CHAPTER FIVE

The nitrogen requirements of potoroine marsupials

5.1 Introduction

ALTHOUGH 78% of the earth's atmosphere is nitrogen, little of the element is in a metabolisable form. Supplementing an animal's diet with nitrogen often enhances growth and increases reproduction and survival rates (for example, Preston and Leng 1988). These findings are supported by various ecological theories. For example, Slansky and Feeny (1977) proposed that a herbivore's nutrient consumption rate should vary to maximise nitrogen intake. Often, this equates with selecting a balanced diet with regard to an animal's requirements for nitrogen and energy (Prins and Beekman 1989).

Plants synthesize their structural components from carbohydrates, and hence contain less nitrogen (0.03-7% of dry matter; Mattson 1980) than do animals (10%; Hafez and Dyer 1969), which use nitrogenous compounds to synthesize structural tissues. Furthermore, as plant growth wanes, nitrogen levels fall sharply; plants, therefore, can vary enormously in the quantity and quality of nitrogen or nitrogenous compounds.

It is apparent that animals inhabiting areas with a variable climate, particularly those varying considerably in their rainfall, will at times face nitrogen shortages. They must, therefore, have elaborate mechanisms for sequestering available nitrogen. Mattson (1980) listed several strategies taken by herbivores to avert nitrogen deficiency. These include 1) increasing food intake; 2) prolonged feeding times; 3) specialized digestion incorporating endosymbionts; 4) occasional carnivory; 5) switching among plant parts and species; 6) evolution of larger body size. The animal may also evolve physiological processes which conserve nitrogen — for example, recycling endogenously synthesized urea to the gut.

The early studies of nitrogen metabolism by macropodids were conducted with species from arid or semi-arid environments. These included the *Setonix brachyurus* (Calaby 1958), *Macropus rufus* (McIntosh 1966), *M. robustus erubescens* (Brown and

Main 1967) and *M. eugenii* (Barker 1968). Not surprisingly, all exhibited mechanisms for conserving nitrogen.

Hume (1977b) recognized this bias towards arid-adapted species and designed studies to measure the maintenance nitrogen requirements of macropodids from diverging habitats. The maintenance requirement is defined as the minimum intake of dietary nitrogen necessary for the maintenance of nitrogen equilibrium. This corresponds to the turnover of tissues, and irreversible nitrogen losses in faeces, urine and hair. The productive animal needs additional nitrogen for pregnancy and lactation. The wet-forest dwelling *Thylogale thetis* which would rarely if ever experience nitrogen shortages, had double the maintenance nitrogen requirement of *M. eugenii*. Another inhabitant of moist forests — *M.* parma (parma wallaby), has similar requirements to those of *T. thetis* (Hume 1986).

These findings suggest that low nitrogen requirements reflect the evolutionary environment of a species rather than phylogeny, and open the possibility that similar differences may occur among the three potoroine genera.

This chapter describes a series of balance studies conducted to estimate the maintenance nitrogen requirements of potoroine marsupials.

5.2 Materials and methods

5.2.1 General

Two experiments were conducted specifically to measure the maintenance nitrogen requirement of *A. rufescens*. The approximate requirement was determined in Experiment 5.1 and this value was used to formulate diets of varying nitrogen content to allow precise measurements in Experiment 5.2. Nitrogen balance data from other experiments involving *P. tridactylus* and *B. penicillata* were then compared with the nitrogen balance-nitrogen intake regression lines for *A. rufescens*. This allowed estimates of nitrogen requirements for the former species.

5.2.2 Specific Procedures

Experiment 5.1 Preliminary studies of maintenance nitrogen requirements

Seven male and two female, adult *A. rufescens* were randomly assigned to three isoenergetic diets ranging in nitrogen content from 0.97 to 2.01% (Table 5.1). One animal refused to eat the low-nitrogen diet and after 3 days of the dietary adaptation period was transferred to the medium-nitrogen diet.

Table 5.1 Composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the diets fed in Experiment 5.1

Dietary ingredient	Level of inclusion		
	Low nitrogen	Medium nitrogen	High nitrogen
Maize	300	600	600
Wheat	170	170	170
Cornflour	500	200	170
HCl-Casein	—	—	30
Mineral mix (Table A1.6)	29	29	29
Mineral/Vitamin premix (Table A1.6)	1	1	1
Analysis			
Organic matter	988	979	980
Ash	12	21	20
Nitrogen	9.7	15.6	20.1
Acid detergent fibre	16	29	27
Neutral detergent fibre	73	102	99
Cellulose	11	22	20
Hemicellulose	57	73	72
Lignin	5	7	7

Experiment 5.2 The maintenance nitrogen requirements of *A. rufescens*

Eight adult male *A. rufescens* were offered, high fibre diets containing 1.70, 1.05 and 0.67% nitrogen and low fibre diets with 1.30, 0.94 and 0.58% nitrogen (Table 5.2) in six collection periods (CP). The animals were assigned to two groups of four in a crossover design such that one group received the high-fibre series first and the other the low fibre. In each half of the experiment, the high nitrogen diets were offered first, followed by the medium and then the low nitrogen diets (Table 5.3).

Table 5.3 The design of Experiment 5.2

	Collection period (CP) and diet					
	CP1	**CP2**	**CP3**	**CP4**	**CP5**	**CP6**
Group 1	HN-HF	MN-HF	LN-HF	HN-HF	MN-HF	LN-HF
Group 2	HN-LF	MN-LF	LN-LF	HN-LF	MN-LF	LN-LF

HN, MN, LN - high, medium, low nitrogen
HF, LF - high, low fibre

Table 5.2 The composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the diets fed in Experiment 5.2.

	Level of inclusion					
Dietary ingredient	**LN-LF**	**MN-LF**	**HN-LF**	**LN-HF**	**MN-HF**	**HN-HF**
Maize	140	410	680	130	410	620
Wheat	100	100	100	100	100	100
Oat-hulls	130	100	70	297	267	235
Cornflour	600	360	120	443	193	—
HCl-Casein	—	—	—	—	—	15
Mineral mix (Table A1.6)	29	29	29	29	29	29
Mineral/Vitamin premix (Table A1.6)	1	1	1	1	1	1
Analysis						
Organic matter	972	964	965	963	953	957
Ash	28	36	35	37	47	43
Nitrogen	5.8	9.4	13.0	6.7	10.5	17.0
Acid detergent fibre	73	65	63	139	142	134
Neutral detergent fibre	161	155	148	298	281	273
Cellulose	58	53	51	111	117	110
Hemicellulose	88	90	85	159	139	139
Lignin	15	12	12	28	25	24

Measurements of the rate of passage of digesta were made on all animals fed the high-nitrogen diets (that is, CP1 and CP4) and these are reported in Chapter 7. Animals were fed the high-nitrogen diets for three weeks; other diets were fed for two weeks.

During the four-week crossover period (that is, between CP3 and CP4), all animals were housed in the outdoor enclosures and were fed the maintenance ration (Appendix 1). This was enough time for animals to regain any condition lost during the previous two weeks on the low nitrogen diets.

Other aspects of the experiments (animal husbandry, collection procedures and analytical techniques) have been described previously in Chapter 4.

5.2.3 Calculations

Metabolic faecal nitrogen (MFN) was determined by the method of Bosshardt and Barnes (1946). In this method, MFN is the intercept on the Y axis of the regression line describing the relationship between faecal nitrogen per 100g dry matter intake (DMI) and the nitrogen content of the food eaten.

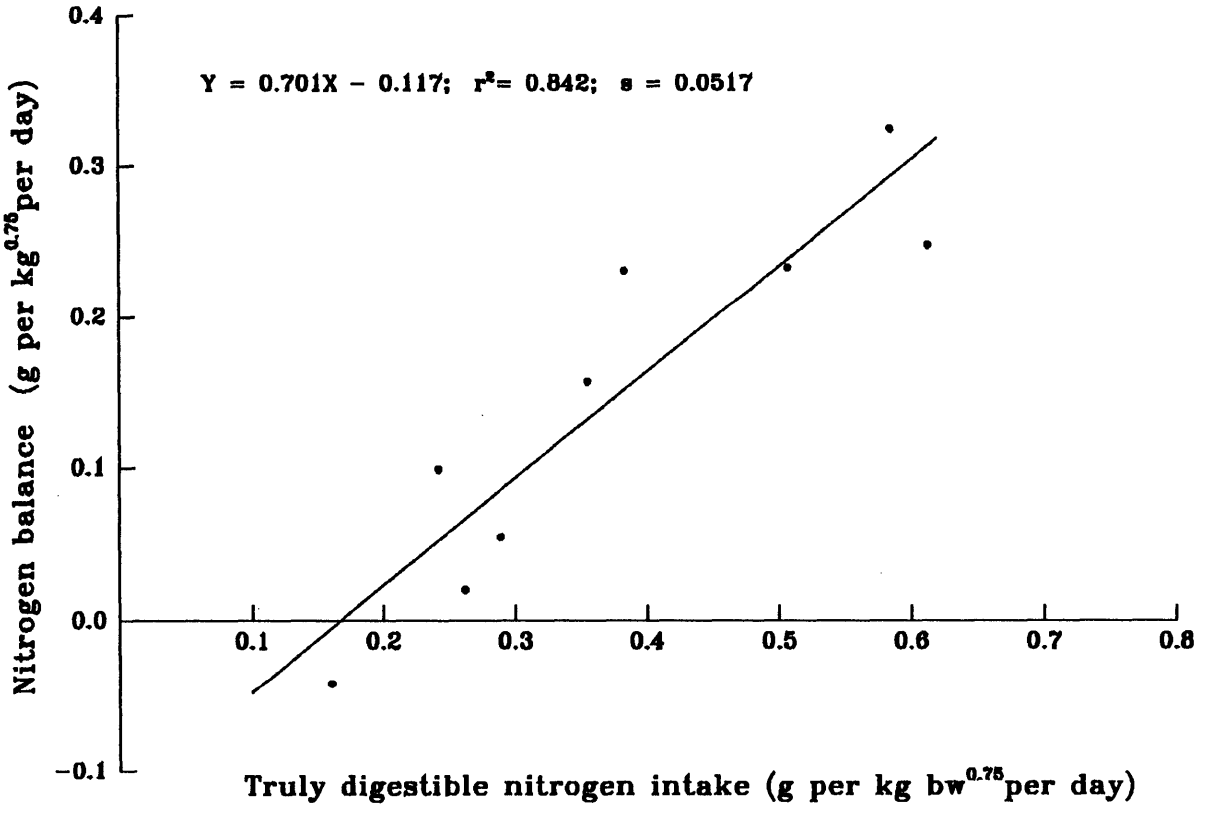

Figure 5.2. The relationship between nitrogen balance and truly digestible nitrogen intake in Experiment 5.1.

5.2.4 Statistical

The maintenance nitrogen requirement was estimated as the intake of nitrogen supporting zero nitrogen balance, by linear regression of nitrogen balance on nitrogen intake. Tests of non-parallelism and differences in elevation between the various regression lines were tested statistically using the computer programme "lines" (T. J. Kempton unpub).

5.3 Results

General

Because the results of Experiment 5.2 reflected the trends observed in the preliminary study (Experiment 5.1), little emphasis is placed on the findings of the latter study. Instead, the results of Experiment 5.1 are referred to only briefly the relationship between nitrogen balance and truly digestible nitrogen intake is presented graphically (Fig 5.2).

5.3.1 Nitrogen balance

In Experiment 5.1, the nitrogen balance values ranged from -0.04 to +0.33 $g.kg^{-0.75}.d^{-1}$; eight of the nine animals maintained positive nitrogen balance. The sole negative value was a consequence of a low DMI rather than a diet of unacceptably low nitrogen content. Diets of lower nitrogen content were offered in Experiment 5.2; these produced a similar range of values for nitrogen balance (-0.09 to +0.33 $g.kg^{-0.75}.d^{-1}$). However, in this experiment, 12 of the 45 data points were animals in negative nitrogen balance.

5.3.2 Metabolic faecal nitrogen

When faecal nitrogen (g per 100g DMI) was regressed against the nitrogen content of feed consumed, the resulting significant ($P<0.05$, $P<0.001$, $P<0.001$) relationships yielded estimates of metabolic faecal nitrogen of 0.26, 0.34, and 0.36g per 100g DMI respectively for the preliminary experiment and the low and high fibre diets in the major study (Fig 5.1). These values were used to calculate intakes of truly digestible nitrogen, according to Mitchell and Bert (1954).

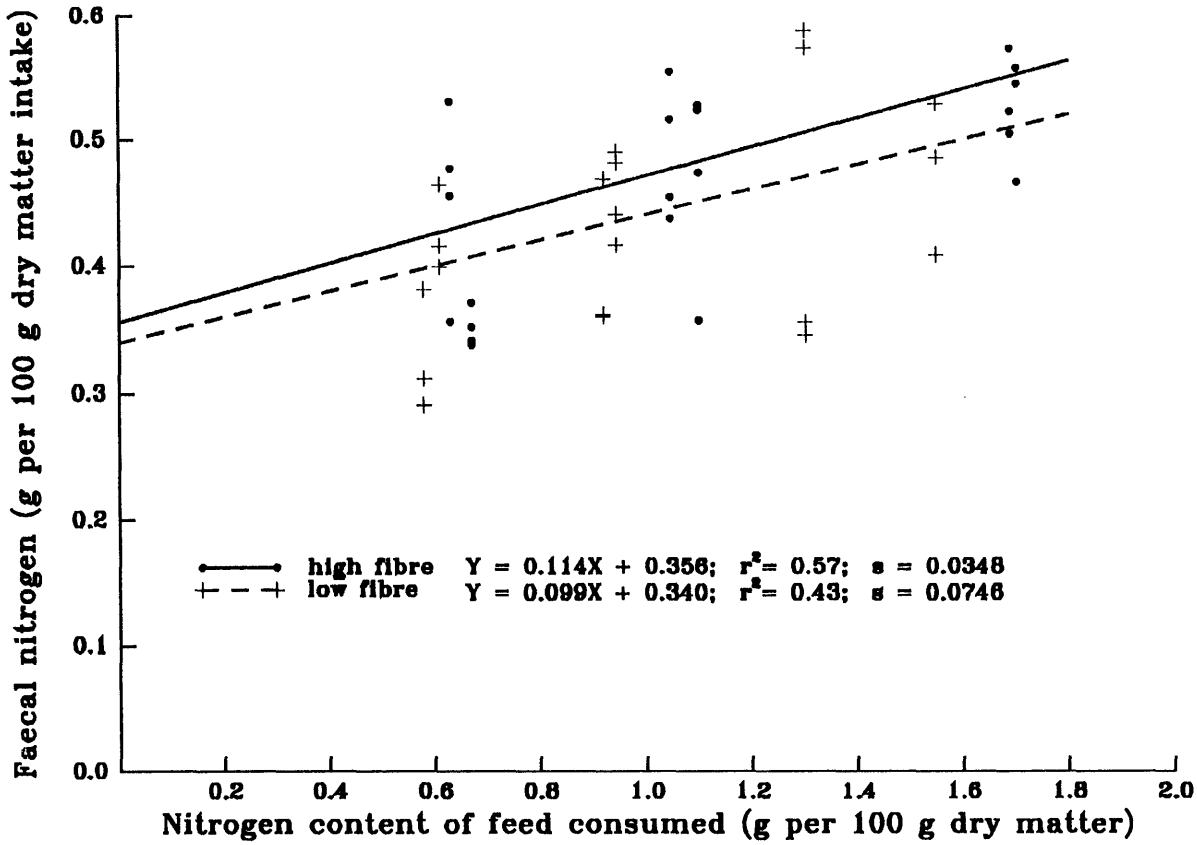

Fig 5.1 The relationship between faecal nitrogen output per 100 g of dry matter intake and the nitrogen content of the diet

5.3.3 Maintenance nitrogen requirements

In Experiment 5.1 the significant relationship between nitrogen balance and nitrogen intake yielded an estimate of maintenance nitrogen requirement of 170 mg of truly digestible nitrogen per $kg^{-0.75}.d^{-1}$ (Fig 5.2). Similar significant relationships between nitrogen balance and the intake of truly digestible nitrogen for the low- and high-fibre diets fed in Experiment 5.2 are shown in Figure 5.3a. The low- and high-fibre regression lines did not differ significantly with respect to parallelism or intercept. This justified the pooling of all data (Fig 5.3b) to yield a single estimate of maintenance nitrogen requirement of 200 mg of truly digestible nitrogen per $kg^{-0.75}.d^{-1}$ for animals fed grain-based diets with neutral-detergent fibre levels between 100 and 300 g per kg

dry matter. As expected, nitrogen balance was related also to the intake of nitrogen and to the intake of apparently digestible nitrogen as shown in the following equations:

Nitrogen balance ($g.kg^{-0.75}.d^{-1}$) = 0.591 x Nin - 0.132; r^2 = 90%; s = 0.0362; P<0.001.....*Equation 5.1*

Nitrogen balance ($g.kg^{-0.75}.d^{-1}$) = 0.734 x ADNin - 0.0682; r^2 = 86%; s = 0.0426; P<0.001 .....*Equation 5.2*

where Nin and ADNin are, respectively, dietary nitrogen intake and apparently digestible nitrogen intake expressed as $g.kg^{-0.75}.d^{-1}$.

In Fig 5.4 nitrogen balance data from the rate-of-passage comparison between *A. rufescens*, *P. tridactylus* and *B. penicillata* (Chapter 7) are compared with the regression of nitrogen balance on apparently digestible nitrogen intake determined in Experiment 5.2. Maize-oat hull diets of similar composition were fed in both studies.

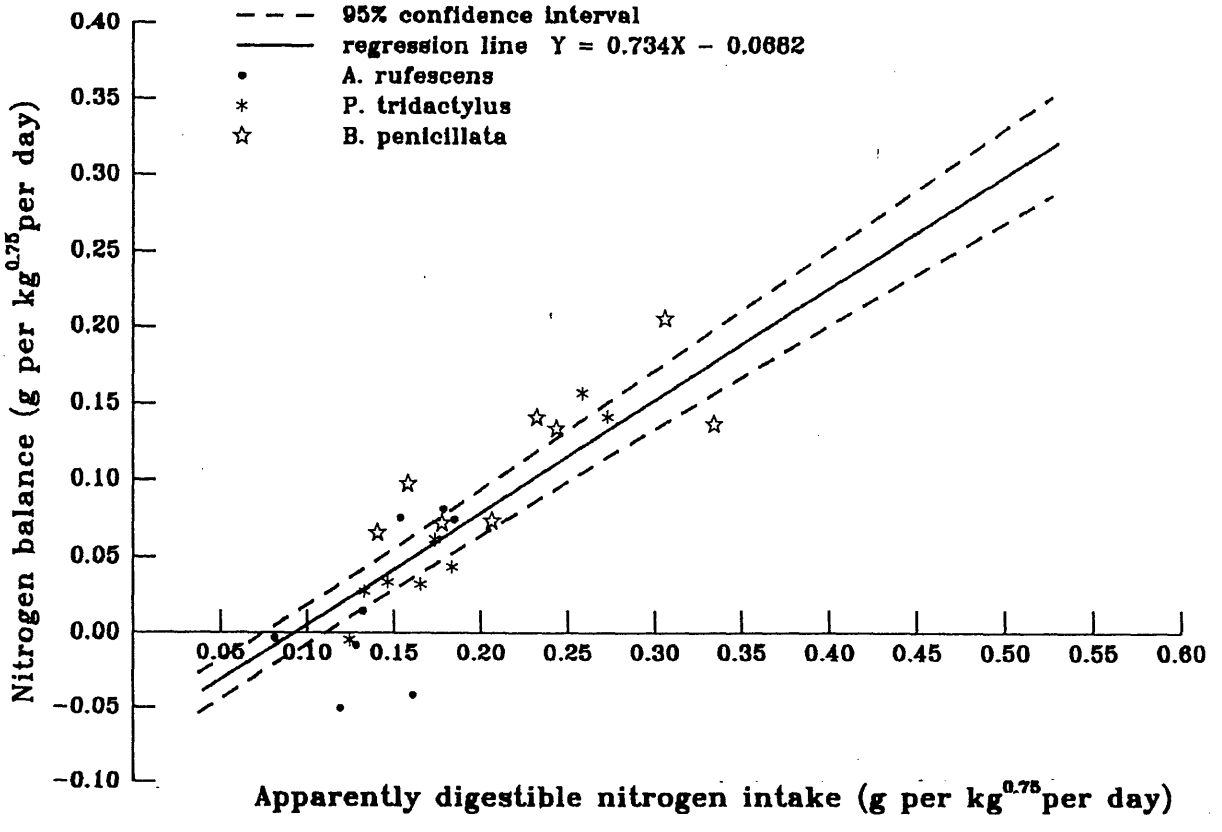

Fig 5.4 Nitrogen balance data from A. rufescens, P. tridactylus and B. penicillata fed a maize-oat hull ration (Chapter 7) compared with the nitrogen balance-apparently digestible nitrogen intake regression line determined in Experiment 5.2 (Equation 5.2)

Many of the data points lie within, or just outside, the 95% confidence interval about the regression line. This comparison suggests that the three potoroine species have similar maintenance nitrogen requirements. However, this conclusion should be treated with caution owing to the narrow range of the *Aepyprymnus* data.

