens, P. tridactylus and B. penicillata fed a lucerne-based diet.

	Aepyprymnus	*Potorous*	*Bettongia*	ems*	sig
number	3	5	2		
Body mass (g)	2650	900	1070		
(sem)	(180)	(60)	(30)		
Change (% CP)**	1.9	-4.9	-0.7		
Dry Matter					
Intake ($g.d^{-1}$)	77	33	46	122.1	***
Intake ($g.kg^{-0.75}.d^{-1}$)	37	35	43	87.6	ns
Apparent digestibility (%)	59	58	57	5.4	ns
Energy					
DE Intake ($kJ.kg^{-0.75}.d^{-1}$)	410	360	460	12.0	ns
Water					
Intake ($g.d^{-1}$)	269	196	165	8999.0	ns
Intake ($g.kg^{-0.80}.d^{-1}$)	123	211	158	5902.9	ns
Intake ($g.100g^{-1}$ DMI)	348	588	355	14389.0	*
Faecal DM (%)	28	31	35	53.2	ns
faecal water ($g.kg^{-0.80}.d^{-1}$)	37	38	40	370.0	ns
urine ($g.kg^{-0.80}.d^{-1}$)	41	74	55	1424.9	ns
Nitrogen					
Intake ($g.kg^{-0.75}.d^{-1}$)	0.95	0.91	1.12	0.058	ns
Faecal N ($g.kg^{-0.75}.d^{-1}$)	0.32	0.35	0.49	0.008	ns
Apparent digestibility (%)	66	61	57	3.0	*
Urinary N ($g.kg^{-0.75}.d^{-1}$)	0.36	0.32	0.41	0.018	ns
Balance ($g.kg^{-0.75}.d^{-1}$)	0.26	0.21	0.23	0.006	ns
ADF					
Apparent digestibility (%)	38	36	36	12.1	ns
NDF					
Apparent digestibility (%)	58	56	61	28.1	ns
Hemicellulose[1]					
Apparent digestibility (%)	76	78	83	39.8	ns
Cellulose[2]					
Apparent digestibility (%)	56	53	57	45.8	ns

* - error mean square
** - body mass change during the collection period (CP).
1 Hemicellulose - calculated as the difference between ADF and NDF.
2 Cellulose digestibility - calculated by assuming that lignin is indigestible.

Data on particle-size distribution in the diet and faeces are shown in Table 8.2.3. The high digestibility of the cell-walls in the lucerne-based diet is reflected in the lower particle sizes in the faeces than those in the diet. There are proportionately more small particles and less large particles in the faeces than in the diet. By comparison, the particle size distribution in a maize-oat hull ration, with low cell-wall digestibility, was not affected by passage through the gut.

Table 8.2.3 The distribution of particle sizes in potoroine diets and faeces. Values are means ± their standard errors.

	Sieve size (μm)					
	1200	**600**	**300**	**150**	**75**	**<75**
Lucerne diet	16	18	13	11	9	33
Faeces						
A. rufescens	5	24	17	11	7	38
(n=3)	1.3	0.7	2.1	2.1	0.4	3.9
P. tridactylus	5	19	15	10	7	45
(n=5)	0.7	0.9	0.8	1.3	0.6	1.7
B. penicillata	5	22	15	8	10	39
(n=2)	0.1	0.8	0.8	0.6	3.3	3.6
Maize-oat hull diet (Chapter 9)	2	13	18	11	14	43
Faeces						
A. rufescens	2	20	24	11	5	38
(n=4)	0.2	1.2	2.7	1.3	1.0	2.3
P. tridactylus	3	23	26	15	5	29
(n=4)	1.2	2.5	2.1	1.1	1.2	3.6

8.2.4 Discussion

The rationale behind feeding a lucerne-based diet to potoroines was to examine whether the low and variable digestion of structural carbohydrates in previous experiments, was related to characteristics of the grain-oat hull diets, or whether it is a distinguishing feature of this marsupial sub-family.

It is apparent from the results that all three potoroines can digest substantial amounts of structural carbohydrates. Furthermore, the low coefficients of variation (ca 9%) for the overall means of NDF and ADF digestibility imply that previous variability was diet-related. There are at least three explanations for low fibre digestion. First,

potoroines cannot digest oat hulls. This is unlikely because oat hulls, although they may contain up to 6% lignin (Rowe and Crosbie 1988), are rich in hemicellulose which, in this experiment, was highly digestible. Second, fibre digestion was limited in previous experiments, because the diets contained only sufficient nitrogen for maintenance. This hypothesis is refuted by the results shown in Appendix 2, in which limited digestion of structural carbohydrates occurred in animals fed diets containing about 2% nitrogen. This level is about double the maintenance requirements established in Chapter 5. Thirdly, the most plausible explanation, is that high levels of soluble carbohydrates, in conjunction with the fine particle size of the diet, favoured non-cellulolytic microbes at the expense of the cellulolytic organisms. This is supported by the results of the *in vitro* digestibility experiment and is discussed further in Chapter 9. Further support comes from the brief study of particle size. The general reduction in the size of lucerne particles during passage through the potoroine gut reflects the cellulolytic activity. By comparison, the distribution of particles in a maize-oat hull ration of low cell-wall digestibility was not affected by passage through the gut.

Because the ratio of total nutrient requirements to gut capacity increases with decreasing body size (Demment and Van Soest 1985), it becomes more and more difficult for smaller animals to obtain their nutritive requirements from the products of fermentation alone. We would expect, therefore, that captive potoroines might face difficulties meeting metabolic requirements on a lucerne-based diet containing 50% of dry matter as structural carbohydrates. However, the present results do not support this contention. Only the *Potorous* had a mean loss of body mass, and this can be attributed to two animals with low feed intakes. Also, the comparison with *M. robustus spp* (Table 8.2.4) fed a pelleted diet of similar formulation but lower fibre content, does not suggest that large body size confers any advantage upon these macropodids in captivity. Of course the situation may be quite different in the wild because animals must assimilate the additional energy-yielding nutrients needed for free existence. Potoroines digested a greater proportion of dietary ADF and NDF than *M. robustus spp*, although this may reflect the lower consumption of digestible dry matter by potoroines.

Table 8.2.4 Food intake and digestibility data for various macropodoids[1] fed pelleted diets[2] containing about 75% lucerne hay and 25% maize.

Parameter	Genus			
	Macropus	*Aepyprymnus*	*Bettongia*	*Potorous*
number	8	3	5	2
Body mass (kg)	17.6	2.7	0.9	1.1
Dry matter intake ($g.kg^{-0.75}.d^{-1}$)	56.8	36.9	35.4	43.4
Digestibility (%)				
DM	63.5	58.8	57.8	56.8
NDF	39.3	59.6	57.9	55.5
ADF	29.3	36.0	37.8	36.0

1 - *Macropus* refers to *M. r. robustus*. The data for this species were provided by D.O. Freudenberger (pers. comm.).

2 - The diet fed to the *M. r. robustus*, although of similar formulation to that fed to potoroines, contained 28% less NDF.

Lucerne has been used as the sole dietary constituent in previous balance studies with macropodids, often in comparison with sheep (for example, Foot and Romberg 1965; Hume 1974, 1977a; Kempton *et al.* 1976; Dellow and Hume 1982a). In the present study, a pelleted lucerne-maize diet was fed which makes direct comparisons with published results impossible. Nevertheless, generalisations can be drawn. For example, potoroines digested ADF as efficiently as the *M. giganteus*, *T. thetis* and *M. eugenii* studied by Dellow and Hume (1982a) and the *M. rufus* of Hume (1974) but less efficiently than the *M. r. erubescens* studied by the same worker. However, potoroines had higher NDF digestibilities than the *M. rufus*, *M. r. erubescens* and sheep studied by Hume (1974). While ADF digestibilities in potoroines were similar to, or marginally lower, than the crude-fibre digestibilities measured in sheep, *M. giganteus* and *M. rufus* (Foot and Romberg 1965; Forbes and Tribe 1970; Kempton *et al.* 1976), NDF digestibilities were considerably higher. In general, it appears that sheep digest structural carbohydrates more efficiently than do either potoroines or macropodids, but there seems little difference between the latter groups.

What was the likely effect of the 25% maize in the diet fed to the potoroines? Because the potoroines ate the 75% lucerne-25% maize diet, but refused the 100% lucerne, one may argue that the maize provided the necessary soluble carbohydrates to stimulate the fermentation system, without the massive disruption (low pH) that

accompanied feeding higher levels of maize. However, lucerne may contain much soluble carbohydrate. This suggests that the maize may have lowered the digestion of cell-wall constituents. Therefore, the digestibility coefficients reported here may underestimate the digestive potential of potoroines.

Of considerable interest in the present study was the increased water consumption over that recorded in potoroines fed grain-oat hull diets. This finding is discussed in detail in Chapter 10.

Another finding of interest relates to the reintroduction of the basal diet. In ruminants, it is known that a sudden switch to a grain-based ration may have catastrophic effects through the proliferation of facultative organisms that produce large amounts of lactic acid (Allison *et al.* 1975). I am unaware of similar findings in macropodids but, as a precaution, pellets of the grain-rich basal diet were reintroduced by mixing them in a 1:2 ratio with those of the lucerne diet. The potoroines selected the basal ration. Most refused the lucerne diet, even though it had constituted their entire ration for the previous month.

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8.3 The influence of the level of maize on the utilisation of lucerne-maize diets

8.3.1 Introduction

THE previous experiment showed that potoroines are as capable as the larger macropodids at metabolising the plant-cell walls in a lucerne-maize diet. However, in that experiment, the diets contained less grain than any of the grain-oat hull diets fed previously. Thus, it is still questionable whether the low and variable digestion of plant-cell walls in earlier studies, was due to the source of fibre or to the level of grain in the diets. The rational approach for answering this question is to increase further the level of dietary oat-hulls. Unfortunately, it proved impossible with our facilities to pellet a diet containing more than 50% oat hulls. Instead, it was decided to examine the utilisation of the cell-wall constituents of lucerne in diets containing two levels of maize.

8.3.2 Materials and methods

Animals and diets

Four adult male *A. rufescens*, housed in outdoor enclosures, were offered a pelleted diet containing 620 g.kg^{-1} lucerne chaff and 350 g.kg^{-1} maize (Table 8.3.1). The animals were slow in accepting the new diet, and losses of body mass ranged from 7-8% in the first week. When masses had stabilized, the animals were moved from the outdoor enclosures to individual metabolism cages. Here, they were fed the diet for a further seven days before the start of an 8-day balance study. By this time they had been offered the diet for 18 days.

The *Aepyprymnus* were fed the low-lucerne diet once the first period ended. They were allowed only an 8-day adaptation before the start of the second balance period. It was thought that this short adaptation would exacerbate any effect of a sudden switch to a high-grain diet. The *Aepyprymnus* were weighed at the start and the finish of each collection and adaptation period.

On the last day of each balance period, the animals were injected intramuscularly with about 1 ml of sterile physiological saline containing ca 280 kBq of ^{14}C-urea. The kinetics of urea metabolism were described in Chapter 6.

Table 8.3.1 Composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the experimental diets.

Dietary ingredient	Level of inclusion Diet 1	Diet 2
Lucerne chaff	620	350
Maize	350	620
Mineral mix (Table A1.6)	29	29
Mineral/Vitamin premix (Table A1.6)	1	1
Analysis		
Organic matter	918.2	937.8
Ash	81.8	62.2
Nitrogen	24.9	18.8
Acid detergent fibre	212.0	144.5
Neutral detergent fibre	327.0	233.8
Cellulose	161.0	113.2
Hemicellulose	115.0	89.3
Lignin	50.9	31.3

Experimental design

Only four animals were available for the experiment. With such low numbers a cross-over design, in which half the animals were fed each diet in each period, seemed inappropriate. This is because any influence of one diet on the next would make interpretation of results difficult. Thus, the high-lucerne diet was fed in Period 1 and the low-lucerne diet in Period 2.

Experimental

The experimental procedures were similar to those described in Section 8.2. Feed spillage was minimal in this experiment and was not analysed. Samples were prepared for analysis and analysed using the standard procedures described in Chapter 4. Data for all parameters were analysed statistically by one-way analysis of variance using the BMDP 1V statistical package.

8.3.3 Results

The means and their standard errors of difference for all parameters are shown in Table 8.3.2. As stated previously, the animals adapted slowly to the high-lucerne diet and all lost about 7% of body mass in the first week of feeding. In contrast, the animals readily accepted the low-lucerne diet. In the adaptation period for this diet, dry matter

Table 8.3.2 Intake, digestibility and balance data from A. rufescens, fed a diet containing 62% lucerne and 35% maize (Diet 1), or 35% lucerne and 62% maize (Diet 2).

	Diet 1	Diet 2	sed	sig
number	4	4		
Body mass (g)	2896	3013		
(sem)	(180)	(60)		
Change (% start-CP)[1]	-7.3	3.4	0.47	***
Change (% CP)[2]	1.4	0.3	0.51	ns
Dry Matter				
Intake ($g.d^{-1}$)	73	79	5.9	ns
Intake ($g.kg^{-0.75}.d^{-1}$)	33	35	2.2	ns
Apparent digestibility (%)	61	71	1.4	***
Energy				
DE Intake ($kJ.kg^{-0.75}.d^{-1}$)	364	439	29.2	*
Water				
Intake ($g.d^{-1}$)	205	159	28.6	ns
Intake ($g.kg^{-0.80}.d^{-1}$)	87	65	8.9	*
Intake ($g.100g^{-1}$ DMI)	278	199	27.6	*
Faecal DM (%)	28	30	1.6	ns
Nitrogen				
Intake ($g.kg^{-0.75}.d^{-1}$)	0.83	0.65	0.053	*
Faecal N ($g.kg^{-0.75}.d^{-1}$)	0.29	0.24	0.028	ns
Apparent digestibility (%)	65	62	1.4	ns
Urinary N ($g.kg^{-0.75}.d^{-1}$)	0.41	0.27	0.032	**
Balance ($g.kg^{-0.75}.d^{-1}$)	0.13	0.14	0.020	ns
ADF				
Apparent digestibility (%)	23	29	3.2	ns
NDF				
Apparent digestibility (%)	31	31	3.2	ns

1 - body mass change from when the diet was first offered until the end of the collection period.
2 - body mass change during the collection period.

intakes often exceeded 100 g per animal per day. During this period, the animals regained about half the mass they had lost during adaptation to the high-lucerne diet. All animals maintained, or gained small amounts of mass during the measurement periods, but this was not affected by diet.

The animals ate similar quantities of each diet, but excreted more ($P<0.05$) dry matter when fed the high-lucerne diet. Therefore, the dry matter digestibilities were lower ($P<0.001$) than the corresponding values for the low-lucerne diet.

Although both diets were isocaloric in terms of gross energy, the low-lucerne diet contained more digestible energy. Animals fed this diet ingested more ($P<0.001$) digestible energy.

The ADF in the low-lucerne diet was digested to a greater extent (29%) than that in the high-lucerne diet (23%), but the difference was not significant. The digestibility of NDF was the same in each diet (31%). The coefficients of variation for ADF and NDF digestibilities were 21 and 13% respectively.

The high-lucerne diet contained more nitrogen than the low-lucerne diet, and this resulted in higher ($P<0.05$) nitrogen intakes on the former diet. There was a trend, although non-significant, for higher outputs of faecal nitrogen from animals fed the high-lucerne diet. The digestible nitrogen content of both diets was similar. The animals excreted considerably more ($P<0.001$) urinary nitrogen when fed the high-nitrogen diet. Nitrogen balance was not significantly different between diets.

Aepyprymnus tended to drink more when fed the high-lucerne diet. In absolute terms this was not significant. However, they drank more per unit metabolic body mass ($P<0.05$) or per unit of dry-matter intake ($P<0.05$). Both diets gave rise to faeces containing about 70% water.

8.3.4 Discussion

The present experiment was planned to provide further information about plant-cell-wall digestion in potoroines. Of particular interest was the influence on fibre digestion of the soluble carbohydrates in grains, and this subject forms the nucleus of this discussion. The experiment was conducted at the end of the entire study of potoroines, when four *Aepyprymnus* only were available. This limitation dictated the form of the experimental design.

Instead of repeating the study reported in Section 8.2 (the first lucerne experiment), in which the diet contained 72% lucerne and 25% maize, the high-lucerne diet was formulated with 62% lucerne and 35% maize. It was thought that the amount of maize would be insufficient to influence cellulolysis to any marked degree and that fibre

digestion on this diet would be much higher than on the maize-rich diet. Surprisingly, the results with both diets showed similar and, relative to the first lucerne experiment, low digestibility of cell-wall constituents. This similarity suggests that there were sufficient rapidly digestible carbohydrates in the high-lucerne diet to disrupt cellulolysis. This hypothesis is supported by the absolute values for ADF and NDF digestibilities, which were 69% and 54% respectively of the values reported in the first lucerne study.