Figure 5.3a

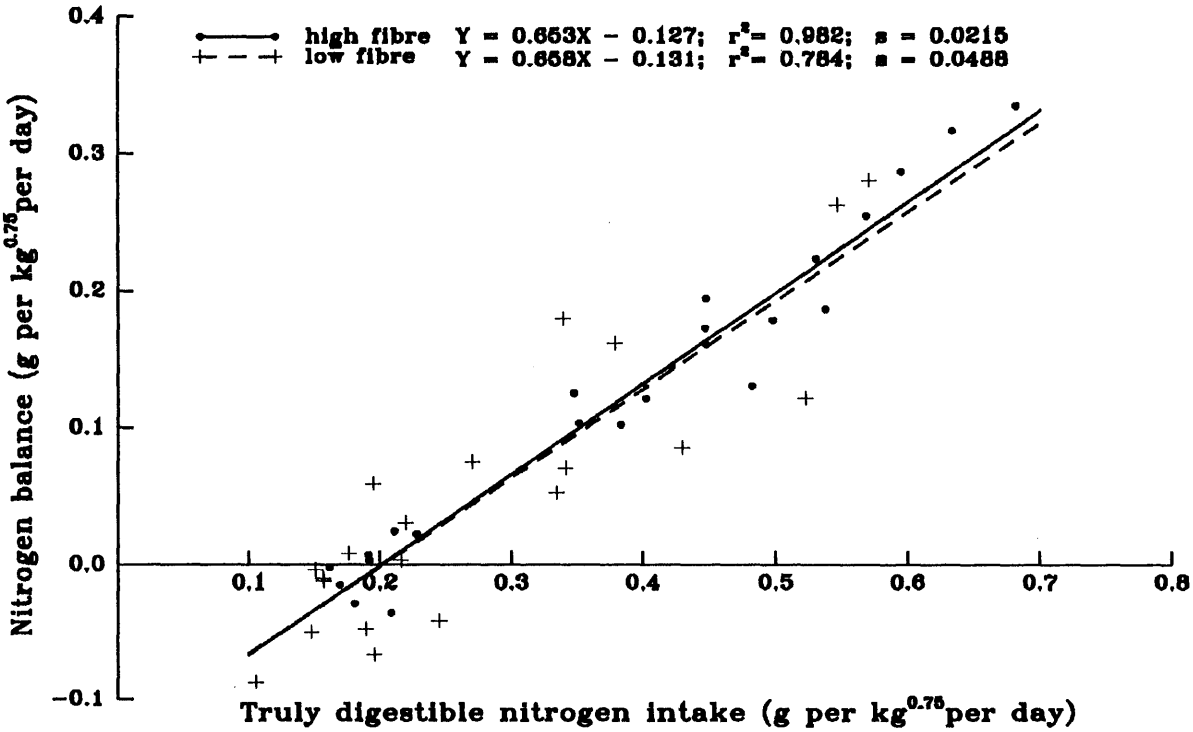

Figure 5.3b

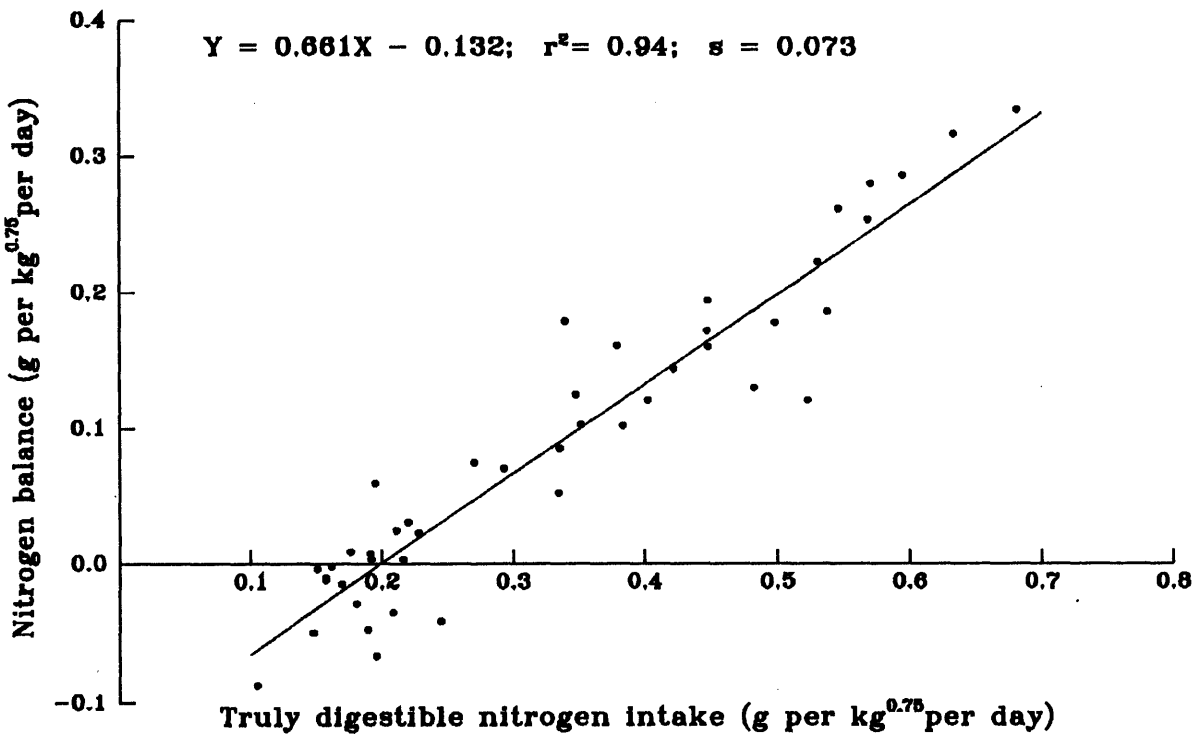

Fig 5.3a,b The relationship between nitrogen balance and truly digestible nitrogen intake (Experiment 5.2)

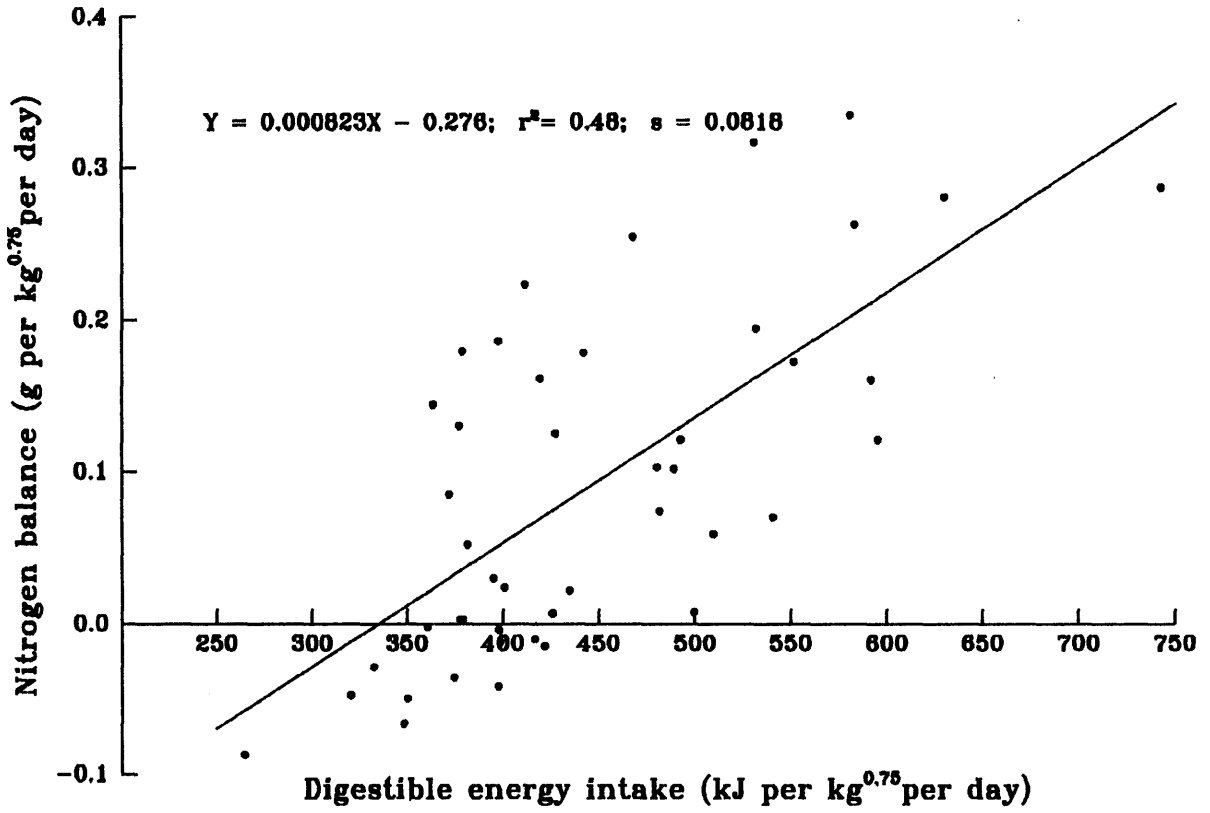

Fig 5.5 The relationship between nitrogen balance and digestible energy intake in A. rufescens

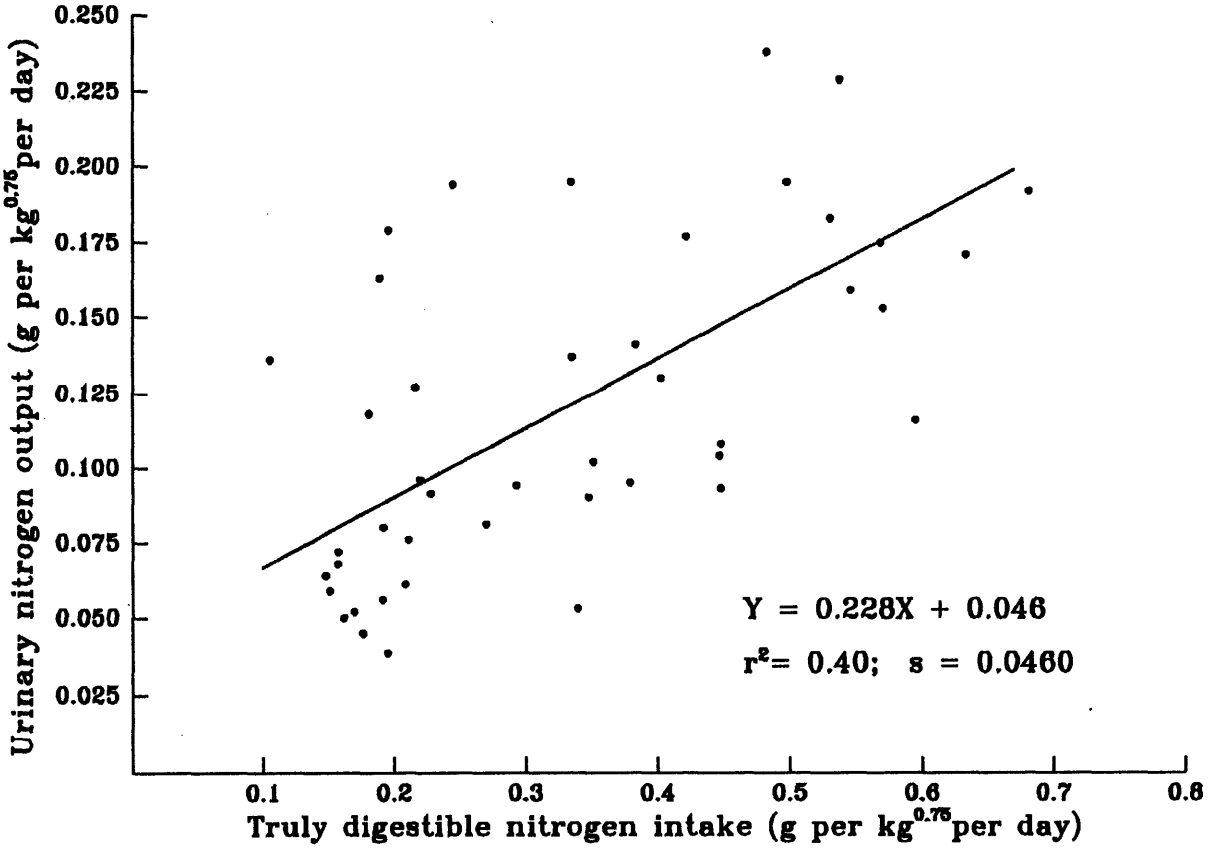

Fig 5.6 The relationship between urinary nitrogen output and truly digestible nitrogen intake in A. rufescens

Although the regressions of nitrogen balance on digestible energy intake (DEI) were significant ($P<0.05$ and $P<0.001$) in the preliminary and major experiments respectively (Fig 5.5), a comparison of DEI with maintenance energy requirements of *A. rufescens* determined by Wallis and Farrell (1988; Chapter 11) indicates that only 2 of the 45 observations were of animals in negative energy balance. Even so, the urinary nitrogen output of these individuals was not so large as to suggest that they were catabolizing body protein to cover any ATP deficit. The fact that most animals were in positive energy balance is supported further by the significant ($P<0.001$) positive relationship between urinary nitrogen excretion and truly digestible nitrogen intake for the major experiment (Fig 5.6). Furthermore, all relationships between nitrogen balance and nitrogen intake were highly significant ($P<0.001$) and showed no tendency towards curvilinearity at low nitrogen intakes. At very low nitrogen intakes, protein catabolism and high urinary nitrogen excretion might be expected in response to depressed DMI and hence low DEI.

Both experiments produced significant ($P<0.05$, $P<0.001$) relationships between nitrogen intake and faecal nitrogen excretion (Fig 5.7).

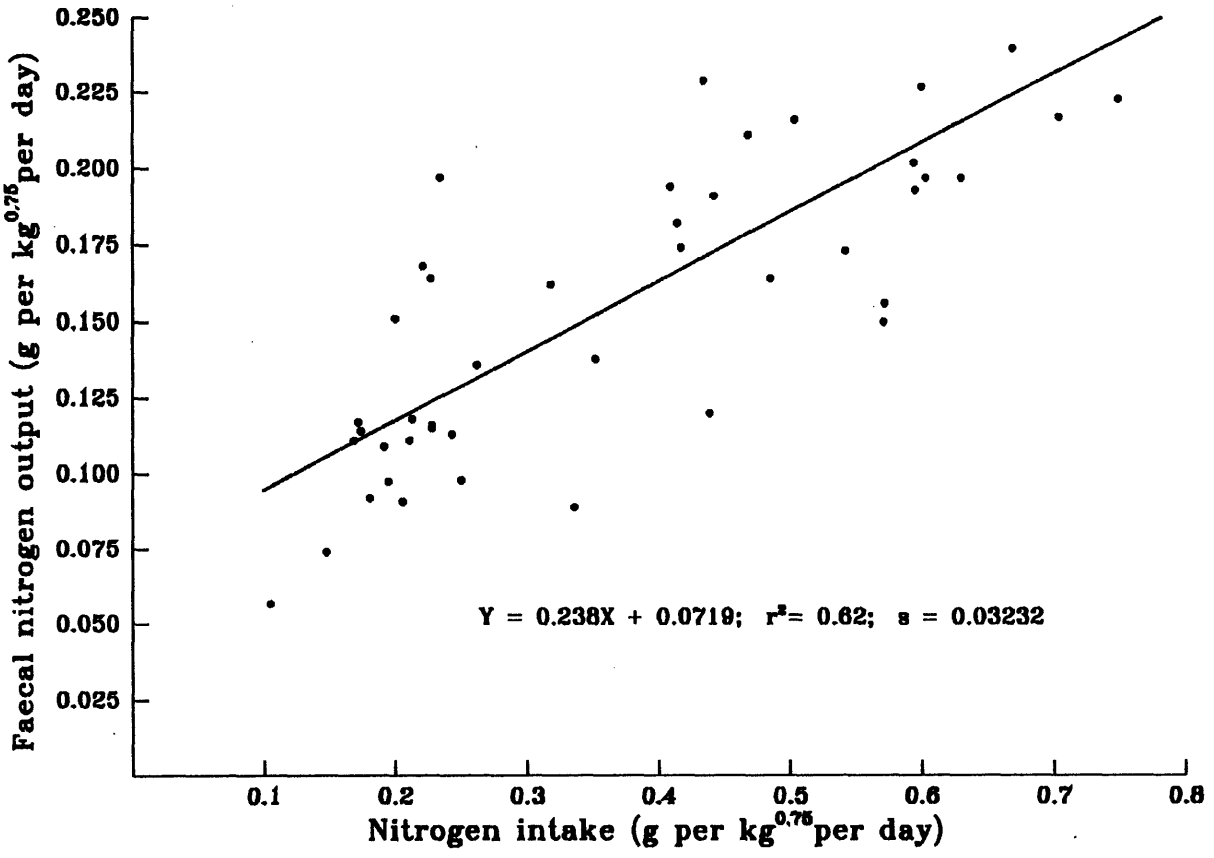

Fig 5.7 The relationship between faecal nitrogen output and dietary nitrogen intake (Experiment 5.2)

5.3.3 Other parameters

Even though animals were given time to recuperate between the two stages of Experiment 5.2, there was still a significant ($P<0.05$) effect of experimental period upon body mass, change of body mass during the experimental period, intake of digestible energy and nitrogen, and water consumption per unit metabolic mass.

The consumption of both dry matter ($P<0.05$) and water ($P<0.01$) per unit metabolic body mass rose in response to increasing dietary fibre level. The latter response appears directly related to the rise in DMI, because it was not evident when water intake was expressed as a percentage of DMI. Apparent digestibility of both dry matter and energy were markedly reduced ($P<0.001$) as dietary-fibre concentration increased.

The greatest influence upon the measured parameters was exerted by dietary-nitrogen concentration. As this declined, there were significant depressions in body mass ($P<0.001$), DMI ($P<0.01$), DEI ($P<0.05$) and water intake per unit body mass ($P<0.001$) and as a percentage of DMI ($P<0.001$). Nitrogen intake, urinary nitrogen excretion and nitrogen balance also declined ($P<0.001$). As dietary nitrogen content fell, the output of faecal nitrogen also fell, but at a lower rate than nitrogen intake. Therefore nitrogen digestibility was much lower ($P<0.001$) on the low nitrogen diet.

A significant interaction between dietary nitrogen and fibre occurred such that body mass ($P<0.001$), DMI ($P<0.001$) DEI ($P<0.05$), N intake ($P<0.001$) and water intake per unit DMI were all increased by simultaneous increases of dietary nitrogen and fibre.

5.4 Discussion

In the present study, 199 mg of truly digestible nitrogen per kg metabolic body mass was sufficient to meet the daily maintenance requirement of *A. rufescens* fed grain-based diets. This requirement is compared with those of other species in Table 5.6. Clearly, *A. rufescens* has similar requirements to those of *M. eugenii* (Barker 1968; Hume 1977b); *M. r. erubescens* (Brown and Main 1967); and *S. brachyurus* (Brown 1968), all of which inhabit semi-arid regions where frequent fluctuations in food quality occur. *Macropus r. robustus* and *M. giganteus* — animals of more mesic but by no means predictable environments — also have similar requirements. All of these macropodids apparently require minimal nitrogen compared with the wet-forest dwelling *T. thetis*.

The preponderance of low requirements for maintenance nitrogen amongst the genus *Macropus* led Hume (1986) to propose that maintenance nitrogen requirements have a phylogenetic basis. However, his later finding that another inhabitant of wet forests, *M. parma*, also exhibits high requirements lends itself to Hume's alternative proposal, that

Table 5.4 Intake, digestibility and balance data from A. rufescens fed diets containing 1.0 (LN), 1.6 (MN) and 2.0% (HN) nitrogen.

	LN	MN	HN	ems	sig
number	2	4	3		
Body mass (g)	2700	2660	2990		
(sem)	(115)	(69)	(87)		
Dry Matter					
Intake ($g.d^{-1}$)	63	67	83	229.3	ns
Intake ($g.kg^{-0.75}.d^{-1}$)	30	32	37	59.9	ns
Apparent digestibility (%)	92	88	88	3.3	ns
Energy					
DE Intake ($KJ.kg^{-0.75}.d^{-1}$)	421	439	518	11391.9	ns
Water					
Intake ($g.d^{-1}$)	147	102	108	1554.2	ns
Intake ($g.kg^{-0.80}.d^{-1}$)	71	49	48	402.0	ns
Intake ($g.100g^{-1}$ DMI)	222	162	131	3079.5	ns
Nitrogen					
Intake ($g.kg^{-0.75}.d^{-1}$)	0.29	0.47	0.74	0.011	***
Faecal N ($g.kg^{-0.75}.d^{-1}$)	0.11	0.16	0.19	0.004	ns
Apparent Digestibility (%)	61	65	74	34.6	ns
Urinary N ($g.kg^{-0.75}.d^{-1}$)	0.15	0.18	0.28	0.003	ns
Balance ($g.kg^{-0.75}.d^{-1}$)	0.03	0.12	0.27	0.007	*

Table 5.5 Intake, digestibility and balance data from A. rufescens fed low (ca 0.6% N), medium (1% N) or high (1.6% N) nitrogen diets.

	Low fibre			High Fibre								
	LN	MN	HN	LN	MN	HN	sed	Fibre	sed	N	N.Fibre	N.CP[1]
number	8	8	8	8	8	8						
Body mass (g)	2880	3020	3080	2840	2930	2880						
(sem)	(190)	(180)	(180)	(110)	(110)	(110)						
Change (% CP)	-1.3	-0.1	1.2	-1.6	1.6	1.8	0.28	ns	1.03	*	ns	*
Dry Matter												
Intake ($g.kg^{-0.75}.d^{-1}$)	28	28	35	32	45	36	3.20	*	1.79	**	**	ns
Apparent digestibility (%)	83	82	84	71	68	68	1.0	***	1.32	ns	ns	*
Energy												
DE Intake ($KJ.kg^{-0.75}.d^{-1}$)	405	409	480	391	538	446	38.9	ns	32.8	*	*	ns
Water												
Intake ($g.d^{-1}$)												
Intake ($g.kg^{-0.80}.d^{-1}$)	18	23	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	***	**	*
Nitrogen												
Intake ($g.kg^{-0.75}.d^{-1}$)	0.17	0.26	0.48	0.21	0.48	0.62	0.033	**	0.019	***	***	ns
Faecal N ($g.kg^{-0.75}.d^{-1}$)	0.10	0.12	0.16	0.13	0.21	0.20	0.031	ns	0.028	ns	ns	ns
Apparent Digestibility (%)	39	53	65	38	55	68	4.2	ns	4.6	***	ns	ns
Urinary N ($g.kg^{-0.75}.d^{-1}$)	0.07	0.13	0.15	0.07	0.11	0.20	0.018	ns	0.020	***	ns	ns
Balance ($g.d^{-1}$)												
Balance ($g.kg^{-0.75}.d^{-1}$)	-0.01	0.00	0.16	-0.00	0.16	0.22	0.024	**	0.029	***	*	ns
ADF digestibility (%)	18	21	37	26	16	20	3.7	ns	3.7	*	**	**
NDF digestibility (%)	26	26	43	20	19	20	6.9	**	4.5	*	ns	**

1 - collection period

high maintenance nitrogen requirements reflect the nutritional environment of a species (Hume 1986).

Further insight into this question comes from examining the maintenance nitrogen requirements of a range of eutherian species, which, if nitrogen and energy metabolism are inter-dependent (Smuts 1935), should have higher requirements than marsupials. Hume *et al.* (1980) used the data of Moir and Williams (1950) to calculate a daily maintenance requirement for sheep of 452 mg nitrogen per kg metabolic body mass, and Prior *et al.* (1974) determined that horses need 331 mg. Unfortunately there are insufficient data on a range of ruminant and horse species to explain this difference. Perhaps of greater interest from an ecological perspective is the similarity between the maintenance nitrogen requirement of *A. rufescens* and *Procavia habessinica*, a 2-4 kg eutherian herbivore from semi-arid and arid parts of northern and eastern Africa and the eastern Mediterranean. If the nutritional environment of a species is the principal determinant of nitrogen metabolism then we would predict this low value. Furthermore, we would predict the depressed basal metabolism and rate of water consumption reported for *P. habessinica* by Rübsamen *et al* (1979). However, all species of the Hydracoidea exhibit levels of basal metabolism below the eutherian mean (Taylor and Sale 1969), even though the genera occupy a wide range of habitats. From this brief discussion it seems reasonable to refute any simple explanation, such as that of Hume (1986), for differing maintenance requirements among different mammalian taxa.

Because the diet is the sole source of all the body's nitrogen, differences in requirements for truly digestible nitrogen reflect differences in the excretion rates of faecal and/or urinary nitrogen. In the present study, excretion rates by both routes were significantly related to nitrogen intake ($P<0.01$, Figs 5.6 and 5.7). Therefore, standardized urinary and faecal nitrogen excretion rates can be computed by substituting the truly digestible nitrogen intake data of various species, into the regression equations describing nitrogen excretion by *A. rufescens*. Standard nitrogen excretion values derived in this way, and expressed as a percentage of the faecal or urinary losses of *A. rufescens* at the same level of nitrogen intake are shown in Table 5.6. These standard values enable differences in nitrogen economy between species to be attributed to the appropriate avenues of nitrogen excretion. For example, the high requirements of the wallabies, *T. thetis* and *M. parma*, and *Petauroides volans* (greater glider) are due to urinary and faecal nitrogen losses which are between 50% and 90% higher than those from *A. rufescens* at the same level of nitrogen intake. While the urinary nitrogen excretion of *Phascolarctos cinereus* is low, its truly digestible nitrogen requirement is still higher than that of *A. rufescens*, due to higher losses of faecal nitrogen.

Table 5.6 The nitrogen requirements and partitioned nitrogen excretion of metatherians. Values are means, expressed as mg.kg$^{-0.75}$.d^{-1}. Values in brackets (%) are N losses relative to those of A. rufescens.

Species	Truly digestible N requirement	Dry matter intake (g)	Truly digestible N intake	Urinary N excretion		Faecal N excretion		Non dietary faecal N		Reference
Foregut fermenters										
M. eugenii	250	31	360	190	(146)	180	(110)	110	(100)	Barker (1968)
M. eugenii	230	28	450	130	(87)	150	(81)	150	(150)	Hume (1977b)
T. thetis	530	38	520	270	(164)	290	(145)	210	(150)	Hume (1977b)
M. parma	480	39	380	220	(169)	310	(182)	220	(157)	Hume (1986)
M. r. erubescens	160	*	*	*	*	*	*	*	*	Brown and Main (1967)
M. r. robustus	240	*	*	*	*	*	*	*	*	Foley *et al.* (1980)
M. giganteus	270	*	*	*	*	*	*	*	*	Foley *et al.* (1980)
A. rufescens	200 [1]									This study

Table 5.6 *continued*

Hindgut fermenters										
T. vulpecula	420	36	440	160	(109)	390	(213)	260	(166)	Foley and Hume (1987a)
T. vulpecula	210	33	440	80	(55)	130	(72)	110	(92)	Wellard and Hume (1981)
T. vulpecula		19	440	180	(55)	110	(61)	60	(90)	Wellard and Hume (1981)
Pseudocheirus peregrinus	290	41	360	220	(169)	190	(116)	100	(67)	Chilcott and Hume (198)
Phascolarctos cinereus	270	41	490	100	(63)	290	(153)	240	(160)	Cork (1986)
Petaurus breviceps	73	54	238	95	(95)	69	(51)	37	(19)	Smith *et al.* (1987)
Petauroides volans	560	44	710	400	(192)	360	(146)	220	(140)	Foley and Hume (1987a)

1 - Data for *A. rufescens* are provided elsewhere in this chapter.
* - insufficient data given

Maintenance nitrogen requirements have invariably been measured in animals fed a very limited range of dietary ingredients. Although existing data span metatherian and eutherian species from different habitats, information is rare describing differences in nitrogen requirements that can be related to differences in dietary composition. The contrasting data published for the *Trichosurus vulpecula* (common brushtail possum) is an exception. It exemplifies the care that must be exercised when interpreting nitrogen requirement data. Wellard and Hume (1981a) determined that, for maintenance, *T. vulpecula* requires 210 mg of truly digestible nitrogen per kg metabolic body mass per day when fed a synthetic diet, whereas Foley and Hume (1987a) reported that *T. vulpecula* consuming *Eucalyptus* foliage requires 420 mg. Most of the difference is accounted for by an increase in MFN excretion on the *Eucalyptus* diet, which is known to be expensive in terms of faecal nitrogen losses (Cork 1986; Foley and Hume 1987a). Indeed, this is not surprising because MFN is derived from undigested enzymes, mucoproteins and sloughed mucosal cells which, being naturally resistant to proteolysis (Gitler 1964), may pass intact to the caudal reaches of the gut and be excreted or further modified by the microbial population in the hindgut (Van Weerden *et al.* 1981). In fact microbial nitrogen is regarded as a major component of MFN in foregut fermenting herbivores (Blaxter and Mitchell 1948) and the same is expected in hindgut fermenters. *Eucalyptus* leaves contain much tannin and lignified cell-wall material, both of which are usually associated with high losses of faecal nitrogen (Whiting and Bezeau 1957; Mason 1971; Mould and Robbins 1981; Wellard and Hume 1981a; Shah *et al.* 1982). More specifically, Hallsworth and Coates (1962) noted that high-fibre diets caused greater destruction of mucosal cells and greater secretion of mucus into the intestine. Furthermore, the species consuming *Eucalyptus* foliage are all hindgut fermenters which, apart from the caecotrophic *Pseudocheirus peregrinus*, have no means for preventing losses of microbial nitrogen.

Other factors known to affect the output of endogenous nitrogen include the kind of protein fed (Rérat 1981; Corring 1982), food intake and body mass (Shannon 1982), metabolic rate, energy intake and duration of starvation (Dale and Fuller 1981) and the age of the animal (Rérat 1981). In the present study, a reduction in particle size through pelleting may explain why faecal nitrogen excretion did not rise in response to dietary fibre concentration. Indeed, Szentmihályi (1977) attributed a similar response in pigs to the unabrasive nature of the powdered cellulose used to supply dietary fibre. However, he mentioned that powdered cellulose is known to increase faecal nitrogen losses under some circumstances.

In conclusion, MFN — the product of many processes — is a major contributor to nitrogen excretion. This places in question single experiments to establish nitrogen requirements, except perhaps with animal species that naturally select few dietary

ingredients. Even then, the nutritional variation within single dietary ingredients may have a profound effect on nitrogen metabolism.

If we are going to question the use of single estimates of MFN, we must, by definition, also question the term true digestibility. Indeed, is there any advantage in expressing requirements in terms of true digestibility? Mitchell and Bert (1954) offered two reasons for measuring MFN: 1) when an accurate knowledge of the wastage of dietary nitrogen both in digestion and metabolism is essential, and 2) in the determination of nitrogen requirements. The MFN represents wastage of body nitrogen that must be replaced to maintain the nitrogenous integrity of the body. Therefore, if a diet affects MFN excretion, this fact should be acknowledged. Thus, requirements expressed in terms of apparently digestible nitrogen are the most appropriate. Detailed information of the diet should be supplied also.

In determining nitrogen requirements by feeding diets to provide a range of nitrogen intakes, it is important that animals are not energy deficient to the extent that they metabolize body protein to furnish ATP. Should this situation arise, maintenance nitrogen requirements will be overestimated through elevated losses of urinary nitrogen.

The presence or absence of a significant relationship between nitrogen balance and DEI has been taken by many workers to indicate energy deficiency and energy sufficiency respectively (for example Hume 1977b; Hume *et al.* 1980; Cork 1986; Foley and Hume 1987a). These thoughts are based on the assumption that zero nitrogen balance coincides with zero energy balance in mature animals (Hume 1974) — an assumption which implies a correlation between energy metabolism and nitrogen balance. Indeed, nitrogen balance and digestible energy intake are usually positively correlated in experiments measuring maintenance nitrogen requirements. This relationship is confirmed over a range of both positive and negative nitrogen balance values of animals showing minor fluctuations in body mass. Examples include sheep, *M. rufus* and *M. r. erubescens* (Hume 1974); *T. thetis* (Hume 1977b); *P. habessinica* (Hume *et al.* 1980); *T. vulpecula* and rabbits (Harris *et al.* 1985); *M. parma* (Hume 1986); *P. volans* and *T. vulpecula* (Foley and Hume 1987a) and *A. rufescens* in the present study.

I contend that this relationship between nitrogen balance and DEI does not imply catabolism of body protein to meet ATP needs but, instead, is indicative of the integration of protein and energy metabolism. Smuts (1935), in discussing a possible relationship between basal metabolism and endogenous nitrogen metabolism, stated: "Both represent the 'idling speed' of the organism, one with reference to the catabolism of a particular class of cellular constituents characterised by their content of nitrogen, the other with reference to the total catabolism of the organism". He demonstrated, in the same paper, that 2 mg of endogenous urinary nitrogen is excreted for every Calorie

(kcal) of basal metabolism. The present study was conducted with animals at maintenance, that is, they were not at rest. Thus, the endogenous urinary nitrogen output at basal metabolism could not be measured. However, based on the studies of energetics reported in Chapter 11, the output of urinary nitrogen by potoroine marsupials approximated Smut's estimate. Brody and coworkers (1934) confirmed Smut's relationship in showing that endogenous nitrogen (EN) is related to the same power of body mass as is the basal metabolic rate:

$$EN = 146W^{0.72}$$

It seems reasonable to believe that as the speed of the organism rises above idling, this relationship should persist.

Blaxter and Mitchell (1948), in discussing protein requirements of ruminants, suggested that 60% of the total requirements of nitrogen in adult animals is determined by metabolic faecal nitrogen. This was confirmed in the present study in which a high proportion of the faecal nitrogen was MFN. Because this factor is invariably correlated with food intake, as the level of metabolism increases with a corresponding increase in food intake so will the rate of excretion of metabolic faecal nitrogen. This increase is removed by expressing MFN output per unit DMI.

As both faecal and urinary nitrogen are correlated with the rate of metabolism, it becomes increasingly clear that a positive correlation between nitrogen balance and digestible energy intake is expected. This is confirmed in the present study in which only two observations were of animals with digestible energy intakes below that required for maintenance (Section 11.1)

Relative to the male animals used in the present study, captive animals and free-living animals have additional requirements for nitrogen: for reproduction in adults and growth of young. Indeed, the relationship between nitrogen balance and apparently digestible nitrogen intake for the lactating *A. rufescens* studied in Chapter 11 (Equation 5.3), suggested that lactating potoroines had nitrogen requirements of 360 mg ADN.kg$^{-0.75}$.d^{-1} or more than three-times the maintenance needs determined for male *A. rufescens*:

Nitrogen balance (g.kg$^{-0.75}$.d^{-1}) = 0.956 x ADN intake — 0.348; r^2 = 81%; s = 0.0566; P<0.001 .....*Equation 5.3*

This highly significant linear relationship was surprising because, presumably, nitrogen requirements are much higher during the last third of pouch-life — the period of rapid growth. It may be that the high nitrogen requirement of the lactating animals reflects their high-nitrogen diet (2.3%, or more than double the level in the medium nitrogen diet fed in Experiment 6.2). Thus, the results for lactating animals should be treated with caution until there is further experimentation.

There is a smaller increment in nitrogen requirements associated with the increased metabolism of wild animals or of captive animals housed in large enclosures. However, the relationship requiring closer scrutiny is that between maintenance nitrogen requirements and diet. The present study was based on the feeding of highly digestible cereal-based rations to potoroines. As previously mentioned, studies with synthetic diets (for example, Wellard and Hume 1981a) grossly underestimated the nitrogen that would be required by *T. vulpecula* consuming *Eucalyptus spp* (Foley and Hume 1987a), mainly because MFN losses were so much lower. The situation may be very complex in potoroine marsupials because they seem to consume a vast array of dietary items. Many of these foods are hypogeous and the grit ingested with them may contribute to sloughing of mucosal cells.

Of what value are maintenance nitrogen requirements determined in the laboratory? For those dealing with the management of free-living animals, knowledge of maintenance requirements determined under a wide range of laboratory conditions, both nutritional and environmental, would be most desirable for 1) predicting conditions under which deficiencies may occur; and 2) as an indicator of the conditions under which a species may have evolved.

5.5 Summary

The maintenance nitrogen requirement of *A. rufescens* was determined in a series of balance studies, with animals fed cereal-oat hull diets varying in their content of nitrogen and plant-cell walls (fibre). Nitrogen balance was not affected by the level of dietary fibre. Consequently, the data were pooled to give a truly digestible nitrogen requirement of 200 $mg.kg^{-0.75}.d^{-1}$ for animals fed diets with neutral-detergent fibre levels between 100 and 300 g per kg dry matter. Nitrogen balance data from experiments with *P. tridactylus* and *B. penicillata* suggested that their nitrogen requirements are similar to those of *A. rufescens*. A comparison of the present results with those published for eutherian and other metatherian species showed that, as expected, the nitrogen requirements of potoroine marsupials are markedly less than those of most eutherians. Less expected was the finding that the maintenance nitrogen requirements of potoroine marsupials are similar to those of some arid-zone macropodids, such as *Macropus robustus erubescens*. The low nitrogen requirements of potoroine marsupials are investigated further in Chapter 6.

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CHAPTER SIX

Urea kinetics in potoroine marsupials

6.1 Introduction

MANY animals recycle endogenously synthesized urea from the blood to the gastrointestinal tract. These animals include most, if not all, herbivores — such as sheep (Cocimano and Leng 1967; Mousa *et al.* 1983), goats (Emmanuel and Emady 1978); *Odocoileus virginianus*, white-tailed deer (Robbins *et al.* 1974); *Camelus dromedarius* (Emmanuel *et al.* 1978); various macropodids (Kennedy and Hume 1978; Dellow and Hume 1982b; Chilcott *et al.* 1985); *Phascolarctos cinereus* (Cork 1981); *Trichosurus vulpecula* and *Petauroides volans* (Foley and Hume 1987a); *Procavia habessinica* (Hume *et al.* 1980); horses (Prior *et al.* 1974); rabbits (Regoeczi *et al.* 1965) and some omnivores — for example, humans (Walser and Bodenlos 1959). This transfer of urea may well signify a frequent shortage of dietary nitrogen for many animals (Mattson 1980).

Recycling of urea to the gastrointestinal tract, particularly the fermentative regions, and the metabolism of this urea by micro-organisms, increases the efficiency of utilisation of dietary nitrogen. Indeed, it is generally accepted that the nitrogen utilised by ruminant micro-organisms has its origin in the rumen ammonia pool. Furthermore, it has been conclusively demonstrated that domestic ruminants can be maintained on diets containing only inorganic nitrogen in the form of urea or ammonium salts (Loosli *et al.* 1949), indicating that the dietary amino acids regarded as essential for survival in monogastric species can be synthesized in gastrointestinal microbial ecosystems. Thus, it is not surprising that the amount of urea recycled to the gut of domestic ruminants has often been linked to the level of microbial metabolism. In wild ruminants and other foregut-fermenting herbivores, the situation is less clear. Their ability to conserve urea, their low requirements for nitrogen and their maintenance of extensive microbial fermentation nevertheless imply a similar link.