The lucerne used in the present study contained much less cell-wall material than that used in the first lucerne experiment. The diet described in Table 8.2.1 contained 10% more lucerne, but had 42% more ADF and 35% more NDF than the high-lucerne diet fed in the present study. However, simply adding more lucerne to the diet would not have solved the problem because potoroines were reluctant to eat the 62% lucerne diet anyway.

Rode and Satter (1987) conducted a similar experiment with cattle. They fed lucerne hay with maize in unpelleted form to maintain forage-to-grain ratios of 25 or 75%. The ADF content of their high-lucerne diet was about 25%. Although their cattle digested more of the ADF than did the *Aepyprymnus* in the present study, the researchers reported similar digestibilities for cell-wall constituents in both of their diets. Perhaps cellulolysis is determined as much by the soluble carbohydrates in the forage, as it is by those in the grain. Van Soest (1982) mentions that ruminants may also suffer acute indigestion if suddenly fed high-quality hay.

It is interesting to compare the fibre digestion in this study with that reported in Section 8.1, in which potoroines were fed diets containing up to 50% oat hulls. The high-fibre diet in that study and the high-lucerne diet in this study both contained about 20% ADF. However, *Aepyprymnus* fed the lucerne digested 23% of the ADF; those fed the diet of maize and oat hulls digested 8%. Other workers have also shown that the source of plant-cell walls is important in their degradation (for example Hsu *et al.* 1987; Kinser *et al.* 1988; Rowe and Crosbie 1988). The latter two studies suggested a major effect of acid-detergent lignin levels on the digestibility of both ADF and NDF. This is not consistent with the present findings. The high-lucerne diet contained 43% more lignin than did the diet with 50% oat hulls.

The *Aepyprymnus* drank more water when fed the high-lucerne diet. This finding is in agreement with results from the first experiment with a lucerne-based diet. In that study animals drank far more than do potoroines fed diets containing maize and oat-hulls. The relationship between diet and water intake is discussed in Chapter 10.

Another result of interest concerns the animal's ready acceptance of the low-lucerne diet. In fact, they ate more of this diet during the adaptation period than they

did during the measurement period. This observation parallels that in Section 8.2. In that experiment, potoroines chose a high-grain diet in preference to a high-lucerne diet, even though they had been eating the lucerne for a month. It is difficult to explain this finding because roughage-selecting ruminants also — for example, sheep and cattle — will eat much grain if it is suddenly made available (Van Soest 1982). However, there seems to be an important difference between potoroine marsupials and roughage-selecting ruminants. Potoroines showed no ill effects of a sudden switch to a high grain diet; but as mentioned in Section 8.2, the ruminants may die under these conditions.

Do the experiments described in this chapter tell us anything about the diet that wild animals might select? One must always take caution when extrapolating from laboratory conditions to those in the wild. This is particularly true in the present study because the potoroines were fed pelleted diets that always contained some cereals. Even so, the study showed that potoroines can digest a large proportion of the dietary-cell-wall constituents, they can counteract increasing dietary fibre levels by eating more, and they can switch suddenly, without ill-effects, to a starch-rich diet. Each of these factors suggests that potoroines are flexible in their feeding ecology. Presumably, this is matched by a metabolism that can switch quickly from using high levels of short-chain fatty-acids to using high levels of glucose. The question of flexibility is discussed more in the following chapter on microbial digestion in potoroine marsupials.

8.4 Summary

A series of three experiments was conducted to investigate the relationships between digestibility, the source of dietary plant-cell-wall constituents and the ratio of grain to plant-cell-wall constituents in potoroine diets. Potoroine marsupials digested a small proportion only (10-20%) of the structural carbohydrates in diets containing maize and up to 50% oat hulls. Furthermore, this digestion was associated with much variation, both within- and between-animals. Potoroines ate more in response to the nutrient-diluting effect of the oat hulls, but this did not affect NDF digestibility. In contrast to their performance on the maize-oat hull diets, potoroines digested 60% of the NDF in a lucerne-based diet containing 50% NDF. However, the digestibility of NDF in a less fibrous (33% NDF) lucerne-based diet was only 30%. It was concluded that the foregut environment of potoroine marsupials is extremely labile and that cellulolysis is related inversely to the level of soluble carbohydrates in the diet.

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CHAPTER NINE

Microbial digestion in potoroine marsupials

9.1 Introduction

POTOROINE marsupials digest as much as 60% of the neutral detergent fibre that they ingest (Section 8.2). Because no vertebrates are known to produce endogenous cellulases, degradation of plant-cell-wall constituents must proceed by microbial fermentation in the animal's foregut and/or hindgut. In potoroines, most microbial activity would be expected in the foregut because it is about 50% larger than the hindgut (Hume and Carlisle 1985).

It was concluded in Chapter 3 that wild potoroines probably select nutritionally rich foods, that is, foods with relatively low concentrations of cell-wall constituents and relatively high concentrations of more digestible material. Examples are the storage carbohydrates found in the tubers and seeds of plants. From the results of Chapter 8 it was concluded that soluble carbohydrates depress cellulolysis in potoroines, in much the same way as they do in ruminants.

This prompts the question: what is the role of the potoroine foregut? The rate of passage studies (Chapter 7) did not detect selective retention of either the particulate or fluid phase of digesta. Thus, any fermentation in the foregut probably involves all of the ingested material. There seems little purpose in fermenting a highly digestible diet solely to obtain energy-yielding nutrients because, in the process, energy is lost as waste products, viz fermentation gases and heat. Perhaps other aspects of microbial metabolism are needed by the potoroines, for example microbial protein, B-vitamins or detoxification of plant secondary compounds. Kinnear *et al.* (1979) reported an active microbial fermentation in the foregut of *B. penicillata*. These workers analysed a fungal species eaten by *B. penicillata* and found it lysine-deficient. They concluded that, because *Bettongia* have otherwise adequate supplies of nitrogen, the purpose of the fermentation was to improve the quality of the ingested protein. This conclusion is precarious because discrepancies appear in their amino-acid data. But, this aside, we do not know enough about *Bettongia's* feeding ecology to assume that potential amino acid deficiencies are common; nor do we know how much of the ingested protein actually bypasses the fermentation region (Chapter 7). Nevertheless, the importance of microbial protein is well documented. Domestic ruminants are known to survive when all of the dietary nitrogen is from non-protein sources. However, the growth of

domestic ruminants may be enhanced by a supply of bypass protein — that which passes to the abomasum undigested (Preston and Leng 1987).

Does the potoroine foregut have another role? One possible function (and one that is also commensurate with the relatively slow rate of passage of digesta through the gut) is that of digesta storage — a predator evasion strategy (Hume 1982). Potential nocturnal predators of *A. rufescens* and *P. tridactylus* include *Canis familiaris dingo* (dingo) and spotted-tailed quolls *Dasyurus maculatus*. *Potorous* are within the size-range of the prey taken by *Ninox strenua* (powerful owl).

It is apparent that the potoroine foregut may have many functions. The purpose of this study was to provide basic data on gut size and microbial metabolism in potoroines. These data should help to explain the findings of Chapter 8 and elucidate the role of the potoroine foregut.

9.2 Materials and methods

This chapter reports measurements of the concentration and production rates of SCFA *in vitro*. It was undertaken in two parts. Part A describes the microbial activity in digesta taken from the foreguts and hindguts of captive *A. rufescens* and *P. tridactylus* fed a high-concentrate ration; Part B describes similar measurements in digesta taken from *A. rufescens* captured while feeding at Drake in northern NSW. This study area is described in detail in Appendix 5.

In a preliminary experiment such as this, the killing of a large number of animals to obtain measurements throughout the whole 24-hour daily cycle, could not be justified. Therefore, measurements were made only during, or immediately after, the peak feeding period (Chapter 4).

9.2.1 Part A SCFA concentrations and production rates in captive *A. rufescens* and *P. tridactylus*.

The concentrations and rates of production of SCFA were measured in digesta taken from four *A. rufescens* and four *P. tridactylus*. All animals were maintained as described in Chapter 4. They were fed the medium nitrogen-medium fibre experimental diet (Table 9.1) for two weeks before a seven-day balance study (Chapter 4).

Table 9.1 Composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the experimental diet.

Dietary ingredient	Level of inclusion
Maize	300
Wheat	100
Oat hulls	350
Cornflour	220
Mineral mix (Table A1.6)	29
Mineral/Vitamin premix (Table A1.6)	1
Analysis	
Organic matter	921.2
Ash	78.8
Nitrogen	9.5
Acid detergent fibre	145.6
Neutral detergent fibre	269.8
Cellulose	124.3
Hemicellulose	124.2
Lignin	21.3

All *in vitro* measurements were made on the two nights that followed the balance study. Two animals of each species were killed on each night with an overdose of pentobarbitone-sodium administered by cardiac puncture, after sedation with Ketalar. The *A. rufescens* were killed at 4, 6, 7 and 9 hours after darkness; the *P. tridactylus* at 5, 7, 8 and 9 hours.

The gut was immediately removed, weighed and then divided into various segments: forestomach, hindstomach, small intestine, hindgut (proximal colon and caecum) and distal colon and rectum. Each segment was weighed and the pH of the digesta in the forestomach, hindstomach and hindgut was measured using a pH meter (Chapter 4). The contents of the forestomach and hindgut were promptly transferred to warm (36°C) 250 ml glass jars for measurement of SCFA production rates. The digesta were mixed and a zero time-sample taken for determination of the initial dry matter and SCFA concentration. Less than 5 minutes elapsed from the time of death until the start of the fermentation. The jar was purged of air using CO_2, capped, and then immersed in a water bath at 36°C without the addition of buffer or substrates. The same procedure was used to obtain further samples of forestomach ingesta at 15, 30, 60, 90, 120 and 150 minutes after the incubation began. There was enough foregut digesta only for single incubations. Insufficient digesta also limited the incubation of hindgut digesta to a zero time-sample and one later sample — at 60 minutes. Thus, the rate of VFA synthesis in the hindgut could not be calculated by regression analysis.

The mass of each subsample depended on the quantity of digesta available. Subsamples were placed in tared scintillation vials containing 0.5 g saturated $HgCl_2$ to stop further fermentation. They were then frozen at -20°C. The total SCFA concentration and the concentration of individual acids was determined by gas-liquid chromatography (Chapter 4). The production rates of acetic, propionic, isobutyric, n-butyric, isovaleric and n-valeric acids were determined by the zero time method (Carroll and Hungate 1954) from the slope of the linear regression of SCFA concentration on time. The total SCFA production rate was calculated by summing the individual production rates. The energy yield of the fermentation was calculated using the calorific values of the individual acids (Blaxter 1962).

During the incubation the remaining gut segments were weighed, and their contents transferred to plastic vials and frozen at -20°C for possible later analysis.

9.2.2 Part B SCFA production rates in wild *A. rufescens*.

The concentrations and rates of production of SCFA were measured in four wild *A. rufescens*. Three of the animals were captured in April while feeding on the property — Cheviot Hills, at Drake, in northern NSW (Appendix 5). The method of capture is described in that paper. The fourth animal was injured by a car and captured on foot by Mr R.R. Ramsay of Cheviot Hills in July. The animal had a hindlimb broken in a collision with his motor vehicle and was killed soon after. The four animals were captured at about 3, 4.5, 5 and 6 hours after feeding started. They were all killed at about 6 hours after the start of feeding (Chapter 3).

All animals were transferred live to a temporary field laboratory. The first three animals were killed by the methods described in Part A. The fourth animal — that captured by Mr Ramsay — died during sedation with Ketalar. The methods used were the same as those described in Part A but with three exceptions:

1. Measurement of pH was made using narrow-range pH papers. However, contamination of the pH papers meant that pH was measured in only one animal (No 4).

2. At the time of collection digesta samples were strained through gauze cloth into glass scintillation vials containing 0.5 g saturated $HgCl_2$.

3. There was sufficient digesta in the hindgut of two of the four animals to calculate fermentation rates by regression analysis using the zero-time rate of SCFA production.

9.3 Results

9.3.1 Results of the balance study

The results of the balance study are shown in Table 9.2. When expressed as a function of metabolic body mass, the *P. tridactylus* ate more than *A. rufescens* ($P<0.05$). However, the results for *A. rufescens* are influenced by two animals whose feed intake declined during the balance study. These animals lost 5.5 and 6.3% of their body mass during the 7-day collection period, whereas most animals maintained mass. The very poor digestion, by both species, of cell-wall constituents (mean NDF digestibility of 11%), is the finding most relevant to the study of SCFA concentrations and production rates. Expressed differently, this is a coefficient of indigestibility of 90%!

In terms of metabolic body mass, the *P. tridactylus* consumed more nitrogen than the *A. rufescens* ($P<0.05$) but excreted less urinary nitrogen ($P<0.05$) and a similar quantity of faecal nitrogen. Thus, the *P. tridactylus* digested more nitrogen than did the *A. rufescens* ($P<0.01$) and had a higher nitrogen balance ($P<0.01$).

In absolute terms *P. tridactylus* and *A. rufescens* drank similar amounts of water. Therefore, *P. tridactylus* drank more than *A. rufescens* when calculated per unit of metabolic body mass ($P<0.05$). Water intake, per unit of feed intake, was similar between species. This finding is discussed in Chapter 10.

9.3.2 Presentation of SCFA results

The results varied widely between animals, a finding that could often be attributed to the time of sampling in relation to feeding patterns. Because of this variation, it seemed that to establish descriptive statistics, for example means and standard errors, might obscure some interesting data. Therefore, for most parameters, the data for each animal are presented.

9.3.3 Comparisons of digestive tract capacity, gut pH and digesta particle size

Data describing the masses and relative proportions of the gut contents from different segments of the digestive tract of wild and captive *A. rufescens*, and captive *P. tridactylus*, are shown in Table 9.3.

Although the wild *A. rufescens* were all captured between 3 and 6 hours after the estimated onset of feeding, the mass of their gut contents varied markedly — ranging from 5.3 to 11.9% of body mass. The animal captured first (3 hours after nightfall) had less digesta, both in absolute terms (135g) and as a proportion of body mass (5.3%), than two of the animals caught later. A third animal had an intermediate level of fill.

Table 9.2 Intake, digestibility and balance data from A. rufescens and P. tridactylus fed a maize-oat hull diet. Values are means ± sed.

	Aepyprymnus	*Potorous*	sed	sig
number	4	4		
Body mass (g)	2970	866		
(sem)	(305.1)	(45.2)		
Change (% CP*)	-3.2	1.8	1.85	*
Dry Matter				
Intake ($g.d^{-1}$)	60	33	7.5	***
Intake ($g.kg^{-0.75}.d^{-1}$)	37	27	3.5	*
Output ($g.kg^{-0.75}.d^{-1}$)	9	13	1.2	*
Apparent digestibility (%)	64	65	1.1	ns
Energy				
Apparent digestibility (%)	66	67	1.1	ns
DE Intake ($KJ.kg^{-0.75}.d^{-1}$)	173	250	24.0	***
Water				
Intake ($g.d^{-1}$)	105	66	20.6	ns
Intake ($g.kg^{-0.80}.d^{-1}$)	44	74	12.1	*
Intake ($g.100g^{-1}$ DMI)	176	199	31.1	ns
Nitrogen				
Intake ($g.kg^{-0.75}.d^{-1}$)	0.25	0.35	0.033	*
Faecal N ($g.kg^{-0.75}.d^{-1}$)	0.16	0.17	0.010	ns
Apparent Digestibility (%)	37	52	2.7	**
Urinary N ($g.kg^{-0.75}.d^{-1}$)	0.14	0.10	0.022	*
Balance ($g.d^{-1}$)	-0.11	0.08	0.063	*
Balance ($g.kg^{-0.75}.d^{-1}$)	-0.05	0.08	0.024	**

CP* - collection period

Table 9.3 Body mass and measurements of gut capacity in wild and captive Aepyprymnus rufescens and captive Potorous tridactylus.

	Wild *A. rufescens*				Captive *A. rufescens*				Captive *P. tridactylus*			
Animal no.	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Body mass (g)	2700	2700	2550	2700	3200	3600	2600	2350	870	980	800	800
Capture time (h after dark)	4.5	5	3	5	-	-	-	-	-	-	-	-
Slaughter time (h after dark)	6.5	5.5	5.5	5.5	4	6	7	9	5	7	8	9
Wet weight of gut contents (g)	320	191	135	312	192	159	174	241	79	98	78	56
proportion of body mass (%)	11.9	7.1	5.3	11.6	6.0	4.9	6.8	10.8	8.9	10.2	9.6	7.4
Forestomach proportion (%)	71	85	59	75	82	50	63	79	82	67	76	68
Hindstomach proportion (%)	3	4	4	5	11	7	9	13	4	3	6	1
Small intestine proportion (%)	2	7	13	7	1	22	24	4	7	12	5	14
Hindgut proportion (%)	24	6	24	14	10	21	13	4	11	21	14	17

Between 59 and 85% of the total gut contents of wild *Aepyprymnus* were found in the forestomach. Only 6-24% of digesta occurred in the hindgut. However, the hindgut digesta were drier than the foregut digesta; thus 46-73% of digesta dry matter occurred in the foregut and 19-36% in the hindgut.