Although hindgut-fermenting herbivores, such as horses, pigs, rabbits, rats and humans retain orally-administered non-protein nitrogen (Block and Mitchell 1946; Slade and Robinson 1970; Hintz and Schryver 1971; Hoover and Heitman 1975; Rérat 1978) they are unable to maintain nitrogen equilibrium. Unless coprophagic, they probably gain little from microbial protein synthesis *per se*. Nonetheless, the recycling of urea to the gut in both foregut- and hindgut-fermenting species probably maintains

microbial populations and hence fermentation rates when dietary nitrogen is limiting. By maintaining a nitrogen supply, animals of both digestive strategies gain from other microbial products, particularly energy in the form of short-chain fatty-acids.

In the preceding chapter it was shown that the nitrogen requirements of potoroine marsupials are low. It was inferred that these species need less nitrogen because they have a low basal metabolic rate. Another reason for their low nitrogen requirements may be that they have evolved mechanisms — for example, recycling urea — to counteract a shortage of dietary nitrogen. Their low nitrogen requirements may reflect also an unpredictable environment in which nitrogen availability is linked to rainfall (Mattson 1980). This possibility was recognized also by Chilcott *et al.* (1985) in studying the effects of water restriction on nitrogen metabolism in *Macropus eugenii* and *Thylogale thetis*. *M. eugenii* inhabit a seasonally harsh environment where both water and nitrogen may be limiting; the environment of *T. thetis* is rarely limiting. A similar situation may have existed in potoroine marsupials, with *Aepyprymnus* and *Bettongia* spp having at times to deal with harsh environments, whereas that of *Potorous* was more favourable (Chapter 3).

The following chapter describes the interaction between water availability, microbial metabolism and urea metabolism in three species of potoroine marsupials.

6.2 Materials and methods

Urea kinetics in potoroine marsupials were studied in two parts. Part A included two cross-over experiments (Experiments 6.1 and 6.2) that examined urea kinetics in animals given free or restricted access to water. In both experiments half of the animals were randomised to each water treatment for the first collection period and then subjected to the opposing treatment during the second period.

Part B describes a single experiment (Experiment 6.3) in which the kinetics of urea metabolism were measured in *A. rufescens*, offered diets that varied in the ratio of maize to lucerne. These measurements were made in conjunction with the experiment described in Section 8.3. Readers are referred to that section for details of animals, diets and experimental procedures.

Part A — Experiments 6.1 and 6.2

Animals and collection periods

Six *A. rufescens* (3 male, 3 female), six *P. tridactylus* (3 male, 3 female) and five *B. penicillata* (2 male, 3 female) were used in Experiment 6.1; eight *A. rufescens* (7 male, 1 female) and eight *P. tridactylus* (6 male, 2 female) in Experiment 6.2. All

animals were adults. During the experimental periods, the animals were managed as described in Chapter 4. Between collection periods, the animals were returned to the outdoor enclosures to provide an opportunity for exercise and to regain any condition lost during the first measurement period. In both experiments the animals were offered *ad libitum* the medium nitrogen-medium fibre diet used in the experiments of Chapter 5 (Table 5.2). Feeding, housing and sampling procedures were similar to those described in Chapter 4. Each collection period consisted of a ten-day general adaptation period, during which water consumption was monitored, followed by a seven-day pre-collection period for adjustment to water regimen. Then followed a seven-day balance study and a final 36-48 hour urine collection period for the urea kinetics study. The animals were weighed when first retrieved from the outdoor enclosures, and at the start and finish of both the pre-collection and collection periods.

Treatments

Both experiments in Part A contained two treatments: 1) *ad libitum* drinking water and 2) restricted drinking water. In Experiment 6.1, animals on the restricted water treatment received daily 50% of their *ad libitum* drinking water intake measured during the final seven days of the general adaptation period. This proved too general an approach so, in Experiment 6.2, *A. rufescens* were allowed 13 $ml.kg^{-0.80}.d^{-1}$ and *P. tridactylus* 23 $ml.kg^{-0.80}.d^{-1}$. This approximated 40% of the mean water intake of all representatives of a species during the pre-collection period and was designed to counteract individuals that apparently drank to excess. Animals on restricted water drank their allowance immediately, negating the need to account for evaporation.

^{14}C-Urea and ^{3}HOH metabolism

On the final morning of the collection period, each animal was weighed and given an intramuscular injection to the hindleg of either 0.5 ml (*Bettongia, Potorous*) or 1.0 ml (*Aepyprymnus*) of a solution containing ca 150 x 10^4 bq ^{3}HOH and ca 300 x 10^4 bq of ^{14}C-urea per ml of sterile saline. The exact dose was determined by weighing the syringe and needle before and after the injection. Samples of the injectate were stored at -10°C for later determination of actual radioactivity.

Because of difficulties in obtaining frequent blood samples from potoroines, in particular dehydrated animals, the decline in specific activity and enrichment of urea with ^{14}C was traced in urine rather than plasma. The validity of this technique has been discussed for rabbits by Regoeczi *et al.* (1965) and for *M. eugenii* by Dellow and Hume (1982b), but no workers have validated the technique in a strictly nocturnal animal, in which feeding and excretion are confined to a few hours after nightfall.

Discrete urine samples from each animal over the next 36-48 hours were collected into small glass vials. It was immediately clear that the volume of each urination was

small and that much urine remained on the collection tray. To counter this problem, trays were washed with ten ml distilled water after each urination and the tray was then dried, ready for subsequent urinations. One ml of glacial acetic acid was then added to the vial, which was stored at -10°C pending analysis. Diluting the urine with the collection-tray washings precluded the measurement of ^{3}HOH activity, so this part of the study was aborted. Spilt feed was continuously removed so that it would not absorb urine.

A similar approach was undertaken in Experiment 6.2 except that the ^{3}HOH was injected at the start of the collection period and the ^{14}C-urea separately at its completion.

In Experiment 6.2, a blood sample (0.5-3.0 ml) was collected from a lateral tail vein of each animal into a heparinized syringe. This was done five hours after the injection of ^{3}HOH and at the completion of the ^{14}C-urea study. Packed cell volume was determined immediately. The remaining blood was centrifuged and the plasma stored frozen in plastic vials for later determination of urea and ^{3}HOH. Blood samples were taken also from unlabelled animals for the determination of background radiation.

Analytical

Dietary and faecal samples were analysed for dry matter, organic matter, nitrogen, ADF and NDF, according to the procedures outlined in Chapter 4.

Serial dilutions (10^{3} and 10^{6}) of the injectates were analysed for ^{14}C and ^{3}HOH by counting a weighed amount (ca 1.00 g) in 10 ml scintillation fluid. In Experiment 6.1 ^{3}HOH was removed by drying a known quantity of urine (ca 0.20 g) over sulphuric acid. The drying was repeated after the addition of 0.2 ml double distilled water. The dried urine was redistilled in 2.00 g double distilled water and a weighed quantity (ca 1.00 g) was removed for counting ^{14}C in 10 ml scintillation fluid. The remaining urine solution was analysed immediately for urea-nitrogen. In Experiment 6.2, 0.1 ml urine was counted with 0.9 ml double-distilled water and 10 ml scintillation fluid. A separate urine sample was diluted for analysis of urea-nitrogen.

Part B — Experiment 6.3

The procedures used in the urea-kinetic study of animals fed the lucerne-based diets (Experiment 6.3) were very similar to those described in Part A. The main difference was that animals were injected with ^{14}C-urea about five hours before night-time. The animals were bled four hours after dosing and again, 48 hours later, at the end of the kinetic study. The plasma was isolated and prepared immediately for ^{14}C and urea analyses. The radioactivity in the urine was determined by mixing 0.1 ml urine with 0.9 ml double-distilled water and 10 ml scintillation fluid.

Calculations

For each animal, the natural logarithm of the specific activity of ^{14}C-urea in each urine sample was plotted against time after dosing, determined as the midpoint between two consecutive urinations.

It was assumed that the injected ^{14}C-urea was distributed uniformly in a single body pool. Therefore, the data points tracing the decline in the specific activity of urinary urea could be described by a single first order exponential function according to the procedures of Cocimano and Leng (1967) and Robbins *et al.* (1974) and following the terminology of Nolan and Leng (1972). The equations are described below:

1) Urea pool size (the mass of urea in which the injected marker was distributed):

$$= \frac{^{14}C\text{-urea injected (dpm)}}{\text{specific activity of urine at time zero (dpm.mg urea N}^{-1})}$$

2) Urea-N space (the volume (ml) of distribution of the urea pool):

$$= \frac{\text{Body urea-N pool (mg)}}{\text{Plasma urea-N (mg N.l}^{-1})}$$

3) Half-time (the time (h) required for the specific activity of urinary urea to drop by half):

$$= \frac{0.693}{k}$$

4) Rate of irreversible loss = urea-N entry rate (the rate ($mg.h^{-1}$) at which urea-carbon left the urea pool and did not return = the rate at which urea-carbon entered the urea pool assuming a steady-state):

$$= \text{Urea pool size.k}$$

$$= \frac{\text{urea pool size}}{1.44 \times \text{half time}}$$

5) Urea degradation rate (the rate ($mg.h^{-1}$) at which urea entered, and was degraded within the gut):

= rate of irreversible loss - rate of urinary urea excretion ($mg.h^{-1}$)

6) Urea-N recycled as a proportion of the rate of irreversible loss (%):

$$= \frac{\text{urea-N degradation rate (mg.h}^{-1}) \times 100}{\text{rate of irreversible loss (mg.h}^{-1})}$$

Statistical

Mean values of intake, excretion, digestibility and urea-kinetic parameters were analysed by two-way analysis of variance using the BMDP 2V statistical package (Part A) and one-way analysis of variance using BMDP 1V (Part B). When statistical differences were found, treatment means were compared using the least significant difference technique. Relationships between parameters were examined by least squares regression analysis.

6.3 Results

In this chapter the results and discussion of the water-restriction experiments are mainly limited to those parameters associated with urea kinetics. The influence of water restriction on other measurements — such as food intake, digestibility, urine osmolality and faecal dry matter, are discussed in Chapter 10.

Experiment 6.1 (Tables 6.1, 6.2)

There were no statistical differences between the two collection periods and therefore the data from the two collection periods were combined. The same applied in Experiment 6.2. 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 beginning of water restriction to the end of the collection period, changes in body mass ranged from -2% to -17% in *P. tridactylus*, +0% to -14% in *A. rufescens* and -2% to -19% in *B. penicillata*. Water restriction reduced ($P<0.05$) dry matter intakes (DMI) which in turn resulted in depression of 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.

In all species, urea pool size as a function of metabolic size was increased by water restriction ($P<0.01$), with *A. rufescens* having larger pools than *P. tridactylus* ($P<0.01$). The other parameters of urea kinetics were not affected by water restriction, a finding that can be attributed largely to the variable response to a 50% water restriction.

Experiment 6.2 (Table 6.3)

The daily drinking water of *A. rufescens* and *P. tridactylus* was restricted to 23 and 13 $ml.kg^{-0.80}.d^{-1}$ respectively. This proved to be a more uniform and harsher restriction than the 50% of *ad libitum* intake practised in Experiment 6.1. In animals given limited water, significant losses of body mass occurred from the beginning of the treatment to the end of the collection period ($P<0.001$) and during the collection period

Table 6.1 Nitrogen and urea kinetic parameters of potoroine marsupials fed a maize-oat hull ration and given free access to water or restricted to 50% of normal intake (Experiment 6.1).

Parameter	*Aepyprymnus*		*Potorous*		*Bettongia*		sed	significance	
	Ad libitum	**Restricted**	**Ad libitum**	**Restricted**	**Ad libitum**	**Restricted**		species	water
Number	3	3	3	3	3	3			
Body mass (g)	3071 ± 225	2617 ± 136	934 ± 56	834 ± 34	1134 ± 69	993 ± 65	0.166	***	*
change (% CP) [3]	2.0	-1.5	1.8	-1.7	4.8	-3.2	3.52	ns	*
change (% PCP-CP) [3]	-0.2	-7.5	0.0	-12.3	1.6	-12.9	5.05	ns	**
Dry matter									
($g.kg^{-0.75}.d^{-1}$)	31.7	26.5	34.9	26.3	50.8	35.1	9.62	ns	ns
Nitrogen									
intake ($g.kg^{-0.75}.d^{-1}$)	0.29	0.24	0.32	0.24	0.47	0.32	0.088	ns	ns
faecal ($g.kg^{-0.75}.d^{-1}$)	0.16	0.12	0.17	0.14	0.22	0.17	0.048	ns	ns
urinary ($g.kg^{-0.75}.d^{-1}$)	0.08	0.09	0.10	0.09	0.12	0.12	0.098	ns	ns
balance ($g.kg^{-0.75}.d^{-1}$)	0.05	0.03	0.05	0.01	0.14	0.03	0.051	ns	ns
Urea-N									
pool (mg)	240	278	42	82	49	148	61.76	**	ns
pool ($mg.kg^{-0.75}$)	100	135	44	95	45	149	41.68	ns	**
proportional turnover (k)	0.074	0.109	0.155	0.094	0.172	0.118	0.0402	ns	ns
Thalf (h)	6.0	8.8	4.8	8.0	3.6	6.6	2.40	ns	ns
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [1]	7.2	15.0	6.3	7.9	6.8	14.2	4.84	ns	ns
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [2]	6.2	11.2	5.5	7.7	9.9	17.0	2.97	*	*
excretion rate ($mg.kg^{-0.75}.h^{-1}$)	2.2	4.7	2.5	3.8	3.1	5.5	1.10	ns	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [1]	5.0	10.3	3.8	4.1	4.7	8.7	4.49	ns	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [2]	4.1	6.5	3.1	4.0	6.9	11.5	2.52	*	ns
recycled (%) [1]	69.4	68.5	59.8	49.9	59.3	59.7	17.66	ns	ns
recycled [2]	66.2	57.3	53.6	50.1	67.6	68.0	12.3	ns	ns
recovery (%)	31.4	43.4	44.0	48.2	46.4	36.3	10.75	ns	ns

1 - calculated from the regression of the specific activity of urinary urea against time
2 - calculated by recovery of the dose in the urine
3 - CP - collection period; PCP - pre-collection period

Table 6.2 Nitrogen and urea kinetic 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**	**species**	**water**
Number	6	6	6	6			
Body mass (g)	2910 ± 128	2721 ± 111	953 ± 43	842 ± 22			
change (% CP) [3]	1.2	-2.6	3.2	-0.83	3.34	ns	*
change (% PCP-CP) [3]	0.6	8.9	1.4	-5.9	3.68	ns	*
Dry matter							
intake ($g.kg^{-0.75}.d^{-1}$)	33.6	25.4	41.0	28.1	6.13	ns	*
Nitrogen							
intake ($g.kg^{-0.75}.d^{-1}$)	0.31	0.23	0.38	0.26	0.056	ns	**
faecal ($g.kg^{-0.75}.d^{-1}$)	0.16	0.12	0.19	0.14	0.030	ns	*
urinary ($g.kg^{-0.75}.d^{-1}$)	0.08	0.11	0.11	0.10	0.031	ns	ns
balance ($g.kg^{-0.75}.d^{-1}$)	0.07	0.01	0.08	0.02	0.049	ns	*
Urea-N pool (mg)	234	316	41.3	96.2	47.3	**	**
pool ($mg.kg^{-0.75}$)	103	148	43	110	28.8	*	**
proportional turnover (k)	0.10	0.11	0.15	0.08	0.037	ns	ns
Thalf (h)	8.5	6.4	4.9	8.7	3.14	ns	ns
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [1]	8.6	15.4	6.0	8.5	4.84	ns	ns
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [2]	8.7	11.4	3.1	5.1	2.92	ns	ns
excretn rate ($mg.kg^{-0.75}.h^{-1}$)	2.2	4.1	2.9	3.4	1.17	ns	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [1]	6.4	11.4	3.1	5.1	4.88	ns	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [2]	6.5	7.4	2.8	4.8	2.66	ns	ns
recycled (%) [1]	74.8	58.4	50.0	57.8	21.1	ns	ns
recycled (%) [2]	74.2	61.6	48.0	55.0	15.4	ns	ns
marker recovery (%)	25.3	40.9	46.3	42.5	12.4	ns	ns

1 - calculated from the regression of the specific activity of urinary urea against time
2 - calculated by recovery of the dose in the urine
3 - CP - collection period; PCP - pre-collection period

Table 6.3 Nitrogen and urea kinetic parameters in A. rufescens and P. tridactylus fed a maize-oat hull ration and given restricted or free access to water (Experiment 6.2)

Parameter	*Aepyprymnus*		*Potorous*			significance	
	Ad libitum	**Restricted**	**Ad libitum**	**Restricted**	**sed**	**species**	**water**
Number	8	8	8	8			
Body mass (g)	2893 ± 111	2680 ± 147	932 ± 40	784 ± 32			**
change (% CP) [3]	1.3	-4.8	-1.0	-3.5	1.43	ns	***
change (% PCP-CP)	-0.1	-14.6	-0.4	-17.6	2.19	ns	***
DMI ($g.kg^{-0.75}.d^{-1}$)	32.2	20.8	37.1	21.8	5.12	ns	***
Nitrogen							
intake ($g.kg^{-0.75}.d^{-1}$)	0.28	0.18	0.33	0.19	0.049	ns	***
faecal ($g.kg^{-0.75}.d^{-1}$)	0.15	0.08	0.15	0.09	0.022	ns	***
urinary ($g.kg^{-0.75}.d^{-1}$)	0.07	0.18	0.12	0.14	0.062	ns	ns
balance ($g.kg^{-0.75}.d^{-1}$)	0.06	-0.08	0.07	-0.04	0.063	ns	**
Urea pool (mg)	204	697	108	213	71.4	***	***
pool ($mg.kg^{-0.75}$)	93	337	110	257	31.2	***	***
plasma urea ($mg.100ml^{-1}$)	7.1	25.6	7.5	28.4	4.28	ns	***
proportional turnover (k)	0.13	0.06	0.08	0.07	0.025	ns	***
Thalf (h)	5.9	13.0	8.7	10.2	3.74	ns	*
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [1]	11.4	18.5	9.2	18.8	4.15	ns	**
irrev. loss ($mg.kg^{-0.75}.h^{-1}$) [2]	9.9	23.0	9.3	24.8	6.09	ns	***
excretn rate ($mg.kg^{-0.75}.h^{-1}$)	2.0	8.7	3.8	9.3	1.90	ns	***
degrad rate[1] ($mg.kg^{-0.75}.h^{-1}$) [1]	9.4	9.8	5.3	9.5	2.96	ns	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [2]	7.8	14.3	5.5	15.4	5.19	ns	*
recycled (%) [1]	81.2	52.0	56.0	50.0	9.66	*	**
recycled [2]	71.7	59.0	58.5	61.5	9.50	ns	ns
marker recovery (%)	27.3	39.9	43.3	38.6	11.80		

1 - calculated from the regression of the specific activity of urinary urea against time
2 - calculated by recovery of the dose in the urine
3 - PCP - pre-collection period; CP - collection period.

($P<0.001$). As expected, DMI was reduced by water restriction ($P<0.001$), as was nitrogen intake ($P<0.001$), faecal-nitrogen output ($P<0.001$) and nitrogen balance ($P<0.001$). Conversely, water restriction significantly increased urinary nitrogen output ($P<0.05$), which suggested that restricted animals were catabolizing body protein to provide maintenance nitrogen, glucogenic precursors and possibly metabolic water.

Water restriction affected most parameters of urea kinetics, but the effect was uniform across species. Circulating levels of plasma-urea nitrogen increased significantly with water restriction ($P<0.001$) as did the size of the urea pool. This was accompanied, in restricted animals, by a much slower turnover of the urea pool ($P<0.001$). Nevertheless, water restriction so increased the size of the urea pool that, although turnover was slower, the rate of irreversible loss (which under steady-state conditions is synonymous with urea-synthesis rate) was still significantly increased by restriction ($P<0.001$). Restricting water increased the urea-excretion rate ($P<0.001$) but, because high rates of irreversible loss were associated with high excretion rates, there was no effect upon degradation rate. The proportion of synthesized urea transported to and degraded in the gut was reduced by water restriction ($P<0.001$), when this was calculated by regressing specific activity against time to yield pool size and turnover rate. However, when calculated by the marker recovery technique, this difference was not realized.

Experiment 6.3

Data describing body-mass changes, nitrogen and water balance, and digestibility parameters are shown in Section 8.3. There were no significant differences between the low- and high-lucerne diets with respect to urea kinetic parameters (Table 6.4). Much higher values were found for all parameters of urea kinetics in *A. rufescens* fed the lucerne-based diets than in those fed the maize-oat hull rations and given free access to water (Experiment 6.2).

Relationships between parameters (Table 6.5)

Part A

Equations describing the significant relationships between parameters of urea kinetics, their coefficients of determination and levels of significance are shown in Table 6.5. In general, nitrogen intake was not an important determinant of urea kinetics. However, in the *Potorous* of Experiment 6.2, the rate of irreversible loss of urea-N calculated by regression ($P<0.01$) or marker recovery ($P<0.05$) was significantly correlated with nitrogen intake.

Table 6.4 Urea kinetics in A. rufescens fed a diet containing 62% lucerne and 35% maize (Diet 1), or 35% lucerne and 62% maize (Diet 2)

Parameter	Diet 1	Diet 2	sed	significance
Number	4	4		
Body mass (g)	2896	3013	228.8	ns
change PCP-CP (%)*	-7.3	3.4	0.465	***
change CP (%)**	1.4	0.27	0.512	ns
Nitrogen intake				
($g.kg^{-0.75}.d^{-1}$)	0.827	0.651	0.0533	*
Urea				
plasma urea ($mg.dl^{-1}$)	19.6	19.6	2.27	ns
Pool [1] (plasma)	260	270	22.7	ns
Pool [2] (urine)	249	207	18.6	ns
space [1] (ml)	3013	3217	471	ns
space [2] (ml)	2848	2725	296	ns
proportional turnover (k)	0.131	0.136	0.0414	ns
thalf (h)	5.4	5.3	0.63	ns
irrev loss ($mg.kg^{-0.75}.h^{-1}$) [1]	34.2	40.1	6.14	ns
irrev loss ($mg.kg^{-0.75}.h^{-1}$) [3]	32.5	30.3	4.36	ns
excretion rate ($mg.kg^{-0.75}.h^{-1}$)	17.7	12.8	1.84	ns
degrad rate ($mg.kg^{-0.75}.h^{-1}$) [3]	14.9	17.6	3.96	ns
recycling (%) [3]	45.9	56.2	5.90	ns
urea recovered (%)	59.5	57.3	4.38	ns
urea unrecovered (%)	40.5	42.7	4.38	ns

* - body mass change from when the diet was first offered until the end of the collection period (CP).

** - body mass change during the collection period (CP).

[1] - determined from plasma pool.

[2] - determined from urine pool.

[3] - determined from marker recovery in the urine.

Circulating levels of plasma urea-N had a large influence on several parameters. In both *Aepyprymnus* and *Potorous*, the size of the body urea-N pool, the rate of irreversible loss of urea-N, and the excretion rate of urea-N were all highly correlated with plasma urea-N. In contrast, the urea-N degradation rate was unrelated to plasma urea-N. Indeed, apart from the expected highly significant relationships between degradation rates and those of irreversible loss, the only other significant correlation involving urea degradation was between urea recycling and irreversible loss ($P<0.05$) in *Potorous* in Experiment 6.1.

The rate of excretion of urea nitrogen was significantly related to both the rate of irreversible loss and N intake in *Aepyprymnus* (Experiments 6.1 and 6.2), *Bettongia* (Experiment 6.1) and *Potorous* (Experiment 6.2).

Part B

Because the values for the parameters of urea kinetics were similar on the high- and low-lucerne diets, the relationships between the various urea parameters were examined after pooling the data from both diets. A number of findings were in accordance with those of Part A. First, there was no relationship between nitrogen intake and irreversible loss of urea. Second, the space in which the injected dose of urea was distributed, as determined from either the specific activity in urine or plasma, exceeded the animal's expected total body water. The plasma and urine values were in reasonable agreement. Thirdly, in contrast to the results of Part A, plasma urea-N concentration was unrelated to other urea kinetic parameters. Indeed, there were two significant relationships only. Urea excretion was correlated with the irreversible loss of urea:

Urea-N excretion rate ($mg.h^{-1}$) = -1.03 + 0.533 x IL; $r^2 = 76.1\%$; $P<0.05$; $n = 8$;

and as expected, degradation rate was correlated with irreversible loss:

Urea degradation rate ($mg.h^{-1}$) = -15.3 + 0.733 x IL; $r^2 = 72.7\%$; $P<0.05$; $n = 8$.

6.4 Discussion

Urea Kinetics — Assumptions and Errors

Isotope dilution studies are based on several assumptions which have been documented by Walser and Bodenlos (1959), Regoeczi *et al.* (1965) and Cocimano and Leng (1967). Essentially, these assumptions rely on steady-state conditions. Under these conditions it is assumed that the quantity of urea in the pool (termed the blood-urea pool) remains constant. Therefore, the amount of urea entering the pool equals the amount leaving it. If urea metabolism in potoroine marsupials is similar to that in sheep, then little of the urea degraded in the gut is recycled to the blood-urea pool

(Cocimano and Leng 1967). Thus, the quantity of urea lost irreversibly from the pool (excreted in the urine and degraded in the gut) equals urea synthesis in the animal.

There is no doubt that several assumptions are violated in the present study. For example, if urea is principally distributed in a single body pool, it should occupy a space more or less equal to the total body water (TBW) of the animal — about 75% of the body mass of a marsupial. This was observed in sheep (Cocimano and Leng 1967), *P. cinereus* (Cork 1981) and in *M. eugenii* and *T. thetis* (Dellow and Hume 1982b). Also, an injected dose of ^{14}C-urea should equilibrate in a similar time as does ^{3}HOH — about four hours in potoroine marsupials. In all of the present experiments, urea space exceeded the expected TBW. Sometimes the space exceeded body mass! This was true whether urea space was calculated from the specific activity of ^{14}C-urea in the plasma, four hours after dosing, or by extrapolating to zero time the regression of specific activity of urinary urea on time. Discrepancies between urea space and TBW have been reported previously. For example, Cocimano and Leng (1967) reported urea spaces of only 20-25% of body mass in sheep fed low-protein diets. Unfortunately, most workers do not give values for urea space. Thus, it is unknown whether high values are typical in some species, particularly those with short feeding bouts. The present results suggest that the size of the urea pool is fluctuating, or that urea is distributed in more than one pool. Based on this finding alone it is pertinent to discuss the possible errors in studies of urea-kinetics. However, because it was not feasible to follow the decline in urea specific activity in urine and plasma simultaneously, nor was it possible to catheterise the bladder, the magnitude of any errors remains unsolved and can be alluded to only by reference to other studies.

Steady-state conditions are difficult to obtain even in domestic ruminants. For example, Cocimano and Leng (1967) and Nolan and Stachiw (1979) maintained sheep under static conditions for weeks, during which body masses remained stable. During experimental periods, the sheep were fed hourly, but fluctuations of up to 30% in plasma-urea and rumen-ammonia concentrations still occurred. Conditions in the present study probably deviated more from a steady-state than those in the cited experiments. There was a constant decline in the body masses of potoroines given limited access to water. Potoroines confined for long periods in metabolism chambers show stress, possibly preventing maintenance of a steady-state. Potoroines are nocturnal and, therefore, feed intake cannot be spread over an entire day (Chapter 7). The fact that water restriction significantly increases body-urea pool size implies that, as long as body mass is declining, this parameter is in a dynamic state.

Walser and Bodenlos (1959) and Regoeczi *et al.* (1965) have discussed the errors associated with tracing the decline in specific activity of urea in urine rather than in

plasma. Regoeczi *et al.* (1965) showed that urea moves between the bladder and the body pool. This was confirmed by Nelson *et al.* (1975 cited by Steffen *et al.* 1980) who found that urea was reabsorbed from the bladder at a rate equivalent to its rate of excretion by the kidney. There is ample time for such a transfer in potoroines, because they wait until nightfall to urinate.

Secondary urea pools are difficult to detect when urine rather than plasma is sampled. This necessitates the approach followed by a majority of researchers (for example, Cocimano and Leng 1967; Robbins *et al.* 1974; Kennedy and Hume 1978; Hume *et al.* 1980; Cork 1981; Chilcott *et al.* 1985; Mousa *et al.* 1983; Foley and Hume 1987a), who assumed that urea is distributed in a single body pool. Evidence from rabbits (McKinley *et al.* 1970), sheep (Nolan and Leng 1972) and *P. cinereus* (Cork 1981) suggests that estimates of urea-pool size are little affected when secondary pools are ignored. This is surprising. Walser and Bodenlos (1959) and Regoeczi *et al.* (1965) studied urea kinetics in humans and rabbits. In comparing urine and plasma, they found higher specific activity of urea (10-20%) in the urine. They attributed this to a secondary urea pool in the kidney, which is not in equilibrium with the primary pool. This secondary pool contained 15% of the body's urea.

In potoroines particularly, one may point to the difficulty in timing urine samples as a potential source of error, because the animals urinate infrequently. However, the high correlation (r^2>0.90) between urea specific activity and time after dosing suggested that the rate of urine formation was relatively stable and, hence, the timing of samples as the midpoint between consecutive urinations is valid.

Finally, difficulties in collecting small volumes of urine particularly from animals given limited access to water must be acknowledged.

In conclusion, the high values for the space in which the ^{14}C-urea was distributed, and the variation between the values for urea kinetic parameters calculated by marker recovery in the urine and by regression techniques, are indicative of errors. Nevertheless, the highly significant influence of water restriction on urea metabolism in Experiment 6.2 and the systematic, rather than random direction of errors, imply that the relationships between urea kinetic parameters will be interpreted correctly. However, the absolute values for each of these parameters should be treated with caution. Therefore, the remainder of this discussion concentrates on the relationships between the parameters of urea kinetics.

Relationships between parameters

Cocimano and Leng (1967) were the first to investigate urea metabolism over a wide range of nitrogen intakes. They fed sheep diets ranging from 3.5 to 27.3% crude

Table 6.5 Relationships between urea kinetic parameters in the water restriction experiments

Relationship	Experiment 6.1	Experiment 6.2
A. rufescens		
DRrg vs ILrg	DR = -2.7 + 0.966.IL, r^2 = 94.1, P<0.001	DR = 3.18 + 0.434.IL, r^2 = 52.7, P<0.001
DRrc vs ILrc	DR = -0.43 + 0.802.IL, r^2 = 97.9, P<0.001	DR = 0.82 + 0.729.IL, r^2 = 90.1, P<0.001
EX vs ILrg	EX = 1.03 + 0.109.IL, r^2 = 54.6, P<0.05	EX = -3.18 + 0.566.IL, r^2 = 65.5, P<0.001
EX vs ILrc	EX = 0.428 + 0.198.IL, r^2 = 74.3, P<0.01	EX = 0.82 + 0.271.IL, r^2 = 55.6, P<0.01
EX vs Nin	EX = 8.19 - 18.8.Nin, r^2 = 79.4, P<0.001	EX = 10.7 - 23.4.Nin, r^2 = 27.3, P<0.001
Nbal vs Nin	Nbal = -0.188 + 0.849.Nin, r^2 = 82.2, P<0.001	Nbal = -0.171 + 0.766.Nin, r^2 = 70.6, P<0.01
PUN vs POOL	not measured	PUN = 2.83 + 0.030.POOL, r^2 = 72.8, P<0.001
ILrg vs PUN	not measured	IL = 8.63 + 0.391.PUN, r^2 = 55.8, P<0.01
ILrc vs PUN	not measured	IL = 4.12 + 0.761.PUN, r^2 = 56.9, P<0.01
EX vs PUN	not measured	EX = -0.100 + 0.367.PUN, r^2 = 85.3, P<0.001
P. tridactylus		
DRrg vs IL	DR = -2.86 + 0.956.IL, r^2 = 84.6, P<0.001	DR = 0.244 + 0.511.IL, r^2 = 85.3, P<0.001
DRrc vs ILrc	DR = -2.83 + 0.951.IL, r^2 = 88.1, P<0.001	DR = -1.06 + 0.676.IL, r^2 = 91.0, P<0.001
EX vs ILrg	not significant	EX = -0.244 + 0.489.IL, r^2 = 84.4, P<0.001
EX vs ILrc	not significant	EX = 1.06 + 0.324.IL, r^2 = 70.1, P<0.001
ILrg vs Nin	not significant	IL = 24.0 -48.1.Nin, r^2 = 61.8, P<0.01

Table 6.5 continued

ILrc vs Nin	not significant	IL = 35.6 - 71.2.Nin, r^2 = 43.5, P<0.05
Nbal vs Nin	Nbal = -0.086 + 0.429.Nin, r^2 = 78.0, P<0.001	Nbal = -0.218 + 0.893.Nin, r^2 = 79.2 P<0.001
PUN vs POOL	not determined	PUN = 4.25 +0.0738.POOL, r^2 = 53.8, P<0.01
ILrg vs PUN	not determined	IL = 4.88 + 0.496.PUN, r^2 = 77.9, P<0.001
ILrc vs PUN	not determined	IL = 5.92 + 0.607.PUN, r^2 = 61.8, P<0.01
EX vs PUN	not determined	EX = 1.63 + 0.270.PUN, r^2 = 81.6, P<0.001
DR/ILrg vs IL	DR/ILrg = 0.175 + 0.0500.IL, r^2 = 42.4, P<0.05	
DR/ILrc vs IL	DR/ILrg = 0.221 + 0.0423.IL, r^2 = 50.3, P<0.05	
Bettongia penicillata		
DRrg vs ILrg	DR = -1.24 + 0.723.IL, r^2 = 98.4, P<0.001	
DRrc vc ILrc	DR = -1.19 + 0.723.IL, r^2 = 97.7, P<0.001	
EX vs ILrg	EX = 1.24 + 0.277.IL, r^2 = 90.2, P<0.001	
EX vs ILrc	EX = 0.021 + 0.311.IL, r^2 = 92.8, P<0.001	
Nbal vs Nin	Nbal = -1.22 + 0.503.Nin, r^2 = 81.0, P<0.01	
DR/ILrg vs ILrg	r^2 = 49.4, P<0.05	

DR - degradation rate (mgN.h^{-1}); IL - irreversible loss (mg.h^{-1}); EX - excretion rate (mg.h^{-1});
Nin - nitrogen intake (g.d^{-1}); Nbal - nitrogen balance (g.d^{-1}); POOL - urea-N pool (mgN);
PUN - plasma urea concentration (mg.100 ml^{-1}; rg determined by regression; rc determined by marker recovery in urine

protein and found significant correlations between several parameters of urea kinetics. For example, plasma-urea concentration was positively related to urea entry and excretion rates, urea-pool size, and the rate of flow of urine; urea-degradation rate was positively correlated with nitrogen intake. These relationships formed the basis of much of their interpretation of urea metabolism in sheep. Because many parameters of urea metabolism were correlated, at least in Experiment 6.2 of the present work, a similar approach is taken here.

Studies with sheep (Cocimano and Leng 1967; Habib 1988), *O. virginianus* (Robbins *et al.* 1974), horses (Prior *et al.* 1974), and two wallaby species from diverging habitats (*M. eugenii* and *T. thetis*; Dellow and Hume 1982b, Chilcott *et al.* 1985) show that urea synthesis is often positively related to nitrogen intake. However, this is not always so. The relationship between urea-entry rate and nitrogen intake was not statistically significant in *C. dromedarius* (Emmanuel *et al.* 1976), *P. habessinica* (Hume *et al.* 1980) and *P. cinereus* (Cork 1981). Similar results were found in the current studies. In Part A, synthesis rates tended to increase as nitrogen intake declined. Indeed, there was a significant negative relationship between synthesis and intake in the *P. tridactylus* in Experiment 6.2. This is explained by the large influence of water restriction which caused a net catabolism of body protein in many individuals. In Part B, there were eight observations only of nitrogen intake and urea entry rate. These values covered a narrow range and it is likely that physiological variation between individuals obscured any relationship between the two parameters.

Because urea is principally distributed in a single body pool, the plasma-urea concentration generally reflects the size of the body-urea pool and the urea-synthesis rate. The three parameters were increased significantly by water restriction. Because potoroine marsupials eat less when their water intake is restricted, two quite different physiological states are apparent: First, that of a dehydrated animal whose food intake is sufficient to meet maintenance requirements; secondly, starvation caused by severe dehydration. Both states affect urea kinetic parameters. In Experiment 6.1, the animals given limited water were dehydrated but maintained nitrogen equilibrium. The urea-pool size was increased significantly in *P. tridactylus* and *B. penicillata*. The harsher water restriction in Experiment 6.2 caused starvation, as indicated by negative nitrogen balance. This produced significantly higher urea pools and plasma-urea concentrations in both *A. rufescens* and *P. tridactylus*. The retention of urea in the body pool on starvation has been shown previously (Packett and Groves 1965). Apparently, in short-term starvation, a mechanism operates that reduces excretion of urea and maintains a high plasma concentration so that the urea pool is maintained or even increased. Some of this urea may be used when the animal eats again (Cocimano and Leng 1967).

The lack of a significant relationship between urea-pool size and the plasma-urea concentration or irreversible loss in Part B is again explained by a small data set that covers a narrow range of values.

In potoroine marsupials, both urea excretion and urea synthesis were increased by water restriction. This was particularly evident in Experiment 6.2 and contrasts with the findings in many other species (for example, Barker *et al* 1970; Maloiy et al. 1970; Hume *et al.* 1980; Bohra and Ghosh 1983; Mousa *et al.* 1983). The difference is probably due to the severe water restriction in the present studies, which caused body mass losses of up to 20%. Because macropodoids store little fat, they readily catabolize protein under starvation conditions (Chapter 11). In keeping with studies of horses (Prior *et al.* 1974), *O. virginianus* (Robbins *et al.* 1974), sheep (Ford and Milligan 1970) and *P. cinereus* (Cork 1981), the rate of excretion of urea was related to its synthesis.

Urea, which is not excreted, returns to the gastrointestinal tract via saliva, and by diffusion across the gut wall (El Shazly 1988). Thus, Ford and Milligan (1970) concluded that the quantity of urea degraded in the gut was mainly determined by the plasma-urea concentration. However, plasma-urea nitrogen and urea-degradation rate were not related in potoroine marsupials, or in several other species (Cork 1981; Prior *et al.* 1974; Robbins *et al.* 1974). Also, in potoroine marsupials, the proportion of synthesized urea recycled to the gut was not related to plasma-urea concentration. These findings support the consensus (see El Shazly 1988) that urea metabolism is dependent on factors other than plasma-urea levels. The level of microbial activity is thought to be particularly important. Whether this is related to an increased permeability of the epithelium caused by products of microbial metabolism — for example, short-chain fatty-acids and CO_2 (Engelhardt and Rechkemmer 1983) — has yet to be determined . The significant absorption of ^{51}Cr-EDTA (Chapter 7) indicated that the gut epithelia were particularly permeable in potoroine marsupials fed the maize-oat hull rations.

The nutritional significance of recycled urea

What is the nutritional value of recycled urea? While the extent of recycling can be substantial, especially at low N intakes, it is not certain that recycling plays a role in nitrogen conservation at high nitrogen intakes (Oddy *et al.* 1983). Theoretically, there are two main reasons why potoroine marsupials might benefit from urea recycling. First, recycling urea to the gut enhances microbial protein synthesis. Because the maize-oat hull diet fed in Part A contained 1% nitrogen only, urea recycling probably enabled the animals to maintain positive nitrogen balance. However, because the diet was rapidly fermented, probably by amylolytic organisms, the forestomach pH dropped

and the overall microbial activity was minimal, at least during the feeding period (Chapter 9). Thus, urea recycled to the foregut, during the feeding period, would be of little value and may even remain intact and be transported along the gut. There was significant microbial activity in the hindgut, and any urea reaching this organ might be of benefit. Secondly, urea recycling decreases the osmotic load on the kidney and conserves water by reducing urine volume. This obviously benefits those animals with restricted access to water.