The body masses of the captive *A. rufescens* varied widely (2350-3600 g); those of the wild animals little (2350-2700 g). However, the mass of gut contents as a proportion of body mass (4.9-10.8%), the proportion of forestomach digesta (50-82%; 37-60% DM) and of hindgut digesta (4-21%; 8-31% DM) covered similar ranges in captive and wild animals. However, the four captive animals had eaten at least 70% of their normal daily intake; at least two of the wild animals probably had not.

The relative capacity of the potoroo gut was similar to that of *A. rufescens*. Between 7.4 and 10.2% of the potoroo's body mass was digesta, of which most (67-82%, 53-76% DM) occurred in the forestomach and much less (10-21%, 11-31% DM) in the hindgut.

The single wild *A. rufescens* for which pH measurements were obtained had readings of 5.9, 2.8, 6.6 and 6.0 for the pH of digesta in the forestomach, hindstomach, ileum and caecum respectively. In contrast, the captive *A. rufescens* and *P. tridactylus* had a forestomach pH of 4.1-4.5 and hindgut pH of about 6. The pH of digesta in other gut segments were similar to those found in the wild *A. rufescens*.

The distribution of particle sizes in the gastrointestinal tracts of wild *A. rufescens* are shown in Table 9.4. The noticeable feature was the high proportion of fine particles in the fermentation chambers — the forestomach, and the caecum and proximal colon.

Table 9.4 Particle size distributions in the gastrointestinal tracts of wild A. rufescens. Values are means ± their standard errors

	Sieve size (μm)					
Organ	**1200**	**600**	**300**	**150**	**75**	**<75**
Sacciform forestomach	9	12	11	7	4	58
(n=7)	2.3	1.5	1.7	1.1	0.9	4.3
Small intestine	3	28	21	15	6	27
(n=2)	0.2	1.1	2.0	2.8	0.8	5.3
Caecum and proximal	5	12	13	8	11	53
colon (n=2)	0.1	0.6	1.2	0.7	0.1	0.1
Distal colon	10	17	14	9	8	4
(n=4)	2.2	3.9	0.6	1.1	0.9	2.4

9.3.4 Total concentration and molar proportions of individual SCFA (Table 9.5)

Of the four wild *A. rufescens*, the digesta of one (No. 3) contained a large quantity of an unknown compound. This compound had a similar gas-liquid chromatography elution time as the C4-SCFA — isobutyric and n-butyric acids. This made it impossible to measure the concentrations and rates of production of the C4-SCFA and the total concentration and rates of production of SCFA. There was insufficient sample to identify the unknown compound. However, it was probably a terpene (T.M. Sutherland pers. comm.). The forestomach digesta of this same animal contained much gum, presumably *Acacia spp.*

In three of the four wild *A. rufescens*, the concentrations of SCFA were higher in the foregut digesta than in the hindgut digesta. Animal 3 was possibly an exception: in its hindgut, SCFA concentrations were apparently higher. However, when allowance is made for the C4-SCFA (which could not be measured in the foregut of this animal), the concentrations were probably similar in the forestomach and hindgut digesta. The fourth of the wild *A. rufescens* — that captured by Mr Ramsay — had lower concentrations of SCFA in the digesta. This animal was different because it was severely injured by a car just before capture.

In captive animals, SCFA concentrations were generally higher in the hindgut than in the forestomach. This finding was pronounced in the two *A. rufescens* killed after the peak feeding period, because they had trace levels only of SCFA in their forestomach digesta (<10 $mmol.l^{-1}$). The two *P. tridactylus* killed at the same time had SCFA concentrations similar to those in the *P. tridactylus* killed earlier in the feeding cycle.

In all animals, the molar proportion of acetic acid was lower in the forestomach than in the hindgut, but propionic acid was higher. This was most apparent in the wild *A. rufescens* in which 27-43% of the forestomach SCFA was propionic acid, but only 12-16% of the hindgut SCFA was propionic acid. In captive and wild *A. rufescens*, a greater proportion of n-butyric acid occurred in the forestomach than in the hindgut. The opposite occurred in the *P. tridactylus*. The proportion of n-valeric acid was higher in the foregut than in the hindgut digesta, which contained little. The forestomach digesta of wild *A. rufescens* contained more n-valeric acid than that of captive animals.

9.3.5 Production of SCFA (Table 9.6)

All animals synthesized SCFA ($mmol.l^{-1}.h^{-1}$) at a higher rate in the hindgut digesta than in the forestomach digesta. A typical fermentation pattern for one wild *A. rufescens* is shown in Figure 9.1. In captive and wild *A. rufescens*, the rates were similar in the hindgut digesta. The mean rate of production of SCFA in the foregut

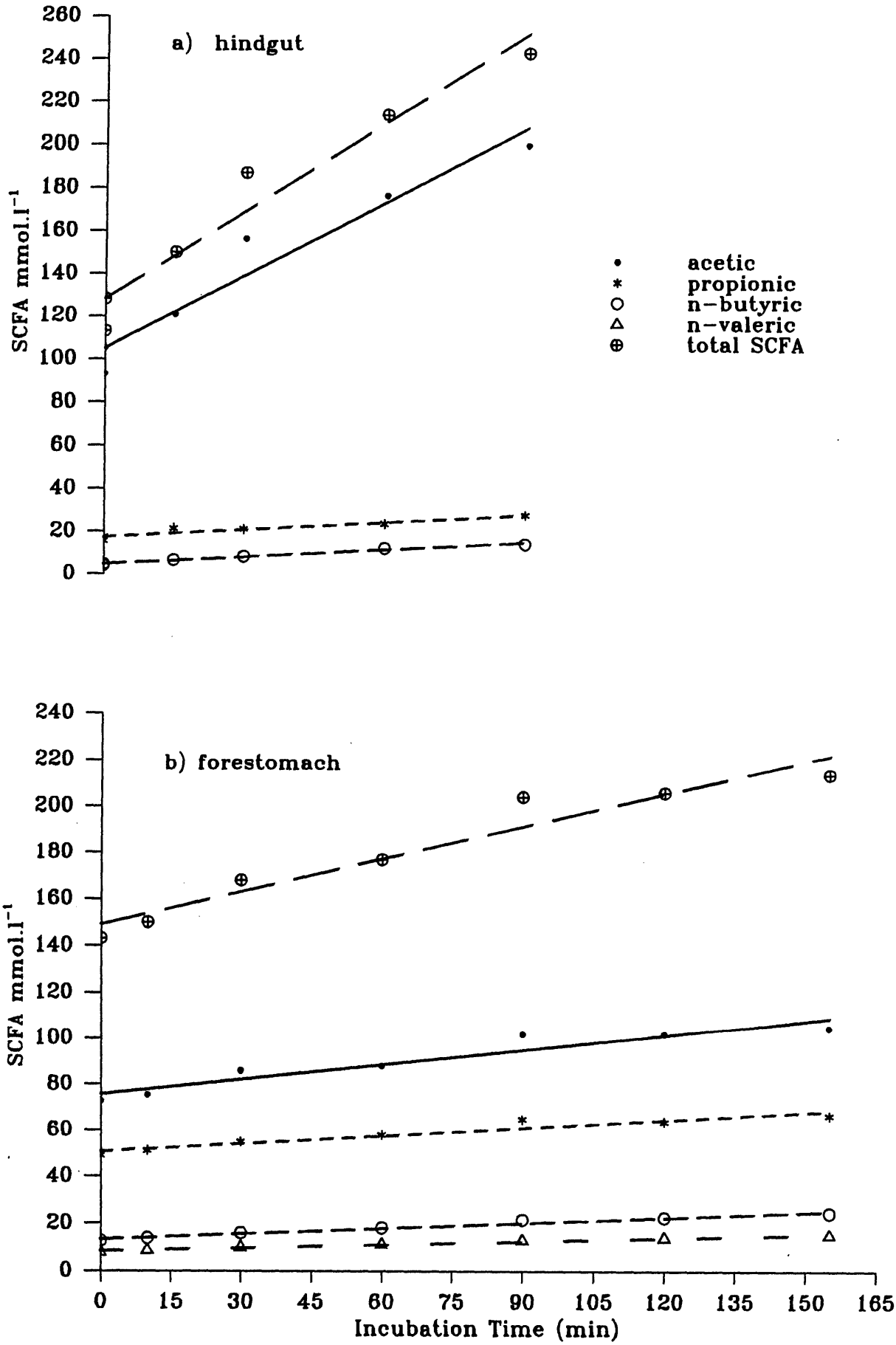

Fig 9.1a,b The change in concentration with time of SCFA in the forestomach and hindgut of one wild A. rufescens

Table 9.5 Total SCFA concentration (mmol.l^{-1}) and molar proportions (%) of individual SCFA in digesta from the forestomach (FG) and hindgut (HG) of wild and captive A. rufescens and captive P. tridactylus.

	Animal number						Animal number					
	1	2	3	4			1	2	3	4		
***Aepyprymnus* (wild)**	**FG**	**FG**	**FG**	**FG**	**mean**	**sem (n=4)**	**HG**	**HG**	**HG**	**HG**	**mean**	**sem (n=4)**
total	143	140	126	104	128	8.9	113	115	147	71	111	15.6
acetic	51	45	50	45	48	1.6	82	82	78	79	80	1.0
propionic	34	41	43	27	36	3.6	14	14	16	12	14	0.8
iso-butyric	—	—	?	—	—	—	—	—	1	—	—	—
n-butyric	9	8	?	13	10	1.5	3	3	4	9	5	1.4
iso-valeric	—	—	—	—	—	—	—	1	—	—	—	—
n-valeric	6	6	7	14	8	1.9	—	—	1	—	—	—
***Aepyprymnus* (captive)**	**FG**	**FG**	**FG**	**FG**	**mean**	**sem (n=4)**	**HG**	**HG**	**HG**	**HG**	**mean**	**sem (n=4)**
total	84	82	2	10	83	1.1	125	123	53	49	88	21
acetic	49	38	—	—	43	5.5	59	63	63	62	62	0.9
propionic	34	40	—	—	37	3.0	29	27	25	28	27	0.9
iso-butyric	—	—	—	—	—	—	—	—	1	1	—	—
n-butyric	13	15	—	—	14	1.0	9	8	8	7	8	0.4
iso-valeric	—	—	—	—	—	—	1	—	4	2	2	0.3
n-valeric	4	6	—	1	5	1.0	2	1	0.5	1	1	0.3
***Potorous* (captive)**	**FG**	**FG**	**FG**	**FG**	**mean**	**(sem) (n=4)**	**HG**	**HG**	**HG**	**HG**	**mean**	**(sem) (n=4)**
total	87	55	74	92	77	8.3	97	119	74	98	97	9.2
acetic	49	39	51	43	46	2.8	73	63	71	71	70	2.2
propionic	45	54	27	49	44	5.9	17	28	21	22	22	2.3
iso-butyric	—	1	—	—	—	—	—	1	—	—	—	—
n-butyric	3	3	15	4	6	2.9	6	6	6	5	6	0.3
iso-valeric	—	—	—	—	—	—	1	—	—	—	—	—
n-valeric	3	4	7	4	5	0.9	2	2	2	1	2	0.3

— - <0.5 mmol.l^{-1}

? - C4-SCFA could not be measured

Table 9.6 Rates of production of SCFA in the forestomach (FG) and the hindgut (HG) of wild and captive A. rufescens and captive P. tridactylus

Animal	Fluid volume (ml)		SCFA production rate ($mmol.l^{-1}.h^{-1}$)		SCFA production rate ($\mu mol.g^{-1}.h^{-1}$)	
***Aepyprymnus* (wild)**	**FG**	**HG**	**FG**	**HG**	**FG**	**HG**
1	199	61	28	83	190	333
2	140	10	25	34	159	120
3	68	26	*	40	*	149
4	196	33	28	34	141	110
mean	150	33	27	48	163	178
sem	30.7	10.6	1.0	11.8	14.3	52.0
***Aepyprymnus* (captive)**	**FG**	**HG**	**FG**	**HG**	**FG**	**HG**
1	136	13	24	54	152	103
2	68	24	36	37	200	85
3	88	15	**	19	**	45
4	172	7	**	66	**	157
mean	116	15	30	44	176	99
sem	23.5	3.5	6.0	10.2	24.0	23.2
***Potorous* (captive)**	**FG**	**HG**	**FG**	**HG**	**FG**	**HG**
1	48	6	43	**	128	**
2	53	14	57	76	233	137
3	47	8	53	83	203	214
4	27	7	63	78	140	161
mean	44	9	54	79	176	171
sem	5.7	1.8	4.2	2.1	25.1	22.7

* - could not be determined

** - negligible production

digesta from wild animals (27 $mmol.l^{-1}.h^{-1}$, n=3) was similar to that in the two captive animals measured during the feeding period (30 $mmol.l^{-1}.h^{-1}$). SCFA production rates were consistently higher in both the forestomach and hindstomach digesta from *P. tridactylus* than in digesta from *A. rufescens*. When the rates of SCFA production were expressed as mmol per gram per hour, there were no clear trends. The mean values were similar in the forestomach and hindgut of wild *A. rufescens* (163 and 178 $mmol.g^{-1}.h^{-1}$ respectively), the forestomach of the captive *A. rufescens* (176 $mmol.g^{-1}.h^{-1}$) and the forestomach and hindgut of the captive *P. tridactylus* (176 and 171 $mmol.g^{-1}.h^{-1}$). The rate was less in the hindgut of the *A. rufescens* (98 $mmol.g^{-1}.h^{-1}$).

Although the rate of SCFA production was higher in the hindgut than in the forestomach, the total hourly production of SCFA during the incubation period was usually 3-10 times higher in the forestomach, because of its larger capacity, than in the hindstomach. The exceptions were the *A. rufescens* killed after the peak feeding period; their forestomachs had negligible SCFA.

Often there were changes in the molar proportions of SCFA during the incubation period. Details are shown in Table 9.7.

Table 9.7. Ratios of the production rates of acetic, propionic and butyric acids to their initial molar proportions, in zero time digesta samples, taken from the forestomach and hindgut of A. rufescens and P. tridactylus (mean ± sem).

Experiment	No.	Acetic	Propionic	Butyric
Ar (wild) FG	3	0.99 ± 0.072	0.84 ± 0.092	1.59 ± 0.276
Ar (wild) HG	4	0.96 ± 0.029	1.06 ± 0.149	1.87 ± 0.214
Ar (capt) FG	2	0.79 ± 0.065	0.87 ± 0.335	1.46 ± 0.828
Ar (capt) HG	4	0.95 ± 0.040	1.17 ± 0.135	1.16 ± 0.118
Pt (capt) FG	4	1.03 ± 0.059	0.87 ± 0.044	1.27 ± 0.172
Pt (capt) HG	3	0.91 ± 0.011	1.20 ± 0.101	1.33 ± 0.127

Ar - *A. rufescens*; **Pt** - *P. tridactylus*

9.4 Discussion

The level of gut fill depends on many factors, among which are body size, diet, metabolic rate and the time of measurement in relation to feeding patterns (Dellow *et al.* 1988). According to the equations of Parra (1978), a herbivore of mass 2.7 kg (the mass of the wild *A. rufescens* in the present experiment) should have about 180 g of fermentation contents. In reality the "normal gut fill" of any species covers a range of values and can be determined only with a large number of animals. The killing of many animals could not be justified in the present study and instead conditions were standardized. Wild animals were captured at least 3 hours after nightfall, and captive animals were killed when they had eaten most of their normal daily intake. Even so, there was still much variation in gut fill. The forestomach, of two of the wild *Aepyprymnus* appeared distended and weighed almost 11% of body mass, which is more than would be expected from the equation of Parra (1978); the forestomachs of the other two animals contained less digesta. Forestomach capacity covered a similar

range of values (5-11% of body mass) in the captive animals. This range is consistent with published values for other small herbivores, for example the browsing wallabies — *S. brachyurus* (Moir *et al.* 1956), *T. thetis* (Dellow *et al.* 1983; Dellow *et al.* 1988) and *M. eugenii* (Lintern-Moore 1973a; Dellow *et al.* 1983), and the "concentrate-selecting" ruminants — *Nesotragus moschatus* (Hungate *et al.* 1959; Hoppe *et al.* 1983), *Madoqua kirki*, *Aepycerus melampus* and *Gazella granti* (Hoppe *et al.* 1983; Hoppe *et al.* 1977a,b). As expected, the gut capacity of potoroines is less than that of the larger grazing macropodids, for example *M. giganteus* and *M. r. robustus* (Dellow *et al.* 1988), and supports the notion that potoroines are probably concentrate selectors. The high proportion of very fine particles in the forestomach of wild *A. rufescens* suggests that the diet is readily triturated. This supports the observations reported in Chapter 3 that *Aepyprymnus* select mainly tubers.