The study reported in Part B was prompted by the finding in Section 8.2 that potoroine marsupials can digest up to 60% of the plant-cell walls in a lucerne-based diet, and by the consensus that the level of urea metabolism is dependent on the extent of microbial activity. It was expected that *Aepyprymnus*, fed the high-lucerne diet, would digest a significant proportion of the plant-cell walls and that the level of urea metabolism would be raised. In contrast, the high level of maize in the low-lucerne diet was expected to suppress both cell-wall digestibility and urea metabolism. Unfortunately, the animals responded similarly to both diets, possibly because the lucerne contained much soluble carbohydrate. It was concluded, in Section 8.3, that both diets may have produced a forestomach environment similar to that in animals fed the maize-oat hull ration — that is, one in which low pH inhibits microbial metabolism. Nevertheless, urea metabolism was much higher in potoroines fed the lucerne-based diets compared with those fed the maize-oat hull rations and given water *ad libitum*. This is probably explained by the higher nitrogen levels in the lucerne diets.

In Table 6.6, the data from the present study are compared with those from other species. The urea-pool sizes and plasma-urea concentrations in *Aepyprymnus* fed lucerne-based diets (Experiment 6.3) were about 50% higher than those in *T. thetis* and *M. eugenii* consuming similar quantities of nitrogen (Chilcott *et al.* 1985). Indeed, the values in *Aepyprymnus* were similar to those in *M. eugenii* and *T. thetis* with much higher nitrogen intakes (Dellow and Hume 1982b). The rates of entry and degradation of urea are similar to the values reported by Kennedy and Hume (1978) and Chilcott *et al.* (1985), but lower than those of Dellow and Hume (1982b).

The main sources of nitrogen for urea synthesis are ammonia absorbed from the gut and the amino-N from deamination in the liver (Preston and Leng 1987). In animals fed the maize-oat hull diet, the low forestomach pH would ionise any ammonia and prevent its absorption from this organ. This, together with the low level of microbial activity, might be expected to depress urea metabolism in animals fed the high-grain diets. However, the comparison with other species (Table 6.6) does not verify this.

Under some circumstances, potoroine marsupials may digest much of the plant-cell wall material in a lucerne-based diet (Section 8.2). One may argue that the high intakes

Table 6.6 Urea kinetic parameters in foregut-fermenting herbivores with free- and restricted-access to water

Species	Water status	N intake mg.kg$^{-0.75}$.d^{-1}	Plasma urea mgN.dl^{-1}	Urea pool mgN.kg$^{-0.75}$	Entry rate mgN.kg$^{-0.75}$.d^{-1}	Degradation rate mgN.kg$^{-0.75}$.d^{-1}	Degradation rate % Entry	Reference
M. eugenii	al	420	8.4	—	706	588	83	Kennedy, Hume (1978)
M. eugenii	al	1210	24.9	284	1200	800	52	Dellow, Hume (1982)
T. thetis	al	1550	33.5	310	1470	630	54	Dellow, Hume (1982)
M. eugenii	al	270	4.3	74	221	168	79	Chilcott *et al.* (1985)
M. eugenii	res	150	6.8	161	384	286	70	Chilcott *et al.* (1985)
M. eugenii	al	780	13.8	126	501	272	55	Chilcott *et al.* (1985)
M. eugenii	res	420	14.0	131	395	249	65	Chilcott *et al.* (1985)
T. thetis	al	860	15.8	156	510	322	62	Chilcott *et al.* (1985)
T. thetis	res	550	19.0	208	541	258	48	Chilcott *et al.* (1985)
Aepyprymnus	al	827	19.6	260	821	358	46	Experiment 6.3
Aepyprymnus	al	651	19.6	270	962	422	56	Experiment 6.3
Aepyprymnus	al	284	7.1	92.6	274	226	81	Experiment 6.2
Aepyprymnus	res	183	25.4	337	444	235	52	Experiment 6.2
Potorous	al	332	7.5	108	220	128	56	Experiment 6.2
Potorous	res	191	28.4	213	451	227	50	Experiment 6.2
Bettongia	al	470	—	45	188	115	59	Experiment 6.1
Bettongia	res	320	—	149	341	210	59	Experiment 6.1
sheep	al	270	5.3	46	156	116	75	Mousa *et al.* (1982)
sheep	res	217	7.0	59	158	139	88	Mousa *et al.* (1982)
goat	al	207	7.1	50	167	131	78	Mousa *et al.* (1982)
goat	res	177	10.1	63	168	151	90	Mousa *et al.* (1982)
Camelus sp	al	203	6.2	914	802	751	94	Mousa *et al.* (1982)
Camelus sp	res	167	9.3	1005	812	779	96	Mousa *et al.* (1982)
bovine	al	970	4.0	—	312	150	48	Wales *et al.* (1975)
Rangifer tarandus	al	940	8.7	—	521	303	58	Wales *et al.* (1975)
Odocoileus virginianus	al	1062	21.3	164	1238	1098	90	Robbins *et al.* (1974)

1 al — *ad libitum* water; res — restricted water

of nitrogen on this type of diet negate the need for urea recycling. However, recycling may be important for supplying nitrogen to the gut during the animal's resting phase. The need for maintaining a continuous supply of ammonia to the gut for cell-wall digestion has been emphasized in a number of studies (see Preston and Leng 1987). Because potoroine marsupials defaecate only while active at night, they retain digesta for up to 24 hours (Chapter 7). Digestion of plant-cell walls during the resting phase may well be enhanced by recycling urea to the gut.

6.5 Summary

The relationships between the various urea kinetic parameters suggested that urea metabolism in potoroine marsupials is similar to that reported in other species. Because the studies were conducted using diets that later proved detrimental to microbial metabolism, it was concluded that urea recycling was probably of little nutritional value. Thus, the low nitrogen requirements of potoroine marsupials reported in Chapter 5 are not necessarily linked to urea metabolism. Severe water restriction significantly increased urea metabolism. This reflects the link between urea conservation and water conservation. In potoroine marsupials with high levels of microbial metabolism, urea recycling is probably important for providing a continuous supply of nitrogen to the gut, so that digestion continues during the resting phase.

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CHAPTER SEVEN

The rate of passage of digesta through the gastrointestinal tracts of potoroine marsupials

7.1 Introduction

DIGESTION, the process that converts food to metabolisable compounds, proceeds through various reactions catalysed by enzymes and, particularly in herbivores, mediated by microbes. The involvement of microbes makes the process autocatalytic, because the rate of digestion increases as the microbial population expands. This growth requires prolonged retention of digesta. Therefore, digestion is a function of reaction rate and of the time available for digestive reactions (Faichney 1975a). It follows that digesta passage rate is an index of gut function.

Herbivores exhibit a wide range of digestive strategies. At one extreme is the relatively fast passage of digesta observed in the horse (Wolter *et al.* 1974, cited by Warner 1981a; Orton *et al.* 1985), *Ailuropoda melanoleuca* (Dierenfield *et al.* 1982) and *Loxodonta africana* (Gill 1960 cited by Warner 1981a), which results in digestion of cell contents and the most accessible cell-wall material. In contrast, other herbivores pass digesta extremely slowly. In this group are *P. cinereus* (Cork and Warner 1983) and sloths — both two-toed, *Bradypus tridactylus* (Honigmann 1936, cited by Warner 1981a) and three-toed, *Choleopus didactylus* (Montgomery and Sunquist 1978)

Digestive strategy is frequently interpreted as a direct consequence of body size (Chapter 2). It is generally accepted that small herbivores, because of their high mass-specific energy requirements, cannot afford the time necessary for digestion of cell-wall materials and, instead, must ingest nutrient-rich foods (Demment and Van Soest 1985). More specifically, Parra (1978) argues that foregut fermentation is clearly disadvantageous in species weighing less than 10 kg. This argument is particularly relevant to macropodids (Chapter 2). Many of these species weigh less than 10 kg, but still display foregut fermentation. Nevertheless, there is a clear relationship between gut structure and body size within the Macropodoididae. In the larger macropodids, the forestomach is predominantly tubiform. However, in smaller macropodid species, the sacciform region is more prominent (Chapter 3). More subtle differences in foregut anatomy occur also between species. These differences include the positioning of the cardia and the degree of the gastric sulcus. Although these differences in forestomach anatomy seem to influence the initial dispersion patterns of radiographic contrast

medium, they have little effect on the passage of solute and particulate markers which, in all species, show marked separation (Dellow 1982).

One extreme of macropodid foregut anatomy occurs in potoroine marsupials, in which about 75% of the foregut is sacciform. The small size of potoroines and the evidence, although scant, that they select a concentrated diet (Chapter 3) implies rapid transit of digesta through the gut. However, the presence of the sacciform forestomach (SFS) — which resembles a mixing vessel — suggests a relatively slow rate of passage. Other suggested roles of the SFS are also associated with slow passage. These roles include 1) synthesis of microbial protein of high biological value (Kinnear *et al.* 1979); 2) microbial production of short-chain fatty-acids and B-vitamins; 3) storage of ingesta (Hume 1982); and 4) microbial detoxification of secondary plant compounds.

Perhaps the major influence on gut transit comes from the orientation of the SFS, which is set apart from the shortest route between the cardia and pylorus (Langer 1980). Although the mechanism is not understood, this anatomy allows some digesta to bypass the SFS. Hume and Carlisle (1985), in studies with *A. rufescens* and *P. tridactylus*, reported that an unknown proportion of both contrast medium (barium sulphate) and radio-opaque particles bypassed the SFS. This may explain the rapid appearance (1-2 h after dosing) of marker in the hindstomach, relative to the 2-8 h reported for *M. eugenii* by Richardson (1980).

The large SFS, the bypass mechanism and the presence — at least in *P. tridactylus* — of a gastric sulcus, all offer potential mechanisms for the separation of digesta phases suggested by Hume and Carlisle (1985). However, the radio-opaque markers they used are not phase specific.

In the present study, dual-phase markers were used in an attempt to elucidate gut function in potoroines. In part A, two experiments are discussed. These describe the kinetics of particulate and fluid phase markers in *A. rufescens*, *P. tridactylus* and *B. penicillata* fed grain-based diets with a range of concentrations of plant-cell walls.

Faichney and White (1988) made the point that the single dose/time sampling procedure yields data that are valid only for the marker and diet studied. Nevertheless, the use of the dual-phase marker approach provides a valuable tool for measuring overall mouth-to-anus transit times of fluid and particulate matter. It gives also a useful adjunct to the radiographic procedures employed by Hume and Carlisle (1985), Frappell and Rose (1986) and Hume *et al.* (1988).

7.2 Materials and methods

7.2.1 General

The passage of digesta through the gastrointestinal tracts of potoroines was investigated in two parts. The two experiments of Part A (May and August, 1984; May and June, 1985) examined the influence of the level of dietary plant-cell walls on the rate of passage of digesta in *rufescens*, *P. tridactylus* and *B. penicillata*.

The single experiment in Part B (October, 1985) investigated the effect on digesta passage of the level of gut fill prior to dosing. This experiment was initiated following the results of Frappell and Rose (1986) which suggested that the passage of contrast medium through the gut was much faster in *P. tridactylus* that had not eaten for 12-15 h. Because wild potoroines are active only at night, this period of fasting occurs each day.

7.2.2 Part A

The first experiment in Part A (Experiment 7.1), conducted during collection periods one and four of the maintenance nitrogen requirement study, involved eight *A. rufescens*. These were fed the high-fibre, high-nitrogen and low-fibre, high-nitrogen diets described in Table 5.2. The cross-over design ensured that every animal received each treatment. Experiment 7.2 resembled Experiment 7.1, except that *A. rufescens*, *P. tridactylus* and *B. penicillata* were all studied. Again, two diets with varying concentrations of cell-wall constituents (Table 7.1) were fed in a cross-over design.

Table 7.1 The composition (g.kg^{-1} ADM) and chemical analysis (g.kg^{-1} ODM) of the diets fed in Experiments 7.2 and 7.3

	Level of inclusion	
Dietary ingredient	**Low fibre**	**High fibre**
Maize	410	410
Wheat	100	100
Oat-hulls	100	267
Cornflour	360	193
Mineral mix (Table A1.6)	29	29
Mineral/Vitamin premix (Table A1.6)	1	1
Analysis		
Organic matter	964	953
Ash	36	47
Nitrogen	9.4	10.5
Acid detergent fibre	65	142
Neutral detergent fibre	155	281
Cellulose	53	117
Hemicellulose	90	139
Lignin	12	25

7.2.3 Part B

In Part B, the rate of passage of digesta was measured in eight *A. rufescens* and eight *P. tridactylus* (Experiment 7.3) fed the same diet as in Experiment 7.2. Half of the animals were dosed immediately before feeding, the remainder when they had eaten ca 40% of their typical voluntary, daily intake. This ensured that animals would have food in their foreguts.

7.2.4 Markers, dosing and collection procedures

Warner (1981a) discussed the various methods for studying the rate of passage of digesta through the gastrointestinal tract, and the associated computations used to describe the kinetics of digesta flow. In the present experiment, the "pulse dose - total collection" was deemed most appropriate, considering the constraints outlined in Chapter 4.

The dual-marker system of the ^{51}Chromium complex of ethylenediamine tetra-acetic acid (^{51}Cr-EDTA), which is believed to associate with the soluble phase in the gut (Downes and McDonald 1964; Teeter and Owens 1983), and the ^{103}Ruthenium-phenanthroline complex (^{103}Ru-P), which binds to particulate matter (Tan *et al.* 1971), were used in all experiments.

The radioactive chromium was obtained from the Australian Atomic Energy Agency at Lucas Heights, NSW. Its specific radioactivity was reduced with unlabelled Cr-EDTA according to the procedure of Binnerts *et al.* (1968). The ^{103}Ru-P was prepared from $^{103}RuCl_2$ obtained from Amersham, UK, using the method of Tan *et al.* (1971).

All animals were dosed with a mixture containing both markers. The *P. tridactylus* and *B. penicillata* were given approximately 185 kBq of ^{51}Cr-EDTA and 74 kBq of ^{103}Ru-P in 0.5 ml; the *A. rufescens* received about 370 kBq ^{51}Cr-EDTA and 168 kBq of ^{103}Ru-P in ca 1 ml. The dose volume varied depending on the activity of the markers. It was usual to lose small amounts of the dose in the fur surrounding the mouth. Other losses occurred through spillage, especially when animals refused to swallow. Hence, the exact dose administered was not known. Instead, the dose was equated with the total amount of each marker excreted in the faeces and urine. The collection of faeces was continued until counts dropped to ca 0.1% of the peak faecal marker concentration (Warner 1981a). Thus, there seemed little benefit in extrapolating the asymptote to the terminal portion of the cumulative marker excretion versus time curves to estimate the dose.

In the first part of Experiment 7.1, the dose was provided on sweet potato prepared by cutting a pellet (25 x 7 mm) of the vegetable and boring a hole in the centre. This was filled with the dose solution. The pellets were then dried at 60°C for 4 h. For the seven days preceding the experiment, each animal was offered a similar undosed piece

of sweet potato, which was readily accepted. However, most animals consumed only part of the sweet potato containing the dose. Thus, in the second half of Experiment 7.1 and in Experiment 7.2, the animals were sedated with ketamine hydrochloride (Ketalar, Parke-Davis) injected intramuscularly at 15 mg per kilogram body mass. The ^{103}Ru-P and ^{51}Cr-EDTA dose was then applied to the back of the mouth using a syringe fitted with a 50 mm length of rubber tubing.

It was later revealed that Ketalar may have a strong effect on gut motility (Hume *et al.* 1988). Thus, in Experiment 7.3, the animals were not sedated. Instead, they were restrained as described in Chapter 4, and the marker was again administered by syringe to the back of the mouth.

In all experiments, all urine and faeces voided during the 130-150 h immediately after dosing were collected, using the apparatus described previously (Fig 4.1). Cages were checked approximately half-hourly for the first 12 h, hourly for the next 24 h, two-hourly for the next 36 h and then four-hourly until completion. During the peak excretion periods (Chapter 4), the cages were checked more frequently. Apart from better timing of defaecation, this allowed also the estimation of faecal water content. When the exact time of defaecation was not known, it was taken to be the midpoint of the collection period.

Faeces were weighed into tared, plastic, gamma-counting tubes, which were capped and refrigerated at 4°C until counting. The total output of each urination was weighed and a sample (ca 5.0 g) retained for counting.

7.2.5 Analysis

The analysis of faecal-marker concentrations is described in Chapter 4.

7.2.6 Calculations

Calculations of mean retention times

Mean retention times (MRT) were calculated two ways. The first was based on the rate of marker excretion with time assuming that defaecation occurred at the midpoint of each time period (Blaxter *et al.* 1956). The marker concentration during the *ith* defaecation (*Mi*) was multiplied by the time of the *ith* defaecation (*Ti*). The sum of *MiTi* values divided by total marker excretion gave MRT, described algebraically as:

$$\text{MRT} = \sum_{i=1}^{n} \frac{MiTi}{Mi}$$

MRT was calculated also using the method of Coombe and Kay (1965) which

allows for variation in the rate of dry matter output. This is described by the expression:

$$MRT = \sum_{i=1}^{n} \frac{MiFi}{Mi}$$

Mi is again the marker excreted during the *ith* time period. *Fi* is the excretion of dry matter from the time of dosing until the time of the *ith* defaecation, divided by the average dry matter excretion rate measured over the entire study. This method is particularly suited to potoroines because defaecation is largely confined to the first few hours of darkness.

7.2.7 Marker Behaviour

The only aspect of marker behaviour examined was absorption. This was determined as the proportion of the total radioactivity excreted that appeared in the urine. Otherwise it was assumed that ^{103}Ru-P and ^{51}Cr-EDTA behaved as they do in other species — for example, sheep (Downes and McDonald 1964; Tan *et al.* 1974), *P. cinereus* (Cork and Warner 1983) and various macropodids (Dellow 1982), that is, ^{103}Ru-P associates mainly with digesta particles and ^{51}Cr-EDTA with the liquid phase.

7.3 Results

7.3.1 Marker Behaviour (Table 7.2)

Regardless of experimental treatment, ^{51}Cr-EDTA was absorbed from the gastrointestinal tracts of all three potoroine species. By comparison, it was unusual to find more than 0.5% of the recovered ^{103}Ru-P in the urine, although up to 2% was found in the urine of some animals. The relatively high levels of ^{103}Ru-P were found in the urine of animals that excreted also faeces of high water content. Thus, it is likely that the ^{103}Ru-P in the urine was excreted in the faeces. These values have been excluded from the statistics describing marker absorption.

Table 7.3 Dry matter intake, body mass and measures of retention time (h) of ^{103}Ru-P and ^{51}Cr-EDTA estimated by faecal collection, in A. rufescens fed a high-fibre or low-fibre diet.

Parameter	High fibre	Low Fibre	sed	sig
Number	8	7		
Body mass (g) (sem)	3080 (180)	2880 (110)		ns
Dry matter intake ($g.kg^{-0.75}.d^{-1}$)	35	36	2.7	ns
MRT Ru	33.9	41.0	5.59	ns
MRT Cr	30.6	36.2	5.14	ns
MRTf Ru	26.6	35.4	6.00	ns
MRTf Cr	24.7	31.7	5.26	ns
t5% Ru	13.2	15.9	4.83	ns
t5% Cr	11.6	15.3	4.71	ns
t50% Ru	21.4	25.4	4.89	ns
t50% Cr	17.9	21.0	4.98	ns
t95% Ru	57.9	68.2	10.53	ns
t95% Cr	59.2	62.4	7.99	ns
t99% Ru	88.2	98.2	12.43	ns
t99% Cr	88.0	91.3	10.21	ns
1/k Ru	22.6	14.9	7.70	ns
1/k Cr	14.6	13.5	1.70	ns
tmax Ru	24.9	32.0	6.73	ns
tmax Cr	22.7	30.1	6.21	ns

Table 7.4 Dry matter intake ($g.kg^{-0.75}.d^{-1}$), body mass, and measures of retention time (h) of ^{103}Ru-P and ^{51}Cr-EDTA estimated by faecal collection, in A. rufescens, P.tridactylus and B. penicillata fed high- or low-fibre diets.