Hume and Carlisle (1985), in a study of captive *A. rufescens*, reported that the stomach constituted 50% of total gut capacity and the hindgut 35%. On the basis of these measurements and the finding that digesta passes quickly to the hindgut, Hume and Carlisle (1985) proposed that "the forestomach may be less important, and the hindgut more important, in fermentative digestion in the Potoroinae than in the Macropodidae". For much the same reasons, Frappell and Rose (1986) concluded that the hindgut is more important than the foregut in the digestive physiology of *P. tridactylus*. Their findings have been discussed already in Section 7.4. The results of the present study contrast with those of the earlier studies. The total mass of digesta in all *A. rufescens* was higher than the mean reported by Hume and Carlisle (1985); the distribution of digesta also was different. The present study found that 71% of digesta occurred in the forestomach and only 14% in the hindgut. The distribution of digesta in *P. tridactylus* was similar. Both findings agree with similar studies on the gastrointestinal tracts of small macropodids: *S. brachyurus* (Moir *et al.* 1956), *T. thetis* (Hume 1977a) and *T. thetis* and *M. eugenii* (Dellow *et al.* 1983). Animals in the present study were sampled during the peak feeding period, so the mass of the fermentation contents often exceeded the predicted values of Parra (1978) and presumably approached the maximum. On the other hand, the *Aepyprymnus* measured by Hume and Carlisle (1985) had minimal gut fill and represented the other extreme — of animals that had fasted for several hours. Thus, based on gut capacity, both the forestomach and hindgut are of similar importance in potoroine marsupials and macropodids.

The *in vitro* technique of Carroll and Hungate (1954) was used to measure production rates of SCFA in the present study, because legal constraints prevented surgical techniques and use of an *in vivo* method. *In vitro* techniques have several limitations. They often involve a significant delay between the animal's last feed and

its death; more time is lost before the incubation begins. Because foregut fermentation rates are very high immediately after the start of feeding (Sutherland *et al.* 1962), any lag will cause *in vitro* techniques to underestimate the *in vivo* fermentation rate in this organ. However, the digesta reaching the hindgut are usually less digestible, and give rise to a slower fermentation than in those entering the foregut. Therefore, lag-time has a minimal effect on *in vitro* methods for measuring SCFA production rates in hindgut digesta (Faichney 1969). Any exposure of digesta to aerobic conditions — for example, during transfer to incubation vessels or subsampling — will depress fermentation. The chance of contamination by oxygen is clearly greater with the limited digesta of small animals. Because of these factors, it is not surprising that, in direct comparisons of *in vitro* and *in vivo* techniques, for example in sheep and cattle (Whitelaw *et al.* 1970), *Procavia habessinica* (Rübsamen *et al.* 1979), and macropodids (Dellow *et al.* 1983), the former method usually gives fermentation rates that are several fold less than rates obtained with the latter method. Nevertheless, *in vitro* comparisons between species should be valid if conditions such as lag phase and time of slaughter relative to feeding patterns are standardized.

Ruminant diets that are rich in soluble carbohydrates give rise to a rapid fermentation leading to a low rumen pH and high molar proportion of propionate (Whitelaw *et al.* 1970). These characteristics were found also in the present experiments. Considerable propionic acid was produced by the forestomach fermentation in both wild and captive *A. rufescens* and in *P. tridactylus*. However, the hindgut fermentations of wild and captive potoroines were different. The relatively low proportion of propionate in the hindgut of wild *A. rufescens* shows that little soluble material escapes the digestive processes in the forestomach and the intestine. Thus, the substrate available to the hindgut microbes is predominantly fibrous. In contrast, much propionate occurs in the hindgut of both captive species. This suggests that some intact starch grains pass to the hindgut. There are at least two explanations for this. Both depend on incomplete degradation of starch in the small intestine. First, part of the diet bypasses the SFS and traverses the gut rapidly (Chapter 7). Secondly, the rapid fermentation in the forestomach and concomitant fall in pH inhibited most microbial activity in the forestomach. Thus, starch grains passed from the forestomach intact and some were eventually fermented to propionate in the hindgut. This may happen also in ruminants. When sheep (Ørskov *et al.* 1970) or cattle (Siciliano-Jones and Murphy 1989) are fed maize, significant amounts of starch escape degradation in the rumen and the intestine, and are ultimately fermented in the caecum.

The concentration of SCFA is the balance between production and absorption. Engelhardt and Rechkemmer (1983) stated that most mammals maintain concentrations of SCFA of about 100 mM in the hindgut, regardless of their diet or type of digestive

system. The same seems to apply in the forestomach of foregut fermenters. Boomker and Van Hoven (1983) listed the SCFA concentrations in the rumen-reticulum of 32 African ruminants, encompassing a range of digestive strategies. The mean SCFA concentration was 132 mM which is probably an overestimate because of the lag-time between death and sampling. Similar values are found in the forestomach and hindgut of various macropodoids (Table 9.8).

Table 9.8 Comparative data on the SCFA concentrations in the forestomach and hindgut of various macropodoids measured after feeding.

Species	**SCFA conc. ($mM.l^{-1}$)**		**Reference**
	FG	**HG**	
Setonix brachyurus	105	66	Moir *et al.* (1956)
Macropus rufogriseus	129	66	Hume (1977a)
Thylogale thetis	120	72	Hume (1977a)
T. thetis	97	61	Dellow *et al.* (1983)
M. eugenii	94	63	Dellow *et al.* (1983)
M. giganteus	111	60	Dellow *et al.* (1983)
M. giganteus	100	68	Kempton *et al.* (1976)
M. r. robustus	103	64	Dellow *et al.* (1988)
Wallabia bicolor	59	49	Dellow *et al.* (1988)
B. penicillata	89[a]	**nd**	Kinnear *et al.* (1979)
A. rufescens (wild)	128	112	This study

nd — not measured

[a] — some animals fasted before sampling

In keeping with most macropodoids (Table 9.8), the SCFA concentrations were higher in the forestomach than in the hindgut of wild *A. rufescens*. This general trend in macropodoids is expected, because the material reaching the hindgut is less digestible than that in the foregut. Also, values for SCFA concentrations in forestomach digesta are probably overestimated due to the lag-time between death and sampling. The higher SCFA concentrations in the hindgut digesta of the wild *A. rufescens*, compared with most macropodoids, are difficult to explain. However, as discussed later, the SCFA production rates were higher in the hindgut of *A. rufescens* than in the hindgut of other macropodoids.

Captive potoroines had lower SCFA concentrations in the forestomach digesta compared with those in their hindgut digesta. All measurements were made on

potoroines that had just eaten. It was clear that, in the forestomach of captive animals, the rate of organic-acid production exceeded the buffering capacity. Thus, the pH of the forestomach digesta fell rapidly to about 4.3, well below pH 6 — the point at which pH stress begins (Mackie and Gilchrist 1979). The low forestomach pH supports the argument in Chapter 8 that the cell-wall constituents in the grain-oat hull diets were poorly digested because of the dominance of amylolytic organisms. Indeed, a pH of 4.3 lies midway between the mean pKa values of acetic acid and propionic acid (4.82), and the pKa of lactic acid (3.86). Moir *et al.* (1956) reported a low pH in the foregut of *S. brachyurus* fed a concentrate diet. They attributed this high acidity to a lactic-acid fermentation.

Why does the fermentation rate exceed the potoroine's buffering capacity? Ruminants may provide the answer. The sudden ingestion of large amounts of starch by domestic ruminants causes severe rumenitis. The absorption of large amounts of lactic acid across the rumen wall causes systemic acidosis and death. However, neither of these symptons arises if the diet is introduced over several days, allowing the rumen flora to adapt. The captive potoroines used in the present study had always been fed starch-rich diets. Because captive animals grow and reproduce on these highly digestible diets, which are known to produce a low forestomach pH, it might be speculated that potoroines have evolved to tolerate a sudden change to high-starch diets. Highly digestible foods are probably not always available to wild potoroines. Instead, potoroines must sometimes obtain more of their nutritive requirements from the products of microbial metabolism. At this time a sudden change to starch-rich foods — for example seeds, could be catastrophic if the animals could not tolerate a low forestomach pH.

In ruminants, buffering capacity is, in part, proportional to saliva flow which, in turn, is related to the rate of chewing (Chapter 2). Furthermore, those species with high fermentation rates have mucosal adaptations that swiftly remove the SCFA (Hofmann 1989). The pelleted diet fed in the present study needed minimal chewing, thus probably suppressing salivary flow and consequent buffering capacity. However, among potoroine marsupials, at least some *A. rufescens* can survive with a minimal flow of saliva. One female continually reproduced, even though its salivary glands had regressed and were not producing saliva (Beale pers. comm.). Despite this being a single observation that must obviously be treated with caution, it prompts the question: how important is saliva in potoroine marsupials? An answer was beyond the scope of the present study, but now forms part of an investigation by Beale at the University of NSW. He may find that these marsupials have developed mechanisms independent of saliva to counter low-forestomach pH.

Do the forestomach contents of wild potoroines ever become highly acidic? The pH of digesta could be measured in only one of the wild animals. However, the pH of the forestomach digesta in this animal (5.9) indicates that the rate of fermentation did not exceed the animal's buffering system. In studies of *B. penicillata*, whose diet was predominantly hypogeous fungi, Kinnear *et al.* (1979) reported that forestomach pH ranged from 5.5 to 6.8. However, most potoroines at times select foods that are rapidly fermentable (Chapter 3) and which might be expected to cause low forestomach pH.

SCFA were produced at a greater rate in the hindgut than in the forestomach of wild *A. rufescens*. However, because of the larger capacity of the forestomach, up to 10 times more SCFA were produced in this organ than in the hindgut.

Table 9.9 The rates of production of short-chain fatty-acids (mmol.l^{-1}.h^{-1}) in several small foregut-fermenting herbivores.

Species	Diet	Site	SCFA production	Reference
Eutherian				
Sheep	lucerne	R	23	Hume (1977a)
		C	16	Hume (1977a)
Sylvicapra grimmia	browse	R	47	Boomker and Van Hoven (1983)
		C	70	
Madoqua kirki	browse	R	89*	Hoppe *et al.* (1983)
Nesotragus moschatus	browse	R	81*	Hoppe *et al.* (1983)
	?	R	70	Hungate *et al.* (1959)
	?	C	16	Hungate *et al.* (1959)
Metatherian				
M. giganteus	grass	SFS	29	Dellow *et al.* (1988)
		TFS	21	Dellow *et al.* (1983)
M. rufogriseus	lucerne	FG	52	Hume (1977a)
		HG	27	Hume (1977a)
T. thetis	lucerne	FG	39	Hume (1977a)
		HG	29	Hume (1977a)
		FG	29	Dellow *et al.* (1983)
M. eugenii	lucerne	FG	19	Dellow *et al.* (1983)
A. rufescens	natural	FG	27	This study
		HG	48	This study
Macropodoid mean		FG	31	
		HG	35	

* - assuming that 0.9 mole SCFA is produced per mole of gas.
R - rumen; **C** - caecum; **SFS** - sacciform forestomach;
TFS - tubiform forestomach; **FG** - foregut; **HG** - hindgut

The rates of production of SCFA in the forestomach and hindgut of wild *A. rufescens* compared with those of other small foregut-fermenting herbivores are shown in Table 9.9. The rate of production in the forestomach of wild *A. rufescens* was similar to the rate reported for sheep and similar also to the macropodoid "mean". All these rates were much lower than those reported for some small African ruminants, in particular *M. kirki* and *N. moschatus*. In African wild ruminants, reticulo-rumen fermentation rates and body mass are inversely correlated (Hoppe 1977a,b). It is interesting to note that the mean temperature of the rumen contents of *M. kirki* was 39.6°C (Hoppe 1977). This is almost certainly higher than that of macropodoids, whose typical body temperature is about 36°C. Thus, the difference between fermentation rates of African ruminants and of macropodoids is probably not just a function of diet. The lucerne hay eaten by the macropodids (Table 9.9) contained 2.5-3.2% nitrogen, which is similar to that in the dicotyledenous leaves eaten by *N. moschatus* and *M. kirki* (Hoppe *et al.* 1983). On the basis of the limited data available, it seems that any relationship between body mass and fermentation rate is not as pronounced in macropodoids as in wild African ruminants.

The rate of production of SCFA in the hindgut of wild *A. rufescens* was higher than the rates reported in macropodids. This may suggest that the digesta reaching the hindgut of *A. rufescens* is more fermentable than that passing to the hindgut of macropodids. However, this is not confirmed by the molar proportions of SCFA in the hindgut of *A. rufescens*. The proportions resemble those in the hindgut of *M. rufogriseus* and *T. thetis* (Hume 1977a) and are dominated by acetic acid. It is of interest that Milton and McBee (1983) reported very high fermentation rates that produced mainly acetate in the hindgut of *Alouatta palliata*. Therefore, some other factor may stimulate cellulolysis in the hindgut of *A. rufescens*. Perhaps the cell-wall constituents reaching the hindgut of *A. rufescens* are more digestible than those reaching the hindgut of the other wallabies; or, perhaps, the hindgut of *A. rufescens* in some way sequesters nitrogen and this promotes higher rates of microbial growth than occurs in the hindgut of other macropodoids. The high proportion of small paricles in the hindgut of wild *A. rufescens* supports this notion. Unfortunately, it is based on few observations and should be treated with caution. Nevertheless, the small capacity of the potoroine hindgut means that fermentation in that organ will contribute relatively little to the animal's energy budget.

Extrapolation to daily production rates of fermentation from rates obtained during a two hour *in vitro* incubation is precarious. Steers exhibit marked diurnal variation in ruminal SCFA concentrations in response to the pattern of feeding (Siciliano-Jones and Murphy 1989). In the four hours immediately after feeding, SCFA production may vary four-fold in cattle (Whitelaw *et al.* 1970) and rabbits (Parker and McMillan 1976).

Thus, extrapolation of SCFA production rates obtained in short-term studies must be restricted to animals that eat for many hours each day, keep gut-fill relatively constant and have a constant intake of fermentable substrates — those animals whose fermentations approach steady-state conditions. These conditions were reported in the rumino-reticulum of several East African ruminants (Hoppe *et al.* 1977a,b; 1983) and in the caecum of the pony (Glinsky *et al.* 1976). Extrapolation cannot be justified in potoroines because, whether captive or wild, they feed for only a few hours each day (Chapter 4). There is evidence in the present study, albeit limited, that wild animals also eat quickly. The animal caught first (three hours after the estimated start of feeding) had much less digesta in its gut than animals caught only two hours later and whose forestomachs appeared distended. From the limited data available, it is clear that steady-state conditions are not a feature of the potoroine gut.

Instead of trying to extrapolate the SCFA production rate to daily energy needs, it seems more pertinent to examine the potential contribution of microbial digestion. The *A. rufescens*, *P. tridactylus* and *B. penicillata* in Section 8.2 digested 57, 53 and 56% of dietary cellulose; and 83, 78 and 76% of hemicellulose respectively. Using the heats of combustion of 16.8 and 15.9 $kJ.g^{-1}$ for cellulose and hemicellulose respectively (Blaxter 1962), and assuming that the efficiency of SCFA synthesis from carbohydrates is 70% (Hungate 1975), it was calculated that *A. rufescens*, *P. tridactylus* and *B. penicillata* obtained 38, 31 and 31% of their digestible energy intake (DEI) from hydrolysis of these cell-wall constituents. At the other extreme are the captive animals in the present study. These animals digested negligible cell-wall constituents. Nearly all of their DEI must have come from enzymatic digestion. The proportion of the ingested soluble carbohydrates that is fermented cannot be determined from the present study. However, a pH of about 4 would severely inhibit most microbial metabolism (Mackie *et al.* 1978) and, indeed, this was noted in two of the captive *A. rufescens*, in which negligible SCFA were detected.

The disparity between the proportional production rates of acetic, propionic and butyric acids and their initial molar concentrations suggests selective absorption of particular SCFA. In the hindgut, the relative rates of apparent absorption were butyrate > propionate > acetate. This was expected, because the rate of flux is dependent on chain length (Vernay 1986). The much slower absorption of propionate from the forestomach was unexpected, because SCFA uptake depends also on concentration (Vernay 1986). However, the same workers identified several other mechanisms by which SCFA are transported from the lumen — for example, the movement of cations. Thus, at least with propionate, it seems that different absorption mechanisms are operating in the forestomach and in the hindgut.