	High fibre			Low fibre				significance	
Species	***Aepyprymnus***	***Potorous***	***Bettongia***	***Aepyprymnus***	***Potorous***	***Bettongia***	**sed**	**species**	**fibre**
	4	4	4	4	4	4			
Body mass (g)	2956	967	1066	3012	943	1058			
(sem)	(352)	(43)	(78)	(314)	(51)	(66)			
change (% CP)*	-1.9	-1.6	2.6	-1.2	-1.3	-0.8	1.89	ns	ns
Dry matter intake	33.7	45.9	43.3	32.7	46.5	43.5	6.30	ns	ns
MRT Ru	34.0	20.2	32.3	36.9	29.9	34.6	6.30	ns	ns
MRT Cr	30.7	19.8	27.4	30.6	27.8	26.7	5.15	ns	ns
MRTf Ru	31.4	22.9	25.3	31.7	36.4	33.5	6.45	ns	ns
MRTf Cr	28.0	22.4	20.3	24.8	32.9	24.8	5.23	ns	ns
t5% Ru	14.2	8.7	14.7	16.8	7.5	14.1	3.58	ns	ns
t5% Cr	13.4	8.3	13.4	15.8	5.6	11.5	3.51	ns	ns
t50% Ru	23.8	15.5	22.4	28.3	20.4	24.7	5.17	ns	ns
t50% Cr	21.7	15.3	16.4	19.8	19.0	19.5	4.36	ns	ns
t95% Ru	62.7	44.8	61.9	73.3	69.9	67.2	10.10	ns	ns
t95% Cr	58.5	44.2	53.0	60.3	65.8	51.6	14.60	ns	ns
t99% Ru	90.1	69.0	89.4	104.3	104.2	96.1	13.11	ns	ns
t99% Cr	84.9	69.6	78.7	89.6	98.5	79.2	6.94	ns	ns
1/k Ru	15.1	21.3	23.7	22.2	32.0	28.0	4.73	ns	ns
1/k Cr	19.5	24.5	19.0	17.5	29.0	18.4	7.77	ns	ns
tmax Ru	20.6	14.9	25.3	27.3	18.6	22.6	4.88	ns	ns
tmax Cr	20.1	14.6	20.9	22.0	17.8	16.3	4.38	ns	ns

* CP - collection period

Table 7.5 Dry matter intake, body mass and measures of retention time (h) of ^{103}Ru-P and ^{51}Cr-EDTA estimated by faecal collection, in A. rufescens and P.tridactylus dosed before feeding, or after eating about 40% of their normal daily intake.

	Aepyprymnus		*Potorous*			significance		
Parameter	**Fed**	**Unfed**	**Fed**	**Unfed**	**sed**	**sp[1]**	**t[2]**	**sp.t[3]**
number	4	4	4	4				
Body mass (g) (sem)	2969 (280)	2884 (233)	882 (48.0)	951 (15.3)				
Dry matter intake ($g.kg^{-0.75}.d^{-1}$)	32	36	42	39	5.0	ns	ns	ns
MRT Ru	32.1	26.3	30.8	27.0	4.93	ns	ns	ns
MRT Cr	28.9	22.7	31.5	26.2	5.62	ns	ns	ns
MRTf Ru	30.4	37.1	34.9	31.8	4.83	ns	ns	ns
MRTf Cr	27.1	33.5	35.2	31.1	4.66	ns	ns	ns
t5% Ru	10.4	6.2	6.7	7.3	3.40	ns	ns	ns
t5% Cr	9.7	4.7	7.8	6.8	3.30	ns	ns	ns
t50% Ru	18.2	13.6	17.3	16.8	4.42	ns	ns	ns
t50% Cr	13.6	11.4	19.9	16.7	4.55	ns	ns	ns
t95% Ru	60.2	54.5	72.6	59.8	10.10	ns	ns	ns
t95% Cr	50.1	51.2	77.6	62.1	11.20	*	ns	ns
t99% Ru	89.3	84.1	111.2	89.8	15.61	ns	ns	ns
t99% Cr	78.9	80.9	117.8	93.9	16.47	*	ns	ns
1/k Ru	20.4	21.5	24.8	20.2	5.87	ns	ns	ns
1/k Cr	21.1	21.1	26.6	22.7	6.43	ns	ns	ns

sp - species; **t** - time of dosing, that is before or after feeding; **sp.t** - interaction

Table 7.2 Urinary excretion, as a percentage of total marker excretion, of ^{103}Ru-P and ^{51}Cr-EDTA from three potoroine species following a pulse dose of the markers. The data, taken from all experiments, are means ± sem, together with minimum and maximum values of n observations.

Parameter	*A. rufescens*	*P. tridactylus*	*B. penicillata*
number	36	16	8
^{103}Ru-P	0.36 ± 0.056	0.19 ± 0.037	0.46 ± 0.217
minimum	0.02	0.05	0.08
maximum	1.93	0.63	1.90
^{51}Cr-EDTA	10.9 ± 1.11	5.2 ± 0.66	5.6 ± 1.06
minimum	1.84	1.23	0.78
maximum	24.36	8.73	10.13

7.3.2 Marker excretion patterns

The means and standard errors of differences between means for several parameters describing the transit of ^{103}Ru-P and ^{51}Cr-EDTA through the gastrointestinal tracts of potoroines are shown in Tables 7.3-7.5.

The coefficients of variation of the MRT for each marker within experiments ranged from 11 to 45% (Table 7.6). Correcting MRT for the rate of dry matter excretion (MRTf) had little influence on these coefficients. Furthermore, no relationship was found between MRT and body mass or dry matter intake.

Table 7.6 The coefficients of variation, for mean retention times, in rate-of-passage studies of potoroine marsupials

	MRT		MRTfi	
Experiment	**Ru**	**Cr**	**Ru**	**Cr**
Experiment 6.1	31	30	40	36
Experiment 6.2				
A. rufescens	13	15	11	12
P. tridactylus	35	35	46	45
B. penicillata	39	35	41	35
Experiment 6.3				
A. rufescens	25	31	20	21
P. tridactylus	23	28	21	22
Pooled data				
(all experiments)	31	30	32	31

In Experiments 7.1 and 7.2, few significant differences appeared between species for any parameter of marker retention. Similarly, the level of dietary structural carbohydrates did not significantly alter the passage of digesta markers. Moreover, the few differences recorded between treatment means were only just significant (ca P=0.05). On biometrical advice (H.I. Davies pers. comm.) they have been ignored.

In Experiments 7.1 and 7.2, which incorporate a crossover design, only three parameters showed a significant difference between measurement periods. These were MRTf (Ru and Cr) and 1/k (Cr) — all in Experiment 7.2. The absence of a significant period effect in Experiment 7.1 was unexpected. The difficulties encountered in Period 1 in making animals eat sweet potato doused in marker prompted the use of a sedative — ketamine hydrochloride — in Period 2. This compound is now known to speed the transit of the contrast medium — barium sulphate — through the potoroine gut (Hume *et al.* 1988).

Providing the marker at different stages of the feeding cycle had no effect on any parameter of retention in either *A. rufescens* or *P. tridactylus*.

Apart from the t95% and t99% excretion times for ^{51}Cr-EDTA, which were significantly longer ($P<0.05$) in *P. tridactylus* than in *A. rufescens*, the two species showed no differences.

In Experiment 7.1, the animals were exposed to 24 h lighting in an attempt to encourage them to feed and defaecate during normal daylight hours. They did not respond. The *A. rufescens* maintained their normal patterns of activity, feeding and excretion, regardless of the day-length imposed. Likewise, MRT was not obviously different from those of other experiments.

In all experiments, both markers appeared in the faeces (t5%) at about the same time, dictated by the frequency of defaecation. Both markers also have similar values for the other measures of retention, implying little separation of digesta phases within the gut.

In *A. rufescens* and *P. tridactylus*, 1/k was usually several hours less than MRTf. There were, however, exceptions. The 1/k values were longer than MRTf in *A. rufescens* fed the high-fibre diet in Period 2 of Experiment 7.1, and in all the *P. tridactylus* in the first period of Experiment 7.2. In contrast, 1/k and MRTf were similar in *B. penicillata*.

There are a number of indicators of the central tendency of marker excretion curves. These include MRT, modal retention time (approximated by tmax) and median retention time (t50) (Warner 1981b). The large differences between these parameters in all experiments, indicate that the distribution of retention times about the MRT is skewed. This distribution can be described by a typical curve whose characteristics

show a delay period, during which there is little excretion of marker, followed by a sharp rise to a peak concentration. After the peak, the curve falls exponentially. Thus, in the present experiment, while there was a difference of 8-10 hours between t5% and t50% excretion times, 30-50 h elapsed between t50% and t95% and between t95% and t99%.

The variation about this typical curve is perhaps as interesting as the basic curve itself. Of the 60 excretion curves for each marker resulting from the present work, 14 have been selected to illustrate the different forms (Fig 7.1 to 7.14). Many of the curves were similar to those describing digesta passage in *M. eugenii* (Warner 1981b). For consistency, results have been described, where possible, under similar headings to those used by Warner.

Early appearance of marker

In about half the marker-excretion curves, the first points indicate defaecations containing radiation from both markers at 2-4 times the background levels. The levels of markers in the succeeding defaecations could not be predicted from those in the initial defaecations. This phenomenon is most apparent in Figures 7.9, 7.10 and 7.12. These first defaecations contained negligible marker, although maximal marker concentrations often occurred within the first five defaecations. More importantly, the maximum was often reached within five hours of the first defaecation, indicating rapid transit of large amounts of marker — Figures 7.2, 7.4, 7.5, 7.6, 7.8, 7.13 and 7.14.

Marker peaks

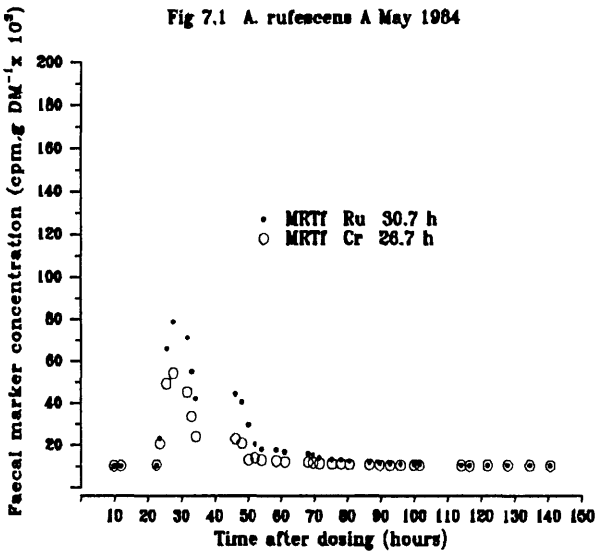
Because it is difficult to typify the shape of marker peaks, a range of forms is shown in the Figures. These include: very sharp (transit time - TT <5h) Figures 7.1, 7.4, 7.8, 7.10 and 7.12; TT of 10-30 h — Figures 7.2, 7.3, 7.6, 7.11 and 7.14; and much longer TT — Figures 7.7 and 7.9. It is particularly interesting that both of these latter examples also have an early appearance of marker.

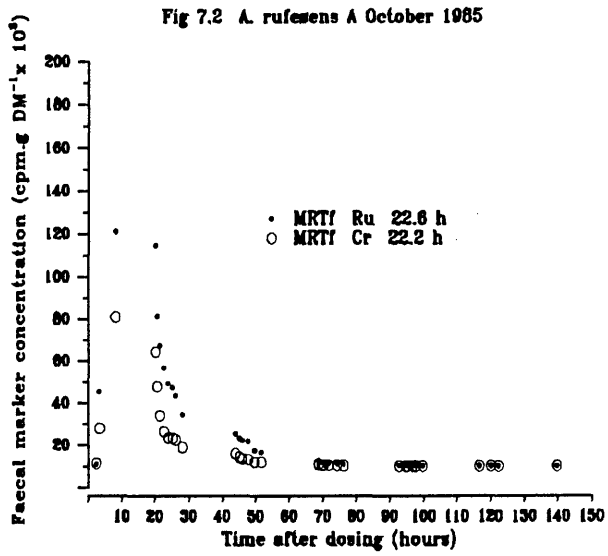
Wave forms

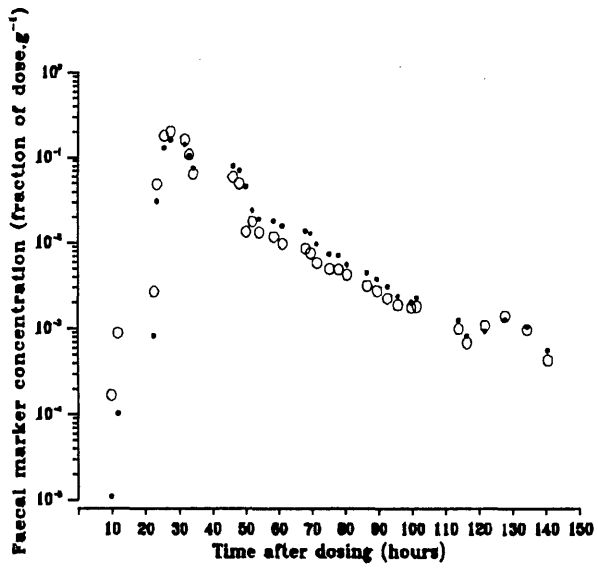
The negative slope portions of about half the marker excretion curves contained "waves", both near to and distant from the peak. There were two forms. The first showed higher than expected faecal-marker concentrations after periods of 12-15 h (the light period) without defaecation. Examples are given in Figures 7.2, 7.4 and 7.6. The second form of waves are those not obviously related to periods without defaecation, as illustrated by Figures 7.8 and 7.12.

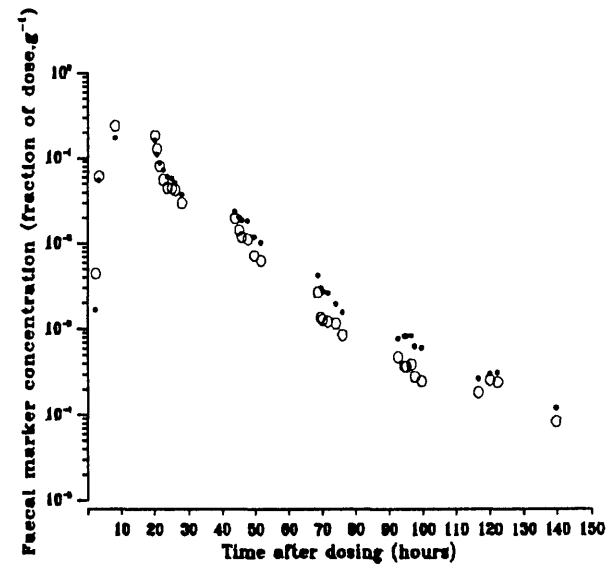
Abrupt changes in the slope of marker excretion curves

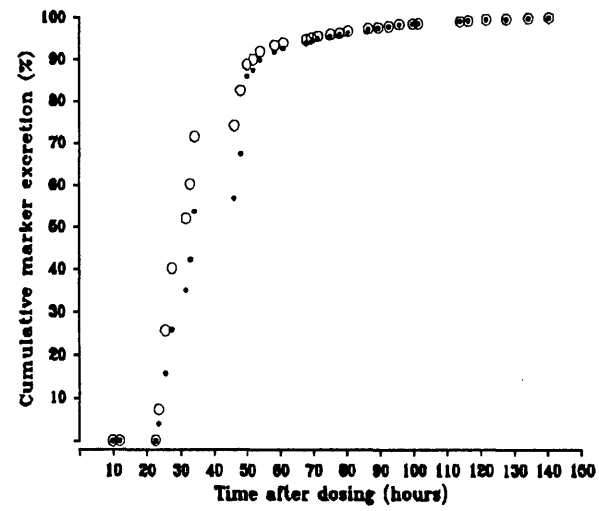
An abrupt change in the negative slope of a marker excretion curve was a feature seen only in *P. tridactylus*. It is most apparent in Figure 7.12 (20 h), in which the portion of the curve with negative slope had two distinct components. The first

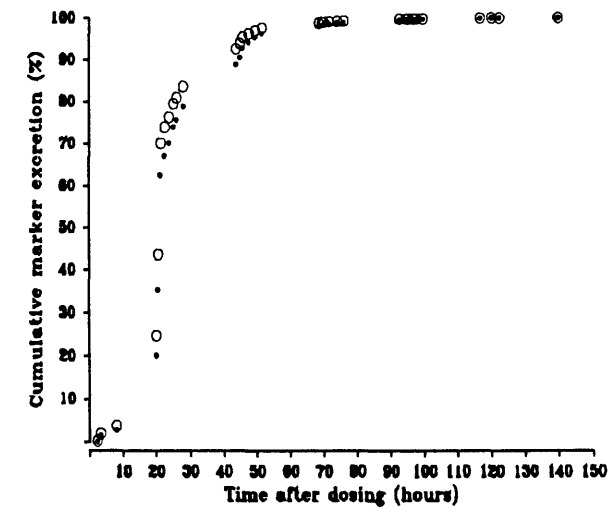






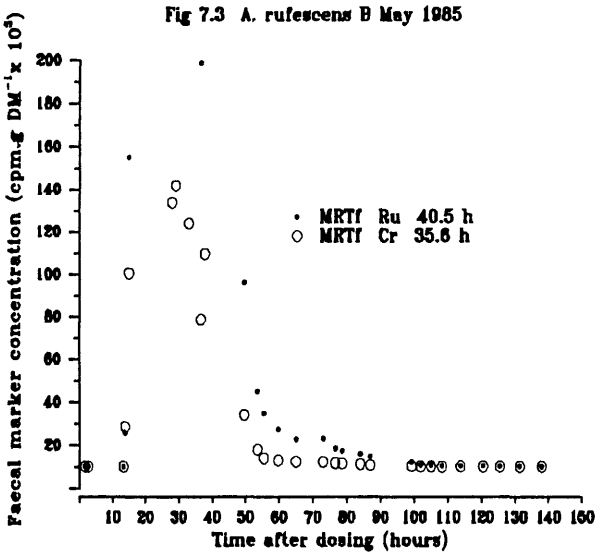


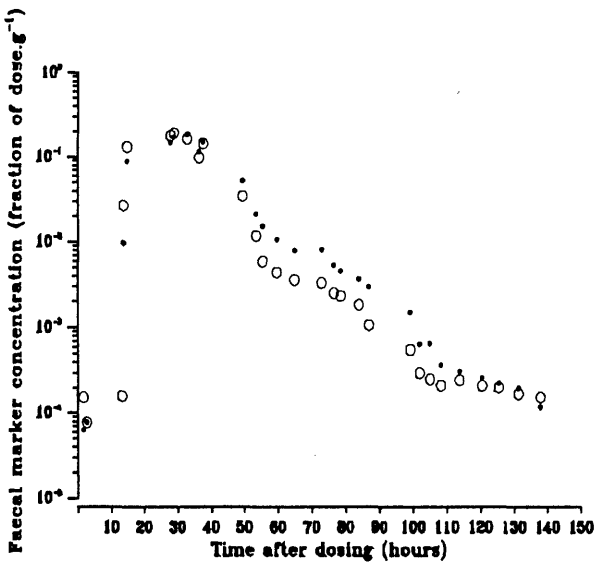




Marker excretion patterns in the faeces of two A. rufescens following a pulse dose.