9.5 Summary

The results from the present study indicate that potoroine marsupials are flexible. Production rates of SCFA in wild *A. rufescens* were similar to those reported for macropodids. Although SCFA were produced at faster rates in the hindgut, the total contribution from this organ is probably much less than total SCFA synthesis in the much larger forestomach. Results from the captive animals showed that potoroine marsupials can tolerate lower pH in the forestomach than can domestic ruminants. Because amylolytic bacteria can tolerate lower gut pH than can cellulolytic organisms, there is the possibility of significant lactate production in the potoroine sacciform forestomach. This possibility was supported by the pH of the forestomach (4.3). However, the high acidity means that microbial growth would be inhibited and that the animal must then obtain all of its nutrient requirements from enzymatic processes. It seems that the forestomach can serve both as a storage organ and as a fermentation chamber. In the latter, microbial SCFA synthesis can make an important contribution to the animal's requirement for energy-yielding nutrients. Accordingly, the animal's metabolism must be equally flexible in switching from relatively high levels of SCFA to one in which glucose is the predominant energy substate absorbed.

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CHAPTER 10

Water metabolism in potoroine marsupials

10.1 Introduction

THE importance of water for the correct functioning of the body is emphasized by the fact that about 72% of the fat-free body mass is water. About thirty percent of this water is found in the "extracellular" compartment — blood plasma, lymph and interstitial fluid and the gut lumen; seventy percent is present in the "intracellular" water — that is in the cells. Water is involved in most, if not all, metabolic reactions (Mitchell 1962). For example, the extracellular water is the "common carrier" in the body moving gases, stored and absorbed nutrients, waste products and hormones from cell to cell and from organ to organ; the circulating fluid exercises an important function in the regulation of body temperature by transporting heat from the deep body tissues for dissipation at the body's surfaces; water provides the high solvent power within which metabolic reactions occur; and water contributes much of the lubrication necessary for processes such as deglutition.

An animal's water supply is provided from its food, both as free water and from water formed in metabolism, and from drinking water. Countering these gains are losses from the body in the urine, faeces and milk and by evaporation from the skin and respiratory surfaces. Because an animal in water balance maintains a more or less constant body water pool, the requirement for water equals the minimal losses. Thus, an animal's water requirement and, to some degree, its ecological distribution are determined by its ability to curtail water losses.

Many Australian marsupials inhabit arid environments that present serious physiological problems. While this does not apply to potoroines in their current distributions, it certainly applied before the influence of Europeans (Chapter 3). At that time potoroines were widespread: one species, *Caloprymnus campestris*, inhabited one of the hottest, driest and most exposed regions of the country. Other species — for example, *Bettongia leseur* and *B. penicillata* — had distributions that included arid regions. Macfarlane (1976) suggested that evolution in wet areas is associated with high water-turnover rates, while low rates are associated with arid regions. Adaptations to arid environments are seen in at least some potoroine marsupials. For example, *B. leseur* has a relative medullary thickness (8.4), as high as any other marsupial yet

measured (Yadav 1979). In contrast, *Potorous spp* have always inhabited mesic areas; the range of *A. rufescens* was intermediate and probably impinged upon arid areas.

In addition to genetic and environmental factors (temperature, humidity etc), the water requirement of animals is influenced by other factors. These include dry-matter intake (Macfarlane and Howard 1972); nitrogen content of the ration (Ritzman and Benedict 1938 cited by More and Sahni 1981); mineral content of the ration (Van Soest 1982); dietary-fibre content and digestibility (Buffenstein 1984); dietary-water content; the level of metabolism (Macfarlane and Howard 1972; Macfarlane 1975); growth; and lactation (Hulbert and Gordon 1972; Hulbert and Dawson 1974; Macfarlane and Howard 1972). Also, water intake varies widely between individuals within a species (More and Sahni 1981).

In the present study, water intake was measured in all experiments. Thus, the influence of a number of the aforementioned factors — dietary nitrogen and cell-wall content, dry-matter intake, cell-wall digestibility and lactation — on water metabolism in potoroine marsupials was established. The tolerance of *A. rufescens*, *B. penicillata* and *P. tridactylus* to water restriction was examined in conjunction with the studies of urea metabolism described in Chapter 6. Water turnover was measured in winter and summer in the same three species housed in outdoor enclosures. Finally, water turnover was measured in winter and summer in free-living *A. rufescens*, as part of the energy metabolism studies described in Chapter 11. All these results are brought together in this chapter on water relations in potoroine marsupials.

10.2 Materials and Methods

Because the data in this chapter are taken from experiments reported elsewhere in this thesis, the reader is referred to the original chapters for details of experimental procedures. The results reported in this chapter are divided into three parts: 1) measurements of water intake/output/turnover made in metabolism cages or respirometers; 2) measurements of water turnover in captive animals housed in outdoor enclosures; and 3) measurements of water turnover in free-living *A. rufescens*.

In this chapter the term *water turnover* refers to the water influx/efflux of an animal whose body mass is more or less stable for the duration of an experiment. Clearly, not all of the body mass lost from animals with restricted access to water is water *per se*; they lose body mass also from catabolism of tissues. Under these circumstances water turnover does not equate with water influx/efflux. Thus, the terms *water efflux* and *water influx* are used when marked changes in body mass occur.

10.2.1 Measurements of water relations in caged potoroine marsupials

The general procedures for measurements of water intake and output are described in Chapter 4. In some experiments — for example, the water restriction, rate of passage of digesta and the lucerne studies — faeces were collected as soon as they were excreted. This made it possible to measure faecal-water loss. In many experiments, water excretion in the urine was not measured, because little urine was produced. Collection trays were washed with water to ensure quantitative collection of urinary nitrogen. However, in the lucerne experiments, the trays were not washed down because potoroines drank much more and produced copious urine. Thus, only a small proportion of the urine was left on the trays.

In the second water-restriction experiment (Experiment 6.2), rehydration was studied in animals which, for the previous two weeks, had limited access to water. Animals were returned to conditions of free water just before the peak feeding period. Water intake was measured in the next 20 minutes and during the following 12 hours. Haematocrit or packed cell volume (PCV) in the blood of potoroine marsupials was measured in both water-restriction experiments. Osmolality of urine was determined by freezing-point depression (osmometer, Fiske Associates Inc., USA) in the second water-restriction experiment.

Measurements of water consumption by captive lactating animals were made in respiration chambers, in conjunction with the studies described in Section 11.2. Because the data were confounded by repeated measures, of which there were insufficient to treat each animal separately, the data were pooled, arbitrarily, into various pouch-young mass classes. This procedure can be criticised on statistical grounds but, because there are few data, it was the only possible approach.

10.2.2 Water turnover in potoroine housed in outdoor enclosures

Water turnover was measured in five *A. rufescens*, five *P. tridactylus* and four *B. penicillata* in June, 1987 (winter); and in four *A. rufescens*, four *P. tridactylus* and four *B. penicillata* in January, 1988 (summer). All animals had free access to the maintenance diet (Table 4.1) and to water. Not all of the animals were available for use in both the winter and the summer study periods. However, the study was standardized by using only adult male animals.

The isotope procedures were similar to those described in Chapter 4. *Aepyprymnus rufescens* were injected intramuscularly with ca 1 ml, and *P. tridactylus* and *B. penicillata* were injected intramuscularly with ca 0.5 ml, of a dose solution containing 3.7 MBq ^{3}HOH per ml of physiological saline. The exact injection mass was

determined by weighing the syringe before and after injection. The animals were then hung, in open-weave hessian sacks, for the four-hour equilibrium period. They were then bled, released and left undisturbed until rebleeding at 5 and 9 days (winter) and 7, 12 and 15 days (summer). Light rain between days 7 and 12 prompted the third measurement in the summer study period.

The four-hour equilibrium period was determined in an earlier study. Five *A. rufescens* were injected intramuscularly with 3.7 MBq of ^{3}HOH and were bled at 2, 4 and 6 h after dosing. The specific activities of tritium in the plasma are shown in the following table.

Table 10.1 The specific activity of ^{3}HOH in plasma taken from A. rufescens 2, 4 and 6 hours after injection

	specific activity (cpm.gHOH^{-1})		
Animal	**2h**	**4h**	**6h**
A	95331	134451	148413
B	138271	151782	145958
C	158172	134881	160790
D	146977	148078	149089
E	129143	126700	119731

The result for Animal C (4h) is difficult to explain. Nevertheless, the data indicate that a single intramuscular dose of ^{3}HOH equilibrates at different times in different animals. Because the equilibration time could not be predicted — that is, it was not related simply to body mass — it was decided to standardize procedures by taking equilibrium blood samples 4 hours after dosing. It is difficult to obtain multiple blood samples from *P. tridactylus* and *B. penicillata*. Thus, equilibration times were not determined in these smaller species. For the purpose of standardization, a 4-hour equilibration time was used in these species also.

10.2.3 Water turnover in free-living *A. rufescens*

Water turnover was measured in free-living *A. rufescens* on three separate occasions. The purpose of the first of these measurement periods was to devise a technique for recapturing *A. rufescens*; field metabolic rates were measured by doubly-labelled water in the second and third periods. Because the measurement of water flux is a necessary part of the study of field energetics, the reader is referred to Section 11.2

for all details of materials and methods. The November/December study period was particularly interesting because it consisted of a dry period, followed by several days of consistent rain. Thus, the influence of rain on water turnover in free-living *A. rufescens* could be measured.

10.3 Results

10.3.1 Results from studies of caged potoroine marsupials

The studies of nitrogen requirements (Chapter 5), rate of passage of digesta (Chapter 7), variability in digestion of plant-cell walls (Section 8.1), the *in vitro* SCFA production measurements (Chapter 9) and the water restriction experiments (Chapter 6) were all based on diets in which the levels of nitrogen and plant-cell wall constituents were altered by changing the ratios of maize, cornflour and of oat-hulls. Thus, the results are presented by examining the influence of the level of nitrogen and plant-cell-wall constituents on water flux under conditions of free access to water. Data from the lucerne experiments (Sections 8.2 and 8.3) and the specific effects of water restriction are examined separately.

As the level of dietary nitrogen increased, the *A. rufescens* reported in Chapter 5 (Table 10.2b) drank more ($P<0.001$). Although the animals also ate more ($P<0.01$), as dietary nitrogen increased, the animals drank more per 100g of dry-matter intake, indicating that the effect was independent of food intake. This result was not found in the preliminary study of nitrogen requirements (Table 10.2a). Indeed, there was a trend, albeit non-significant, for animals in the preliminary experiment to drink more when fed the low-nitrogen diet.

As the concentration of plant-cell-wall constituents increased, potoroine marsupials usually drank significantly more per unit metabolic body mass (Tables 10.2b, 10.3a). However, when results were calculated as a function of dry-matter intake, it was clear that fibre had no effect on water intake. This contrasts with the animals' reaction to increasing nitrogen in their diet.

In absolute terms ($g.d^{-1}$), *P. tridactylus* usually drank as much as *A. rufescens* (Tables 10.3a-d; 10.6a), but when expressed per unit of metabolic body mass or, particularly, per unit dry-matter intake, *P. tridactylus* drank significantly more than *A. rufescens* (Tables 10.3a-d; 10.6a). An exception was the second water-restriction experiment (Table 10.6c). In that study, *A. rufescens* and *P. tridactylus* given free access to water, had similar water intakes per unit metabolic mass. In the two studies involving comparisons among three species (Table 10.3b; 10.4a), *B. penicillata* drank

Table 10.2a Water intake by A. rufescens fed cereal-based diets containing 1.0 (LN), 1.6 (MN) and 2.0% (HN) nitrogen (Chapter 5).

	LN	MN	HN	ems	sig
number	2	4	3		
Water					
Intake ($g.d^{-1}$)	147	102	108	1554.2	ns
Intake ($g.kg^{-0.8}.d^{-1}$)	71	49	48	402.0	ns
Intake ($g.100g^{-1}$ DMI)	222	162	131	3079.5	ns

Table 10.2.b. Water intake by A. rufescens fed low (ca 0.6% N), medium (1% N) or high (1.6% N) nitrogen diets (Chapter 5).

	LOW FIBRE			HIGH FIBRE							
	LN	MN	HN	LN	MN	HN	sed	fib	sed	N	N.fib
number	8	8	8	8	8	8					
Water											
intake ($g.d^{-1}$)	59	82	90	66	97	105	7.6	**	7.4	***	ns
intake ($g.kg^{-0.8}.d^{-1}$)	18	27	26	20	29	32	1.9	**	1.7	***	ns
intake ($g.100g^{-1}$ DMI)	93	119	114	93	98	132	4.2	ns	4.8	***	**

similar amounts per unit metabolic mass as did *A. rufescens*, but significantly less than *P. tridactylus*.

In the studies described in Section 8.1 (Table 10.3a), a significant interaction occurred between species and level of plant-cell-wall constituents. *Aepyprymnus* did not drink more in response to increased dietary fibre, but *P. tridactylus* drank significantly more per unit metabolic mass and per unit dry-matter intake as dietary fibre increased. The latter was not without complication. On the high-fibre diet, *P. tridactylus* digested more of the cell-wall constituents than they did when fed the low- and medium-fibre diets.

In Tables 10.3a and 10.4a, partitioned water losses are shown for *A. rufescens* and *P. tridactylus* fed a maize-oat hull diet (Section 8.1), and for *A. rufescens*, *P. tridactylus* and *B. penicillata* fed a lucerne-based diet (Section 8.2). Losses are expressed as a proportion of free-water intake. When water was available *ad libitum*, *A. rufescens* distributed its water losses equally among faeces, urine and evaporation. Compared to *A. rufescens*, the smaller species — *P. tridactylus* and *B. penicillata* — tended to lose proportionately more water via evaporation, and less in their faeces. For example, when fed a maize-oat hull diet, *Potorous* excreted proportionately less water in their faeces than *Aepyprymnus* ($P<0.001$), but proportionately more via evaporative processes ($P<0.001$).

The intake of drinking water was much higher in all three species fed the lucerne-based diet (Table 10.4a). This is best illustrated by *P. tridactylus*, which drank 588 g.100g DMI^{-1}. The data were also extremely variable. This variation, together with the low and unequal replication, meant that water intake per day, or per unit metabolic mass, was not significantly different between species. However, the trend for *P. tridactylus* to drink more than *A. rufescens* or *B. penicillata* persisted, and was significant per unit dry-matter intake ($P<0.05$). In the first lucerne experiment (Section 8.1; Table 10.4a), all species excreted similar volumes of faecal water. The potoroos excreted more urine than did either *A. rufescens* or *B. penicillata*, but the difference was not significant. Respiratory water losses, which were calculated by difference, were higher in *P. tridactylus* than in *A. rufescens* ($P<0.05$), but were not significantly different from those of *B. penicillata*. Respiratory water is underestimated because measurements of water intake do not include metabolic water. If the animals metabolised only starch, the yield of metabolic water would be about 13 $ml.kg^{-0.80}.d^{-1}$.

Only *A. rufescens* were studied in the second lucerne experiment. Animals drank more when they were fed the high-lucerne diet than when they were fed the low-lucerne diet (Table 10.4b). This was significant when expressed per unit of metabolic body

Table 10.3a Water flux in A. rufescens and P. tridactylus fed maize-oat hull diets containing 1% nitrogen and 18%, 28% or 40% NDF (Section 8.1).

	Aepyprymnus			*Potorous*				significance		
Parameter	**LF**	**MF**	**HF**	**LF**	**MF**	**HF**	**sed**	**Sp**	**Fib**	**Sp x Fib**
number	3	3	3	3	3	3				
Water intake										
($g.d^{-1}$)	116	117	123	70	90	138	21.4	ns	ns	ns
($g.kg^{-0.80}.d^{-1}$)	51	54	52	72	99	143	17.1	***	*	*
($g.100g\ DMI^{-1}$)	145	148	128	192	214	327	43.1	**	ns	ns
Faecal dry matter (%)	34	44	48	53	49	44	3.6	**	ns	**
Water partitioning (% Water intake)										
Faecal water	34	29	37	13	17	19	4.7	***	ns	ns
Urinary water	30	40	25	39	27	27	5.8	ns	ns	ns
Evaporative water	36	31	38	48	55	55	6.4	***	ns	ns

Table 10.3b Water intake and faecal dry matter of A. rufescens, P.tridactylus and B. penicillata fed "high-fibre" or "low-fibre" diets (Chapter 7).

	Low fibre			High fibre				significance	
	Aepyprymnus	*Potorous*	*Bettongia*	*Aepyprymnus*	*Potorous*	*Bettongia*	**sed**	**sp**	**fib**
number	4	4	4	4	4	4			
Water intake									
($g.kg^{-0.8}.d^{-1}$)	30	83	38	34	69	41	16.8	**	ns
($g.100g^{-1}$ DMI)	112	250	108	110	181	86	19.3	**	ns
Faecal dry matter (%)	39	38	54	47	49	54	4.0	**	**

Table 10.3.c. Water intake in A. rufescens and P.tridactylus fed a maize-oat husk diet containing 1% nitrogen and 40% NDF (Chapter 7).

	Aepyprymnus	*Potorous*	sed	sig
number	8	8		
Water				
intake ($g.d^{-1}$)	105	79	13.0	ns
intake ($g.kg^{-0.8}.d^{-1}$)	45	85	8.9	***
intake ($g.100g^{-1}$ DMI)	157	242	25.8	**
faecal dry matter (%)	38	43	2.7	ns

Table 10.3.d. Water intake by A. rufescens and P. tridactylus fed a maize-oat husk diet containing 1% nitrogen and 29% neutral detergent fibre (Chapter 10).

	Aepyprymnus	*Potorous*	sed	sig
number	4	4		
Water				
Intake ($g.d^{-1}$)	105	66	20.6	ns
Intake ($g.kg^{-0.8}.d^{-1}$)	44	74	12.1	*
Intake ($g.100g^{-1}$ DMI)	176	199	31.1	ns

Table 10.4a Water flux in A. rufescens, P. tridactylus and B. penicillata fed a lucerne-based diet (Section 8.2).

	Aepyprymnus	*Potorous*	*Bettongia*	ems	sig
number	3	5	2		
Water					
Intake ($g.d^{-1}$)	269	196	165	8999.0	ns
Intake ($g.kg^{-0.8}.d^{-1}$)	123	211	158	5902.9	ns
Intake ($g.100g^{-1}$ DMI)	348	588	355	14389.0	*
Faecal DM (%)	28	31	35	53.2	ns
Water partitioning (% water intake)					
Faecal water	30	18	25	370.0	ns
Urinary water	33	35	35	1424.9	ns
Evaporative water	37	46	40	593.5	*

Table 10.4b Water flux in A rufescens fed diets containing 62% lucerne and 35% maize (diet 1), or 35% lucerne and 62% maize (diet 2) (Section 8.3).

	Diet 1	Diet 2	sed	sig
number	4	4		
Water				
Intake ($g.d^{-1}$)	205	159	28.6	ns
Intake ($g.kg^{-0.8}.d^{-1}$)	87	65	8.9	*
Intake ($g.100g^{-1}$ DMI)	278	199	27.6	*
Faecal DM (%)	28	30	1.6	ns

mass (P<0.05) and per unit dry-matter intake (P<0.05). The faecal dry-matter content was about 30% on both diets.

The intake of water by lactating *A. rufescens* fed the maintenance ration was measured until the young emerged from the pouch. Lactating *Aepyprymnus* did not drink more to compensate for losses of water in their milk (Table 10.5).

Table 10.5 Water consumption (mean ± sem) by lactating A. rufescens fed a cereal-based ration (Section 11.2).

		Water intake		
Pouch young mass (g)	**number**	**g.d^{-1}**	**g.100g^{-1} DMI**	**g.kg$^{-0.8}$.d^{-1}**
0	5	118 ± 17.1	187 ± 16.4	53 ± 6.8
1-30	5	123 ± 12.2	177 ± 10.9	55 ± 5.8
31-60	6	113 ± 14.3	163 ± 10.7	46 ± 4.4
61-110	4	94 ± 13.6	176 ± 21.0	39 ± 5.5
111-150	4	133 ± 23.2	189 ± 9.3	53 ± 7.6
151-300	5	128 ± 19.3	173 ± 10.8	49 ± 5.2
301-400	4	139 ± 15.2	184 ± 7.2	50 ± 2.9
>400	5	125 ± 6.5	175 ± 7.5	46 ± 0.8

10.3.2 Results of the water-restriction experiments

The influence of water restriction on urea kinetics was discussed in Chapter 6. The following sections present results of the effect of water restriction on intake and digestibility parameters.

Results from Experiment 6.1 (Table 10.6a,b)

Although restricting water to 50% of *ad libitum* intake had a significant effect upon losses of body mass (P<0.05), the effect was variable. From the start of water restriction to the end of the collection period, changes in body mass ranged fron -2% to -17% in *P. tridactylus*, +0% to -14% in *A. rufescens* and -2% to -19% in *B. penicillata*. Water restriction reduced dry-matter intake (P<0.05), which in turn depressed nitrogen intake (P<0.05), faecal-nitrogen output (P<0.05) and nitrogen balance (P<0.05). The output of urinary nitrogen was not affected by water availability (Table 10.6.a).

Table 10.6a Water intake and digestibility parameters in A. rufescens and P. tridactylus fed a maize-oat hull diet and given free access to water or restricted to 50% of normal intake (Experiment 6.1)

Parameter	*Aepyprymnus*		*Potorous*			significance		
	Ad libitum	**Restricted**	**Ad libitum**	**Restricted**	**sed**	**sp**	**wat**	**sp x wat**
number	6	6	6	6				
Body mass (g)	2910	2721	953	842				
(sem)	(128)	(111)	(43)	(22)				
Water								
intake ($g.d^{-1}$)	104	57	107	33	69.0	ns	***	ns
intake ($g.kg^{-0.8}.d^{-1}$)	45	24	112	33	20.3	**	**	ns
intake ($g.100g^{-1}$ DMI)	139	102	275	138	55.9	**	**	ns
Packed-cell volume (%)	62	61	44	45	1.7	***	ns	ns
Dry matter								
digestibility (%)	66	67	68	68	1.5	ns	ns	ns
Energy								
digestibility (%)	65	66	68	67	1.6	ns	ns	ns
DEI ($kJ.kg^{-0.75}.d^{-1}$)	350	290	460	300	27	ns	**	ns
NDF digestibility (%)	8	10	18	15	3.8	**	ns	ns
ADF digestibility (%)	7	4	15	11	3.7	**	ns	ns

Table 10.6b Water and digestibility parameters in potoroine marsupials fed a maize-oat hull diet and given free access to water or restricted to 50% of normal intake (Experiment 6.1).

Parameter	*Aepyprymnus*		*Potorous*		*Bettongia*			significance	
	Ad libitum	Restricted	Ad libitum	Restricted	Ad libitum	Restricted	sed	sp	wat*
Number	3	3	3	3	3	3			
Body mass (g)	3071	2617	934	834	1134	993			
(sem)	(225)	(136)	(56)	(34)	(69)	(65)			
Water									
intake ($g.d^{-1}$)	33	28	121	34	63	30	21.4	*	**
intake ($g.kg^{-0.8}.d^{-1}$)	98	60	116	29	69	30	26.4	ns	**
intake ($g.100g^{-1}$ DMI)	135	105	350	134	146	80	54.8	***	**
Faecal dry matter (%)	39	47	38	49	54	56	4.0	**	**
Packed-cell volume (%)	61	61	43	43	53	51	2.3	***	ns
Dry matter									
digestibility (%)	65	66	69	65	64	66	2.0	ns	ns
Energy									
digestibility (%)	64	66	68	65	64	65	1.9	ns	ns
DEI ($kJ.kg^{-0.75}.d^{-1}$)	330	330	380	270	430	360	41	ns	ns
NDF digestibility (%)	16	15	14	17	14	14	3.6	ns	ns
ADF digestibility (%)	7	6	5	14	9	6	3.5	ns	ns

* - The species by water interaction was non-significant for all parameters.

Packed-cell volumes were not affected by water restriction. Similarly, digestibility parameters were little affected by water restriction. However, in the comparison of *A. rufescens* and *P. tridactylus* analysed as a split-plot in time (Table 10.6.b), digestibility of cell-wall constituents was higher in *Potorous*.

Both *A. rufescens* and *P. tridactylus* produced drier faeces when access to water was limited ($P<0.01$). The dry-matter content of faeces from *B. penicillata* did not change with water restriction. However, the faeces from *Bettongia* were always drier than those of the other species ($P<0.01$).

Results from Experiment 6.2 (Table 10.6c)

The daily drinking water of *A. rufescens* and *P. tridactylus* was restricted to 13 and 23 $g.kg^{-0.8}.d^{-1}$ respectively. This proved to be a more uniform and harsher restriction than the 50% of *ad libitum* intake imposed in Experiment 6.1. Animals given limited water suffered significant losses of body mass from the beginning of the treatment until the end of the collection period ($P<0.001$). As expected, water restriction reduced dry-matter intake ($P<0.001$), nitrogen intake ($P<0.001$), faecal nitrogen output and nitrogen balance ($P<0.001$). Conversely, water restriction significantly increased urinary-nitrogen output ($P<0.05$), which suggests that animals with limited water intake were catabolizing body protein to provide maintenance nitrogen and possibly metabolic water. In contrast to the first water-restriction experiment, potoroines digested more dry matter, ADF and NDF when water was limited ($P<0.001$). Also, *Potorous* digested more of these dietary constituents than did *Aepyprymnus*.

Water kinetics were traced in both urine and plasma. Estimates of total body-water (TBW) obtained by regressing the specific activity of tritium in the urine, against time, were generally higher than values calculated from a plasma sample obtained 4 h after dosing. However, this difference was significant ($P<0.05$) only for *P. tridactylus* on the water-restriction treatment. In both species and with both methods of calculation, TBW, as a proportion of body mass, was not changed by water availability.

The rate of water efflux calculated from the decline in specific activity of tritium in the plasma was very low. This method grossly underestimated the water influx determined by measuring total water intake. By comparison, the regressions of specific activity of tritium in the urine against time were significant for all animals. Furthermore, the coefficients of determination were usually greater than 0.85. Thus, values of water efflux determined in this way seem to have some biological meaning: if animals with free access to water are placed in order of drinking-water intake, the ranking is the same regardless of whether values are determined by measuring the decline in specific activity of tritium in the urine, or by measuring drinking-water intake alone. Water efflux obtained from the specific activity of tritium in the urine of animals

with free access to water are 30%-40% higher ($P>0.05$) than the animal's intake of drinking water. In animals with limited access to water, water efflux estimated with tritium was about double the intake of drinking water ($P<0.001$).

Both *A. rufescens* and *P. tridactylus* responded to water restriction by producing drier faeces ($P<0.001$). This was more pronounced in *P. tridactylus*, which produced drier faeces than *A. rufescens* under *ad libitum* and restricted water conditions ($P<0.001$). Because the urine collection apparatus was washed with water to ensure the quantitative collection of nitrogen, the actual volume of urine could not be measured accurately. Nevertheless, it was apparent that the urine volume was lower in animals with restricted access to water. This coincided with their higher ($P<0.001$) urine osmolality.

In agreement with the first water-restriction experiment, packed-cell volume was not changed by water restriction.

Aepyprymnus and *Potorous* rehydrated quickly. In the first 20 minutes after water was reintroduced, both species drank a volume equivalent to about 7.5% of their body mass. However, during the night that followed the reintroduction of water, the *P. tridactylus* drank about 22% of their body mass which was more ($P<0.05$) than *A. rufescens* which drank 16% of body mass over the same period. Between 48% and 90% of this mass gain was retained.

10.3.3 Results of the studies in the outdoor enclosures (Table 10.7)

Since the regressions of specific activity against time were based on few data points, some caution should be taken when interpreting the results. However, the lowest coefficient of determination was 0.99, suggesting that WTR was stable in all animals.

Ambient temperatures were lower and relative humidity higher in the June study period, but this had a negligible effect on water-turnover rates. Likewise, the light rain that fell between the second and third recaptures in the summer measurement period, did not affect water turnover.

Although there was a tendency for total body-water expressed as a percentage of body mass to be higher in June than in January, this did not yield inter-specific differences. However, it must be emphasized that not all the same individuals were used in each study period. In both winter and summer, *B. penicillata* tended to have a higher percentage of body water.

In contrast to the lack of seasonal effects, for most parameters of water metabolism, *Aepyprymnus* had a slower rate of turnover ($P<0.001$), a longer turnover time and half

Table 10.6c Water and digestibility parameters in A. rufescens and P. tridactylus fed a maize-oat hull diet and given free or restricted access to water (Experiment 6.2).

Parameter	*Aepyprymnus*		*Potorous*		significance			
	Ad libitum	**Restricted[1]**	**Ad libitum**	**Restricted[2]**	**sed**	**sp**	**wat**	**sp x wat**
number	8	8	8	8				
Body mass (g)	2893	2680	932	784				
(sem)	(111)	(147)	(40)	(32)				
Water								
intake ($g.d^{-1}$)	111	28	54	19	22.5	**	***	ns
intake ($g.kg^{-0.8}.d^{-1}$)	51	12	57	23	7.7	ns	***	ns
intake ($g.100g^{-1}$ DMI)	166	76	157	110	24.0	ns	***	ns
total body water (%)	68	65	74	77	3.0	***	ns	ns
efflux ($g.d^{-1}$) [3]	176	71	123	51	16.9	**	***	ns
efflux ($g.kg^{-0.8}.d^{-1}$) [3]	76	36	134	60	12.8	***	***	ns
Faecal dry matter (%)	41	56	47	64	2.6	***	***	ns
Packed-cell volume (%)	61	61	48	49	2.7	ns	***	ns
Rehydration (% body mass)								
(20 min)		7.6		7.4	0.98	ns		
(overnight)		16.1		22.1	2.51	*		
Urine osmolality ($mosm.kg^{-1}$)	1156	2584	963	2491	66.5	ns	***	ns
Dry matter digestibility (%)	66	70	69	74	8.1	**	***	ns
NDF digestibility (%)	19	30	27	39	3.5	**	***	ns
ADF digestibility (%)	7	20	16	30	4.1	**	***	ns

1 - *A. rufescens* given 13 g $water.kg^{-0.8}.d^{-1}$.

2 - *P. tridactylus* given 23 g $water.kg^{-0.8}.d^{-1}$.

3 - Estimated by measuring the decline in specific activity of tritium in the urine.

life (P<0.01) and a slower rate of turnover expressed per kg body mass (P<0.01) than did *P. tridactylus* or *B. penicillata*. However, when turnover was expressed per unit metabolic body mass, there were no differences between species.

10.3.4 Water-turnover in free-living *A. rufescens* (Tables 10.8a,b)

Data describing water flux in individual, free-living *A. rufescens*, before and after a period of heavy rain, are shown in Table 10.8a. The rainfall during the same period is graphed in Fig 10.1. There were insufficient data to justify statistical analysis. However, values of water turnover during the wet period (Animal 1 T2, Animal 5 T1, Animal 6 T1,T2) are about double those measured during the dry period (Animal 1 T1, Animal 2 T1,T2 Animal 3 T1, Animal 4 T1).

There was not enough rain in the other study periods to affect water flux. All parameters were similar in both winter and summer (Table 10.8b). There was no significant difference (paired t-test) between estimates of total body-water from a single equilibration plasma sample, or from the regression of the decrease, with time, of the specific activity of tritium in plasma.

Most females carried pouch young; four measurements were made on animals at peak lactation, that is with young-at-foot. However, there were no significant differences in any parameter of water kinetics between males and females, or between females with young-at-foot and those with small pouch young or without young.

Fig 10.1 Rainfall during the December study period

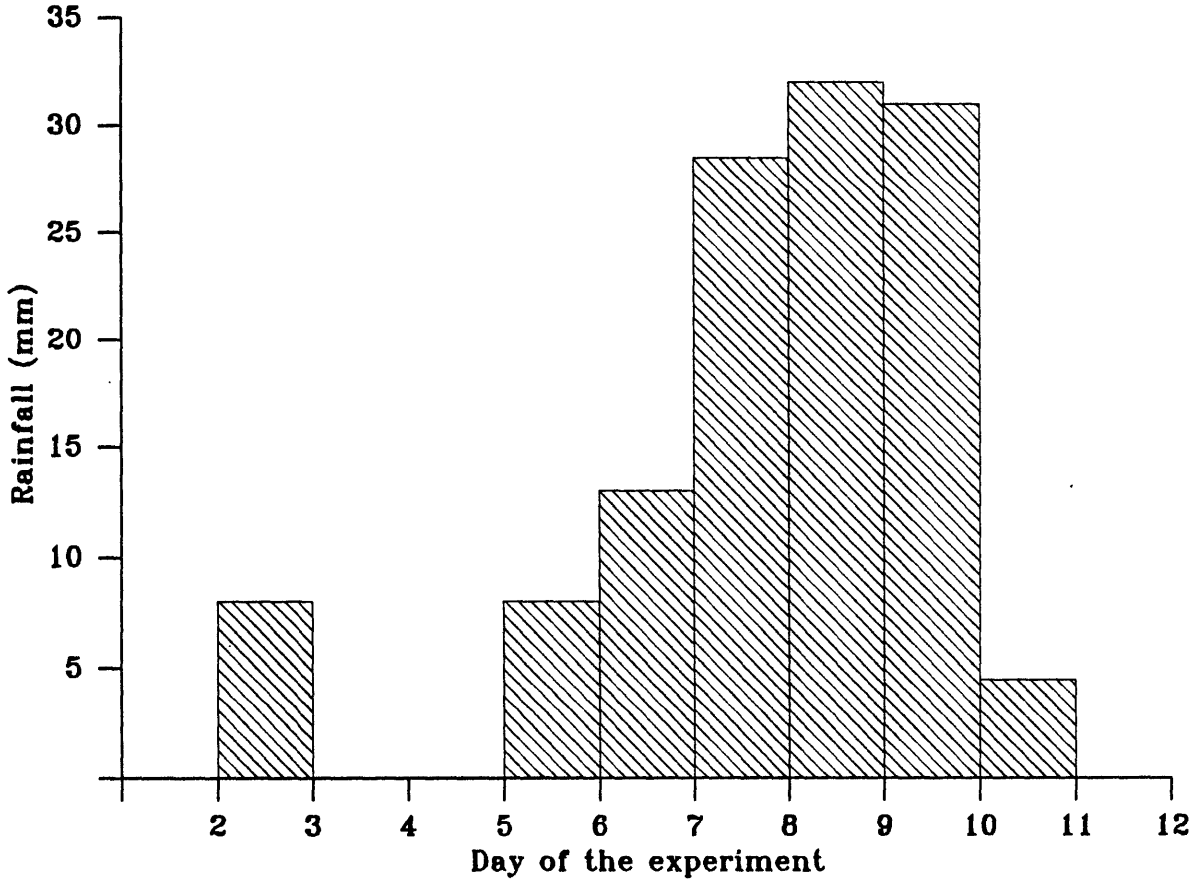

Table 10.7 The winter and summer water fluxes of captive potoroine marsupials in outdoor enclosures.

	Summer			Winter				
Parameter	***Aepyprymnus***	***Potorous***	***Bettongia***	***Aepyprymnus***	***Potorous***	***Bettongia***	**ems**	**sig**
number	4	4	4	5	5	4		
body mass g (sem)	3450 (171)	1088 (80)	1106 (48)	3242 (96)	965 (50)	1088 (17)		
body water g (sem)	2525 (110)	794 (73)	852 (22)	2492 (58)	740 (44)	905 (40)		
body water (%)	73	73	77	77	76	83	24.3	ns
Water								
proportional turnover (k) (d^{-1})	0.120	0.194	0.178	0.127	0.180	0.180	0.0296	**
turnover (1/k) (d)	8.5	5.1	5.9	7.8	5.6	6.5	1.72	***
$T_{1/2}$ (d)	5.9	3.6	4.1	5.4	3.9	4.5	0.82	***
turnover								
($g.d^{-1}$)	301	155	151	319	134	164	2028	***
($g.kg^{-1}.d^{-1}$)	88	142	136	98	137	149	1005	**
($g.kg^{-0.8}.d^{-1}$)	112	145	139	124	137	152	1094	ns

ems - error mean square

Climatic Data

	Summer	Winter
Mean minimum temperature (°C)	4	13
Mean maximum temperature (°C)	14	27
Mean precipitation ($mm.d^{-1}$)	0	4
Mean relative humidity (%)	84	70

Table 10.8a Water flux in wild A. rufescens measured before and during a period of heavy rain (bolded data) in early summer.

Animal	Mass	TBW	Teq	T1	Water turnover		$T_{1/2}$	T2	Water turnover		$T_{1/2}$
	g	%	Day	Day	$g.d^{-1}$	$g.kg^{-0.8}.d^{-1}$	d	Day	$g.d^{-1}$	$g.kg^{-0.8}.d^{-1}$	d
1	2800	78	1	4	185	81	7.9	**10**	**451**	**198**	**3.2**
2	2900	78	1	4	288	123	5.4	6	343	146	4.6
3	2750	80	1	5	352	157	4.3	-	-	-	-
4	2500	77	1	3	393	189	3.4	-	-	-	-
5	2650	82	5	**10**	**543**	**249**	**2.8**	-	-	-	-
6	2700	76	5	**9**	**587**	**265**	**2.4**	**11**	**624**	**282**	**2.3**

Teq, T1, T2 refer to the day that the animal was injected, recaptured and recaptured for a second time respectively.

$T_{1/2}$ - biological half life.

Table 10.8b Water flux in wild A. rufescens measured in winter and summer at Drake.

Parameter	Summer	Winter	sed	sig
number	8	7		
Body mass (g)	2875	2961*	87.6	ns
Body water[1] (g)	2180	2284*	85.5	ns
Body water[2] (g)	2183	2215	95.0	ns
Body water[1] (%)	75.9	77.1*	1.73	ns
Body water[2] (%)	76.0	74.9	2.96	ns
Proportional turnover (k) (d^{-1})	0.185	0.178	0.0126	ns
Turnover (1/k) (d^{-1})	5.6	5.8	0.49	ns
$T_{1/2}$ (d)	3.9	4.0	0.34	ns
Water turnover[2]				
($g.d^{-1}$)	404	392	32.8	ns
($g.kg^{-1}.d^{-1}$)	141	133	11.9	ns
($g.kg^{-0.8}.d^{-1}$)	174	165	14.4	ns

1 - total body water determined using the specific activity of water at equilibrium.

2 - total body water determined by extrapolating to zero the regression of specific activity on time.

* - n=9

10.4 Discussion

The water influx of captive animals given free access to water has been shown to vary with body mass to the exponent 0.8. There is much debate about phylogenetic differences in water needs under these conditions. Seven eutherian species, ranging in size from the mouse to the horse, required on average 123 ml $H_2O.kg^{-0.8}.d^{-1}$ (Richmond *et al.* 1962). By comparison, Denny and Dawson (1975a) showed that five macropodoids (*M. giganteus, M. rufus, M. r. robustus, M. eugenii* and *P. tridactylus*) needed only 90 $ml.kg^{-0.8}.d^{-1}$ or about 30% less water than did the eutherians studied by Richmond *et al.* (1962). Denny and Dawson attributed the water conservation in metatherians to their basal metabolism, which is also about 30% lower than in eutherians. For several reasons the studies are not directly comparable. First, Denny and Dawson used species from a range of habitats; the study of Richmond *et al.* included only one animal from an arid environment, *Dipodomys deserti* (desert

kangaroo-rat), and this species was given limited water. Secondly, Denny and Dawson's animals were housed in outdoor enclosures. Their data presumably overestimate standard water-turnover rate (SWTR) (Nicol 1978) due to water turnover associated with activity. Richmond *et al.* did not mention their animals' housing conditions. In both studies few animals were used, but broad conclusions were drawn about all metatherians and all eutherians.

Other metatherians also seem to need less water than do eutherians. For example, the perameloids — *Macrotis lagotis* (bilby), *Isoodon macrourus* (northern brown bandicoot) and *Perameles nasuta* (long-nosed bandicoot) — needed 37%, 79% and 59% respectively of the amount predicted for eutherians (Hulbert and Dawson 1974). The koala, *P. cinereus*, needs 51%-75% of the predicted requirement for eutherians (Degabriele *et al.* 1978); and two dasyurid marsupials — *Dasycercus cristicauda* (mulgara) and *Dasyuroides byrnei* (kowari), used 71% and 67% respectively of predicted needs (Kennedy and Macfarlane 1971). However, another dasyurid — *Sminthopsis crassicaudata* (fat-tailed dunnart) — has water requirements (200-250 $ml.kg^{-0.80}.d^{-1}$) far exceeding those expected (Haines *et al.* 1974; Morton 1980). Other dasyurids — *Sarcophilus harrisii* and *Dasyurus viverrinus* (Green and Eberhard 1979) and *Antechinus swainsonii*, dusky antechinus, (Cowan *et al.* 1974) — have water requirements similar to those of eutherians.

Although the studies cited above generally suggest that captive metatherians need less water than do captive eutherians, Nicol (1978) refuted this claim. Indeed, he compared statistically the data of Denny and Dawson (1975a) and Richmond *et al.* (1962) and found no differences. He subsequently examined a larger data set comprising 41 species of metatherians and eutherians from divergent habitats. Their WTR was described by the equation $WTR = 102.2 \times W^{0.82}$ ($r = 0.966$). Again, analysis of covariance detected no difference between the marsupial and eutherian phylogenetic groups, but it did detect a strong influence of habitat. Thus, contrary to Denny and Dawson's conclusions, measurement of WTR under standardized laboratory conditions may detect species with physiological adaptations to withstand an arid environment. This was confirmed by the study of Chilcott *et al.* (1985) of wallabies from diverging habitats. When both were fed a medium-nitrogen diet (ca 1.1%), the arid-adapted *M. eugenii* drank (per unit dry-matter intake) about 60% of that drunk by the wet-forest dwelling *T. thetis*.

In the present study of caged animals, *P. tridactylus* consistently drank more water per unit metabolic body mass than did either *A. rufescens* or *B. penicillata*. Measurements of drinking water alone underestimate water requirements because no allowance is made for preformed water in the food, or water derived from metabolism.

Nevertheless, because the three species were kept under similar conditions and ate, per unit metabolic body-mass, similar quantities of standard diets, the intake of drinking water probably reflects the water requirements of each species. The higher water intake by *P. tridactylus* would be expected from its moist coastal habitat (Chapter 3). The differences between *Aepyprymnus* and *Bettongia* on one hand and *Potorous* on the other may also reflect the habitat in which each genus evolved. This was emphasized by Macfarlane (1976):

"Evolution in wet areas is associated with high turnover rates and low salt tolerance, while desert derivation goes with low rate functions and high salt tolerance. This basic ecophysiology changes slowly, and animals that migrate to different environments may retain ancient patterns in areas where they seem inappropriate — so that cattle keep their high rates of energy and water use in arid zones, or llamas remain low in energy and water turnover after three million years in cool or wet environments".

Did the different potoroine marsupials evolve under different environments? As discussed in Chapter 3, this question has no definite answer, because the fossil record is poor. Perhaps the best clue is found in the genus *Potorous*. The species in this genus have proportionately much shorter hindlimbs and longer forelimbs, relative to those of *Aepyprymnus* and *Bettongia*. Not surprisingly, they are also almost quadripedal in their movements. Do these characteristics indicate that *Potorous* are long-term inhabitants of areas with dense vegetation and abundant water?

The influence of dietary constituents on water consumption

Water intake has often been monitored in nutritional studies, but the influence of individual nutrients on water intake has usually been overlooked. In the present study, water intake was measured in all experiments. This enabled an appraisal of the effect that dietary cell-wall-constituents and nitrogen have on water intake. Enriching dietary nitrogen caused the potoroines to drink more water. This was observed also in *M. parma* (Hume 1986) and to a lesser degree in *M. eugenii* and *T. thetis* (Hume 1977a). His study showed also a difference between species; *T. thetis* and *M. parma* drank more than *M. eugenii* at each nitrogen level. Indeed, *M. eugenii* fed a low-nitrogen diet, drank about as much as did *T. thetis* and *M. parma* fed the high-nitrogen diet. This finding and those of the present study suggest that measurements of standard water needs should also consider dietary composition. Why do increases in dietary nitrogen cause animals to drink more? One explanation is that the animal must excrete the additional nitrogen dissolved in water. This water is known as osmotically-obligated water.

Potoroine marsupials fed diets rich in cell-wall constituents drank more and ate more. However, water intake per unit of food intake did not change. Diets which are

higher in fibre cause greater faecal water losses. This was reported also in desert rodents by Buffenstein (1985).

The lucerne-based diets provided the dietary influence that had the most pronounced effect on water flux. Of particular note were the high water intakes by animals fed the 75% lucerne-25% maize diet containing 50% NDF (Section 8.2). However, the high water intakes were not simply a function of fibre content. *Aepyprymnus* and *Potorous* fed a maize-oat hull diet containing 40% NDF (Section 8.1) drank respectively only 37% and 56% per unit dry-matter intake of those fed the lucerne-maize diet. Nor were the high water intakes a function of the high nitrogen in the lucerne; *Aepyprymnus* and *Potorous* fed the basal ration (Table 4.1; Appendix 1) had relatively low water intakes also. Instead, the high water flux is more likely to be related to digestion of the dietary cell-wall-constituents. The fact that water intake is related, in some way, to degradation of plant-cell walls is illustrated in other experiments. In the second lucerne experiment (Section 8.3), NDF digestibility and water intake were both lower than in the first lucerne experiment. In Section 8.1, *P. tridactylus* fed a high-fibre diet digested more NDF and drank more water than they did when fed diets lower in fibre.

The large differences in water intake between potoroines fed lucerne-based diets and those fed maize-oat hull diets suggest that the diet as well as activity, temperature and food and water intakes should be considered when determining SWTR. This is illustrated well by Denny and Dawson's (1975a) study. They compared water metabolism in *P. tridactylus* and *M. eugenii* offered fruit and hay. It is unlikely that *Potorous* ate hay or that *M. eugenii* ate fruit. Not surprisingly, the *Potorous* had a higher WTR. However, it must be conceded that standardizing the diet presents enormous practical problems.

Partitioned water losses

It is difficult to remark on the partitioned water losses because the results from Section 11.1 show clearly that, in metabolism cages, *P. tridactylus* and *B. penicillata* were far more active than *A. rufescens*. Indeed, when potoroines were fed a maize-oat hull diet (Table 10.3a), the evaporative water losses (EWL) of *A. rufescens* are similar to those predicted (45 ml per 3kg.d^{-1}) by Hinds and MacMillen (1986); EWL from *Potorous* were about 50% higher than expected on the low-fibre diet, and increase progressively to 300% of the expected on the high-fibre diet. Similarly, in *Aepyprymnus*, *Bettongia* and *Potorous* fed a lucerne-based diet, the apparent EWL were respectively about 200, 300 and 400% higher than predicted. The high EWL can be explained only if the animals are in positive water balance and thus, EWL cannot be estimated by difference. Because the digestion of cell-wall constituents appears to be

positively correlated with water intake, the foregut may be acting as a water reservoir (Denny and Dawson 1975b). However, as a proportion of total water excretion, the proportional water losses in the urine, faeces and by evaporation were similar to those expected under cool conditions: 20, 30 and 50% respectively (Mitchell 1962).

It is interesting to note that, in *P. tridactylus* and *B. penicillata*, the additional EWL were offset by lower faecal-water losses; urinary water losses were similar in all three species. Both *Potorous* and *Bettongia* produce smaller faecal pellets than do *A. rufescens*. The higher surface area of the small pellets may explain why *Potorous* and *Bettongia* often produce drier faeces than *A. rufescens*..

Haematocrit

Packed cell volumes (PCV) differed between species, in all studies, but the ranking remained the same: *A. rufescens* > *B. penicillata* > *P. tridactylus*. The values recorded for the captive and wild *A. rufescens* (ca 60%) appear to be the highest reported for an adult of any mammalian species. The closest value is from *Halichoerus grypus* (grey seal) which had a PCV of 57% (Greenwood *et al.* 1971). Chilcott *et al.* (1985) reported a high value (59%) for *M. eugenii*, but presumed that the animals were stressed and that blood was released from the spleen; a much lower value had been reported in earlier studies (Hume and Dunning 1979). The high PCV for *A. rufescens* are probably not due to stress because:

1. *Wild animals were often chased around an enclosure before capture; captive animals were usually netted quickly.* However, both captive and wild *A. rufescens* had similar PCV.

2. *The time to capture and bleed animals varied.* Even so, successive bleedings of individuals from both captive and wild populations always gave consistent values.

3. *Blood from the spleen has been reported to haemolyse quickly* (Wright 1953). This was not observed in the present study.

The value for *B. penicillata* is also higher than that for most other metatherians. The physiological significance of these unusually high values is unclear. In a study of the haemogram of *P. tridactylus*, Moore and Gillespie (1968) reported PCV ranging between 40% and 53% with a mean value of 47% — close to the values in this study.

Water metabolism during lactation

In the present study, the effects of lactation on water metabolism, in wild and captive animals, could not be compared directly but, nonetheless, have features in common. Four of the wild *A. rufescens* supported "young-at-foot", but this did not influence TBW or WTR measured using tritiated water. Likewise, lactation did not

cause captive *A. rufescens* to drink more. Grubbs (1980) observed that wild lactating desert rodents also did not have higher WTR.

There have been few studies of water metabolism in lactating marsupials. Those that have been published are based on few observations, and generally contrast with the findings for potoroines. Kennedy and Heinsohn (1974) reported that lactating rock-wallabies (*Petrogale inornata*) have WTR 17% higher than non-lactating animals. Hulbert and Dawson (1974) measured the water economy of two lactating and five non-lactating captive *Isoodon macrourus*. Total body water was about 7% higher, and WTR about 36% higher in the lactating animals. Hulbert and Gordon (1972) noted gross changes in two lactating wild *I. macrourus*. When compared with non-lactating animals, the TBW in the two lactating animals was 32% and 36% higher, and the WTR 10% and 40% higher. In New South Wales, *I. macrourus* is a seasonal breeder. Thus, differences in TBW are probably explained by animals gaining fat when not breeding and then mobilizing the fat in the breeding season. The higher water turnover presumably reflects not only the water content of the milk but higher water demands of the increased metabolism of the lactating animal (Macfarlane and Howard 1972). It is worth noting that peramelids have the shortest lactation of any metatherian yet studied. Several eutherian mammals respond in a similar way. For example, lactation leads to high TBW and increases in WTR of 50-100%, in *Camelus dromedarius* (Macfarlane 1965) and goats (Maltz and Shkolnik 1980). Studies of captive *Sarcophilus harrisii* by Nicol (1978) contrast with the above data. The TBW of two females with pouch young was about 30% lower, but water turnover was 10% higher, than corresponding values for a male and a non-lactating female. Perhaps the low TBW in these females reflects a fat store that will be mobilized during the 15 weeks of lactation after the young emerge from the pouch.

Aepyprymnus is not a seasonal breeder. Furthermore, studies of wild *Aepyprymnus* found no seasonal variation in body mass; also, it was rare to capture a non-reproductive female (Section 11.3). These observations suggest that *Aepyprymnus* can usually sequester adequate nutrients and that fat reserves are unnecessary. Thus, TBW should remain constant. It is much harder to explain why WTR does not increase in animals at peak lactation. However, this finding is in agreement with those in Section 11.3, which showed that field metabolic rates also were not higher in lactating animals. As discussed in Section 11.3 this suggests that the potoroine lactational strategy is "tuned" to spreading, over time, the energy and water burden of reproduction.

Water restriction

Maloiy *et al.* (1979) divided mammalian herbivores into three physiological ecotypes in terms of their water and salt-handling functions:

1. Wet tropical or wet temperate origins, with high WTR and energy use, poor renal concentration and low salt tolerance — for example, buffalo, cattle, horse, pig, *Taurotragus oryx* (eland), *Nesotragus moschatus*, *Kobus ellipsiprymnus* (waterbuck), *Loxodonta africana*, *Alces alces* (moose), *Rangifer spp* (reindeer and caribou).

2. Warm dry savannah, semi-arid. Intermediate rates of water and energy use, good urine concentration, moderate salt tolerance — for example, sheep, donkey, *Connochaetes taurinus* (wildebeest), *Dasyprocta spp* (kongoni), *Procavia spp* and *Dendrohyrax spp.*

3. Arid-zone animals with low rates of energy and water turnover but tolerant of salt, and with medium to high urine concentration — for example, goats *Camelus spp*, *Oryx spp*, *Gazella spp*, *Dipodomys spp*, *Notomys spp.*

To which of the above categories do potoroine marsupials belong? The following discussion attempts to answer this question.

A loss of body mass is an inevitable result of water restriction. The *Aepyprymnus* and *Bettongia* in the present experiment lost up to 20% of their body mass; two *Potorous* in the second water restriction experiment lost 22 and 26% of their original body mass. It could not be determined whether potoroine marsupials are able to tolerate the 30% loss of body mass that *Camelus spp*, *Macropus r. erubescens* and goats are able to survive when given limited access to water (Schmidt-Nielsen *et al.* 1955; Ealey *et al.* 1965; Shkolnik *et al.* 1972). However, potoroines are at least as tolerant as sheep (Macfarlane *et al.* 1961) and donkeys (Maloiy 1970) which survived losses of their body mass of 23 and 20% respectively.

Restricting the water intake of potoroines did not affect the TBW expressed as a percentage of body mass. This result was expected because, in animals such as potoroines which have little stored fat, there is limited scope for change in body composition with loss of body mass. It is, however, debatable to use isotope-dilution methods to measure water relations in animals losing body mass, because the long isotope equilibration period may cause overestimation of TBW. This was shown in macropodids (Denny and Dawson 1975b) and camels (Siebert and Macfarlane 1971) and almost certainly occurred in the present study. Several dehydrated animals had high apparent TBW when this was determined from plasma samples obtained four hours after the isotope was administered; values within the expected range (70%-80%) were obtained when TBW was estimated from the regression of the specific activity of

tritium in the urine against time — measured over several days. Therefore, it was assumed that the plasma estimates were high because the isotope had not reached equilibrium in four hours.

Minimum water requirements are typically measured by reducing the drinking water from the *ad libitum* value to the point where the animals are able to maintain their mass at about 85% of the initial level (Taylor 1968). Some values cited by Taylor were 50 ml, 80 ml and 180 $ml.kg^{-0.8}.d^{-1}$ for *Oryx spp* and *Bos indicus* and *Bos taurus* respectively. By comparison, Rübsamen *et al.* (1979) reported values of about 30 ml per $kg^{-0.80}.d^{-1}$ for *Procavia habessinica*. It was not possible to estimate minimum water requirements in potoroines, because they were losing body mass. Therefore the WTR of 36 and 60 $ml.kg^{-0.8}.d^{-1}$ for *A. rufescens* and *P. tridactylus* respectively are underestimates. Nevertheless, it is probably reasonable to suggest that *Aepyprymnus* needs more water than *P. habessinica* but about the same as *Taurotragus oryx*. The large difference between *Aepyprymnus* and *Potorous* is discussed later.

Australia's animals have responded to the frequent low availability of water in many ways. These include morphological, behavioural, physiological and reproductive strategies (Archer 1984). However, before considering their physiology, we should remember that potoroine marsupials have several characteristics that may be expected to enhance water conservation. First, they are small and may avoid high temperatures by sheltering among vegetation. Second, they are nocturnal and thus avoid the high water losses that result from activity during the heat of the day. Third, they may terminate reproduction if water or nutrients are limiting. Fourthly, they often choose succulent food.

The two water restriction experiments identified several physiological processes commensurate with water conservation. In both experiments, water restriction caused the potoroines to reduce significantly the water content of their faeces to levels reported in a variety of arid-adapted herbivores. These include goats (Shkolnik *et al.* 1972), *C. dromedarius* and *Madoqua kirki*, Kirk's dikdik, (Maloiy 1972, 1973) and fat-tailed sheep (Maloiy and Taylor 1971). Also, potoroines ate less food, and thus water was saved by the lower production of drier faeces and by a proportionate reduction in heat production and thus lower EWL (Mitchell 1962). This reduction in gut water spares water for other needs — for example, maintenance of interstitial water.

In the second water-restriction experiment, limiting water caused a slight increase in apparent digestibility of dry matter. Again, this would tend to save water because the faecal output is further reduced. However, because the animals given limited water were not in a steady state, it is possible that the increased digestibility is an artefact of underestimating faecal output. In a review of the effect of water intake on feed

digestibility, More and Sahni (1981) concluded that water restriction often enhanced digestibility. However, the effect is usually small (for example, 3-5%; Silanikove 1984). It is probably insignificant relative to the water saved by other means, for example production of drier faeces, lower urine production and reduced EWL during conditions of water stress.

The urine produced during water restriction was about three times more concentrated than that excreted when water was freely available. *Aepyprymnus* and *Potorous* had maximum concentrations of 3060 and 3390 mosm.kg^{-1} respectively. These values are similar to those reported for some arid-adapted species — for example, sheep (Macfarlane *et al.* 1961), goats (Maloiy and Taylor 1971), *M. r. erubescens* (Denny and Dawson 1975b), some desert rodents (Grubbs 1980), *C. dromedarius* (Maloiy 1972) and *P. habessinica* (Rübsamen *et al.* 1979), but less than for some other arid-dwelling species — for example, *M. kirki* (Maloiy 1973) and *Macropus rufus* (Denny and Dawson 1975b).

The maintenance of plasma volume by dehydrated potoroine marsupials, is a characteristic also of *Camelus spp* (Macfarlane *et al.* 1963). Macfarlane (1968) explained this finding, in the camel, by their unusually thick capillary walls that retain albumen, and thus high osmotic pressure, during dehydration.

Results from the present study suggest that potoroine marsupials are most characteristic of the second group defined by Maloiy *et al.* (1979), — that is, animals that live in semi-arid regions and have intermediate rates of water and energy use, good urine concentration and moderate salt tolerance. This comparison should be treated with some caution, because the potoroines were subjected to a severe water restriction and were catabolising body solids when measurements were made. Furthermore, measurements were made under cool conditions. Potoroine marsupials may react quite differently to a hot environment.

In the second water-restriction experiment *P. tridactylus* were given 75% more drinking water than *A. rufescens*. Under these conditions, *Potorous* had a WTR that was about 65% higher than that of *Aepyprymnus*. But both species excreted faeces of similar dry-matter content and both concentrated their urine to a similar degree; both species lost similar proportions of their body mass. How do we explain the different water needs of *Aepyprymnus* and *Potorous* in the present experiment? In the studies of metabolic rate described in Section 11.1, it was shown that *P. tridactylus* doubled their rates of metabolism at night. By comparison, *A. rufescens* increased their metabolic rates by only 30% — probably because in the small metabolism chambers their activity is more limited than that of *Potorous*. Thus, it is suggested that the additional water

needs of *Potorous* are partly explained by higher evaporative water losses associated with a higher rate of metabolism.

The three potoroine species studied in the current project have low resting metabolic rates — a characteristic of most metatherians (Chapter 11). From this finding it is concluded that part of the low overall water consumption by potoroines is a secondary effect reflecting the low energy metabolism. Apart from this low metabolism, the constant PCV under *ad libitum* and restricted water conditions, the ability to produce dry faeces and concentrated urine, and the ability to rehydrate rapidly without ill effect, all indicate that potoroines, regardless of habitat, have attributes that would enhance their survival chances during droughts. This finding raises the question of when potoroines became arid adapted. Unfortunately, this question cannot be answered reliably, because little is known about potoroine ancestry in relation to climate. However, Flannery (1984) suggested that, unlike macropodids, potoroines did not undergo major radiations during the late Miocene to early Pliocene. At this time, the Australian climate was becoming drier and the forests were being replaced by vast areas of arid and semi-arid grasslands (Bartholomai 1972). The present study implies that potoroines did not display a parallel radiation because they were unable to utilise plant material high in structural carbohydrates, such as mature grass, not because they could not survive droughts.

Water turnover in the outdoor enclosures

The study in the outdoor enclosures was designed to compare water metabolism among the three genera of potoroine marsupials housed under similar conditions. Of secondary importance was the wish to compare the results with those published for other species. When confined to metabolism cages, the smaller species — *P. tridactylus* and *B. penicillata* — are far more active than *A. rufescens* (Section 11.1). Thus, it was deemed preferable to conduct the study in the outdoor enclosures. Here, activity was less restricted and disturbance to the animals was minimal.

The combined winter and summer results from the present study fall close to the regression line proposed for eutherian species by Richmond *et al.* (1962). Consequently, the potoroine data lie above the regression lines of Denny and Dawson (1975a) and Nicol (1978) for metatherians. The conditions in this study deviated from those recommended by Nicol (1978) for the determination of standard water-turnover rates. First, the animals were active in the outdoor enclosures. Secondly, the ambient temperatures in both seasons fell below the expected thermoneutral zone. Both of these factors raise metabolic rates and hence, presumably, water-turnover rates. Because animals housed in outdoor enclosures probably have higher metabolic rates and thus

higher WTR than caged animals, there is little reason to believe that the standard WTR for potoroines are different from the values predicted from the equation of Nicol. However, Denny and Dawson also obtained their data from macropodids housed in outdoor enclosures. Thus, the data from the present study suggest that the WTR of potoroine marsupials exceeds those of the macropodoids studied by Denny and Dawson (1975a). It should be emphasized that the equation of Nicol includes Denny and Dawson's data, and thus violates Nicol's own recommendations for measurements during minimal activity.

Perhaps the major finding of the present study was that, although *Aepyprymnus* tend to have lower values for most water kinetic parameters than do *P. tridactylus*, there are no major differences when values are expressed as a function of metabolic body mass. This is contrary to the cage studies in which there were consistent differences between the two species.

In conclusion, the results suggest that there are no inter-specific differences in water metabolism. Alternatively, if there are differences, they would be detected only through studies under restrictive conditions. This was the conclusion drawn by Denny and Dawson (1975a) and contradicts Macfarlane's (1965) thoughts that arid-adapted animals could be detected under *ad libitum* conditions.

Water flux in free-living *A. rufescens*

Because *A. rufescens*, at Drake, have not been observed drinking free water, food intake can be estimated by water flux, provided the water content of the diet is known. This is discussed in detail in Section 11.3. Clearly, food intake cannot be estimated if the animal passively imbibes large volumes of "non-food" free water while feeding or grooming. That this does occur after heavy rain was shown conclusively in the November/December study period at Drake (Table 10.8a). A week of constant rain saturated the ground and caused WTR in *A. rufescens* at least to double. The additional water is presumably excreted by a higher flow of urine and in wetter faeces.

Green (1990) reviewed water usage by free-living macropodoids. After rain, the rates of water influx were often three times those measured during dry periods. He explained these findings in terms of the increased water content of herbage which, after rain, may constitute 90% of the fresh mass of grass (Macfarlane and Howard 1972). Herbivores eating this herbage have a high obligatory water intake. In extreme circumstances (for example *M. eugenii*; Bakker *et al.* 1982) this may cause a loss of body solids, presumably because the animal cannot eat enough dry matter to obtain its nutrient requirements.

During dry periods, free-living *A. rufescens* have a rate of water flux that is 2.5 times the mean of 11 macropodoid species listed by Green (1990). Because *Aepyprymnus* appear to select very wet foods (Table 10.8c), much of this water intake is obligatory and provides no indication of their ability to survive droughts.

Table 10.8c The composition, expressed on a dry matter basis, of the tubers of three plant species eaten by A. rufescens at Drake.

Food item	GE ($MJ.kg^{-1}$)	DM (%)	Water (%)	ADF (%)	NDF (%)	Cell contents (%)	N (%)	Ash (%)
Hypochoeris radicata	15	20	80	40	40	60	0.57	18
Murdannia graminea	16	14	86	33	76	24	0.62	14
Trachymere incisa	15	16	84	38	40	60	0.91	22

Because free-living *Aepyprymnus* often seek foods of high water content, it is improbable that their high WTR after heavy rain are due to a higher than usual intake of free water in their food. Instead, the high WTR are best explained by the feeding behaviour of *Aepyprymnus*. This includes much digging and food processing, and is likely to cause "passive" water intake when the ground is saturated.

Green (1990) showed that the rate of water influx in free-living macropodoids scales to body $mass^{0.92}$. A similar exponent (0.94) relates gut capacity to body mass (Van Soest 1982; Chapter 2). Efficient fermentative digestion needs much water, because it depends on large volumes of saliva and a very liquid digesta (ca 80% water). Green (1990) recognized the relationship between gut capacity and the water needs of fermentation. He suggested that the rate exponent of 0.92 in free-living macropodoids may result from the superimposition of digestive requirements for water over predicted standard water requirements. This explanation would seem plausible if the water influx of free-living macropodoids was much greater than that of captive animals. This, however, is not the case. In dry conditions (defined as those in which no rain fell) WTR of free-living macropodoids were much higher than the standard WTR of their captive counterparts. One exception was *A. rufescens* from the current study which, as discussed already, has a high obligatory water intake from its succulent diet. Green

(1990) did not remark on the diets of the other species with high WTR — *M. giganteus* and *Petrogale inornata.*

The fact that there are differences between WTR in captive and free-living macropodoids leads us to question further the value of standard water requirements. It appears that both free-living and captive animals tailor their rates of water influx to the water available. The difference is that captive animals always have access to water. Perhaps the standard WTR would be better determined as the minimum water intake that allows an animal to maintain its body mass.

10.5 Summary

Potoroine marsupials drank more water when the levels of nitrogen and cell-wall constituents in maize-oat hull diets were increased. However, potoroines drank most water when fed lucerne-based diets. When measurements were made in metabolism cages, *P. tridactylus* consistently drank more water per unit metabolic body mass than did *B. penicillata* or *A. rufescens.* The smaller species had higher EWL and this was attributed to their high activity. In the outdoor enclosures the water-turnover rates were similar between species. All three species, when dehydrated, tolerated losses of body mass of about 20%. Under these conditions, their constant PCV, their ability to concentrate urine and produce dry faeces, and their ability to rehydrate rapidly, all suggest some drought tolerance. Lactating animals, captive or wild, did not drink more than non-lactating animals. Free-living *A. rufescens* had similar WTR in summer and winter. However WTR increased during periods of rain.

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