Fig 7.3 A. rufescens B May 1985





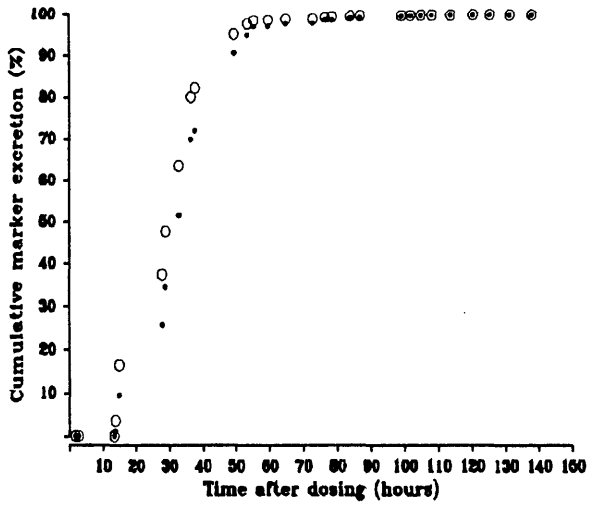
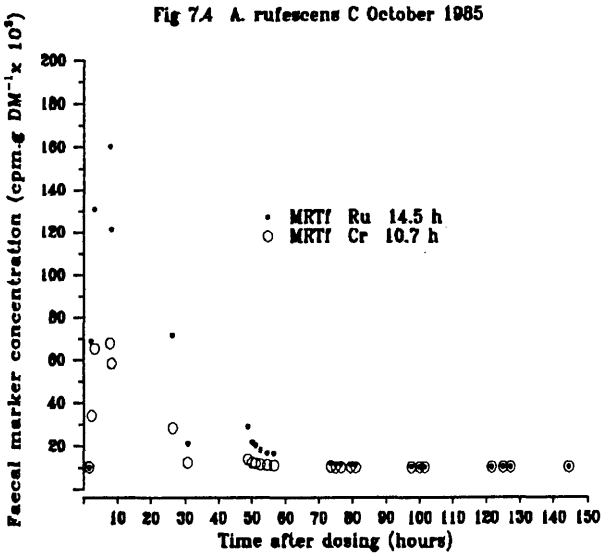
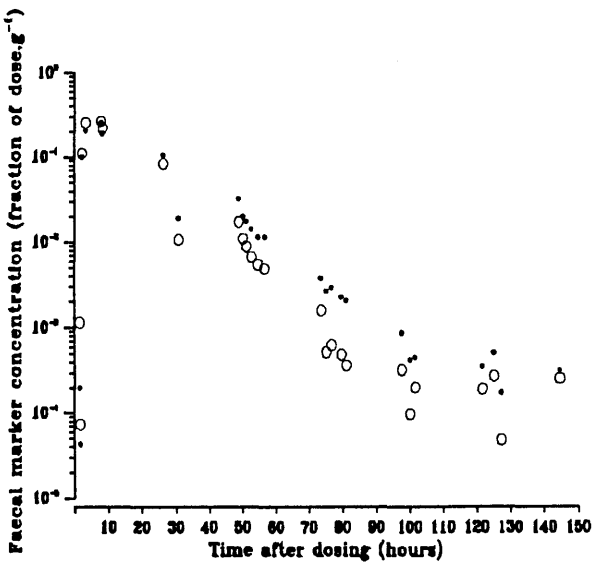
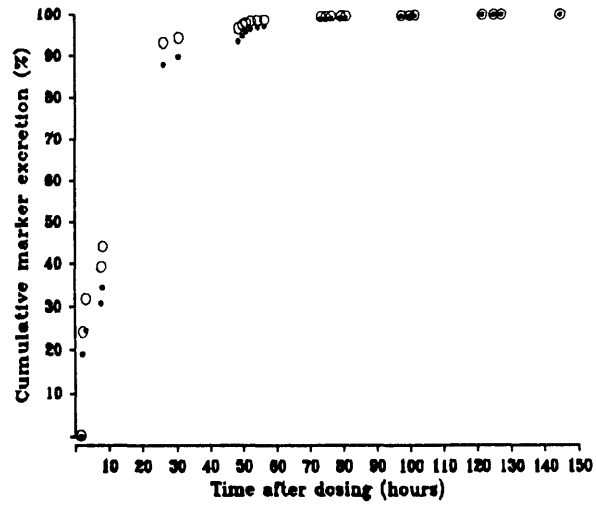


Fig 7.4 A. rufescens C October 1985

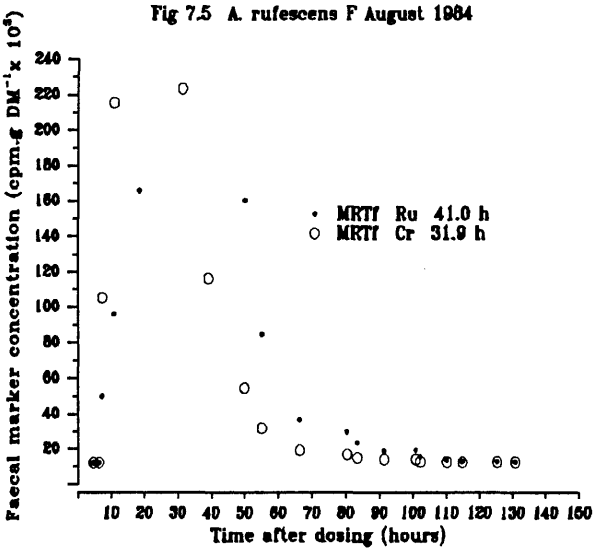


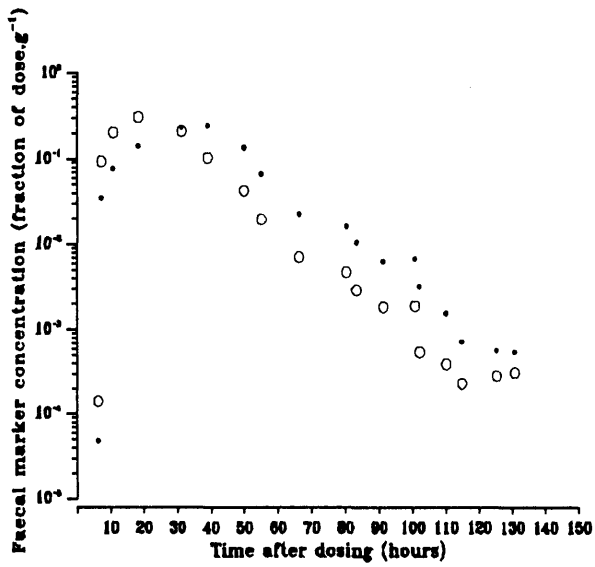




Marker excretion patterns in the faeces of two A. rufescens following a pulse dose.

Fig 7.5 A. rufescens F August 1984





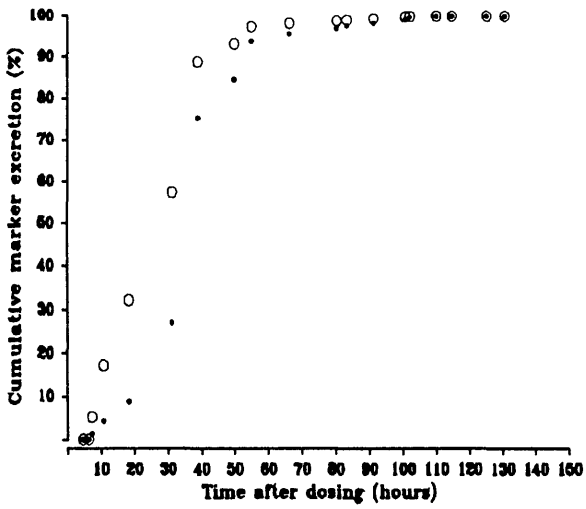
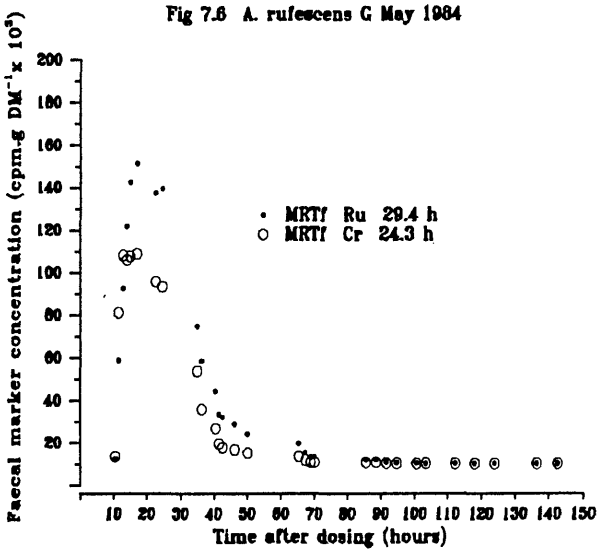
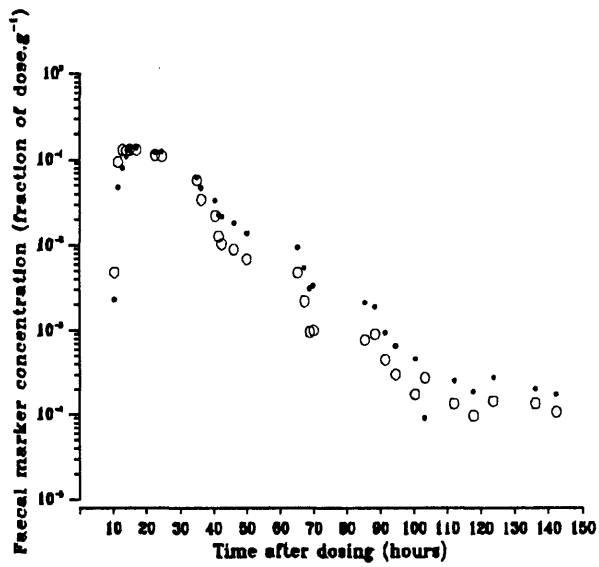
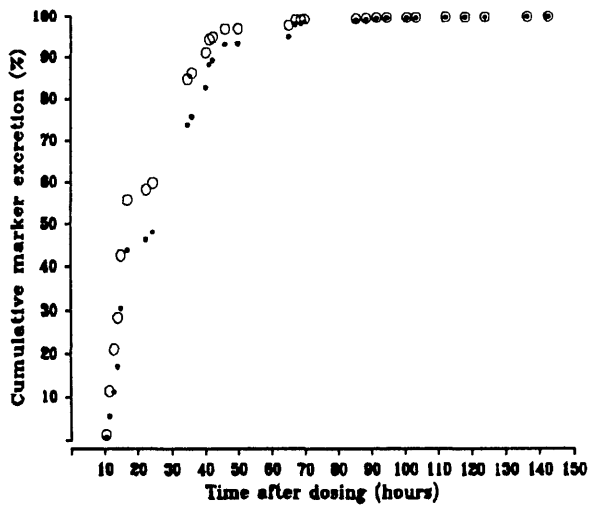


Fig 7.6 A. rufescens G May 1984

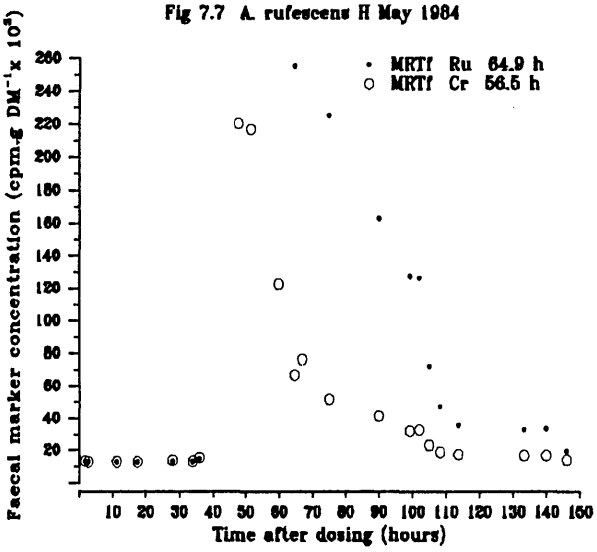


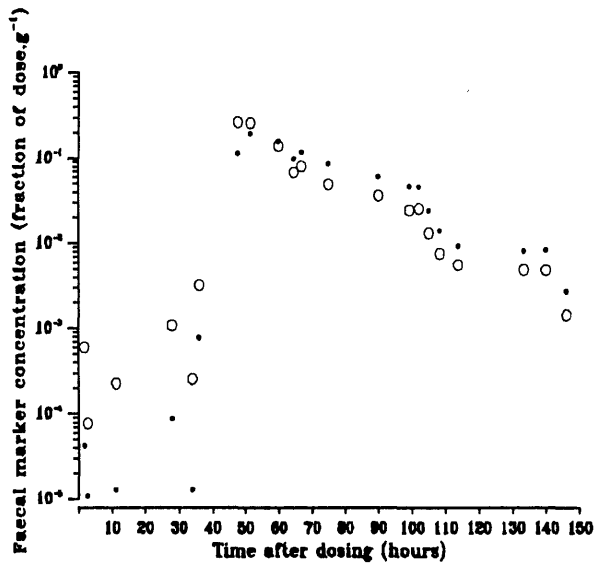




Marker excretion patterns in the faeces of two A. rufescens following a pulse dose.

Fig 7.7 A. rufescens H May 1984





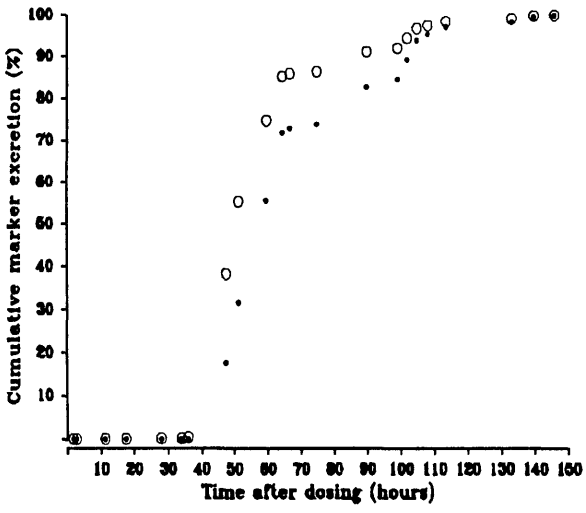
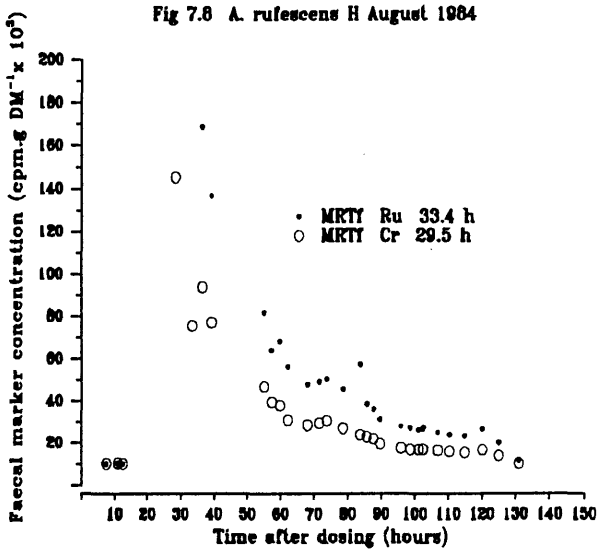
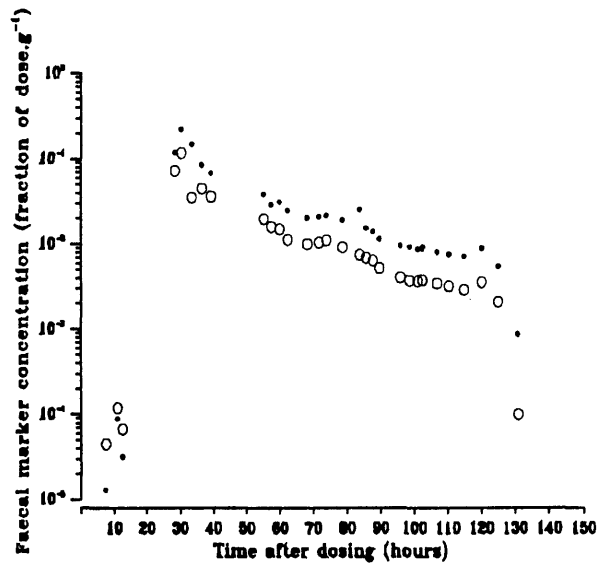
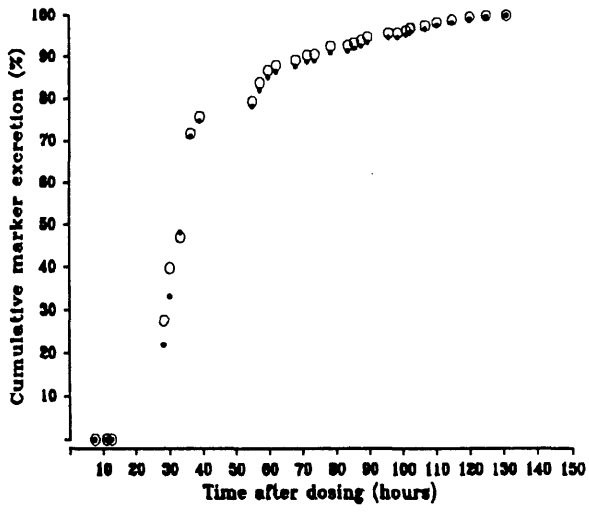


Fig 7.8 A. rufescens H August 1984

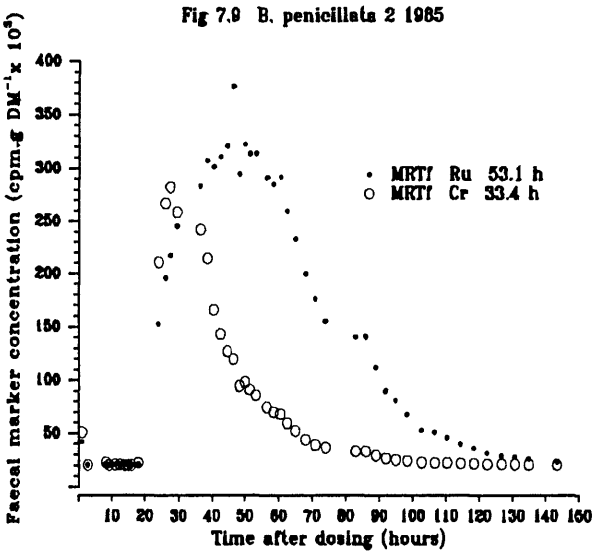


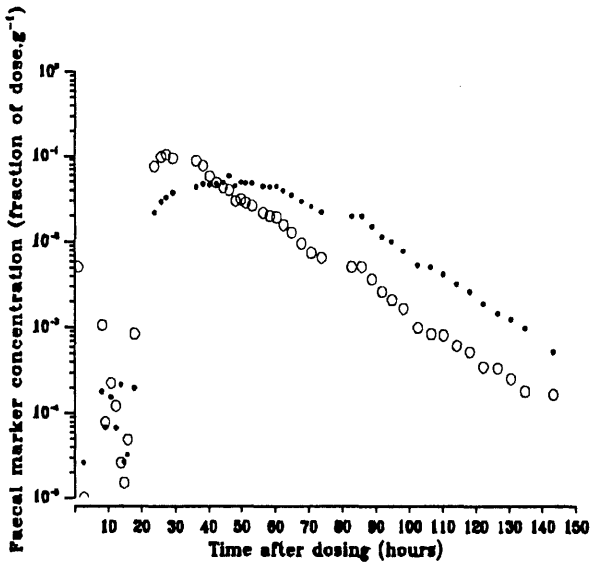




Marker excretion patterns in the faeces of two A. rufescens following a pulse dose.

Fig 7.9 B. penicillata 2 1985





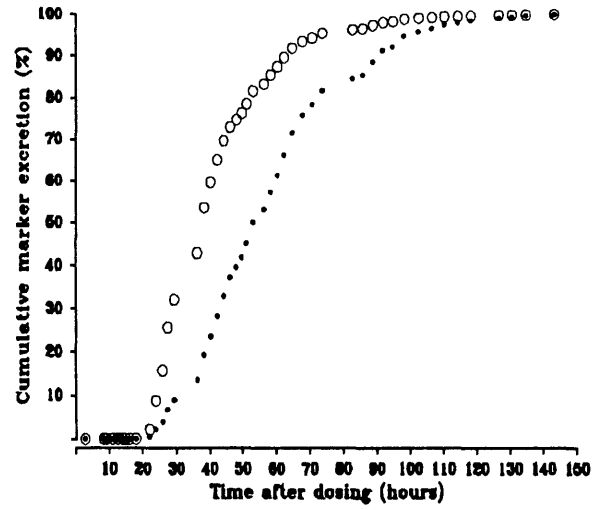
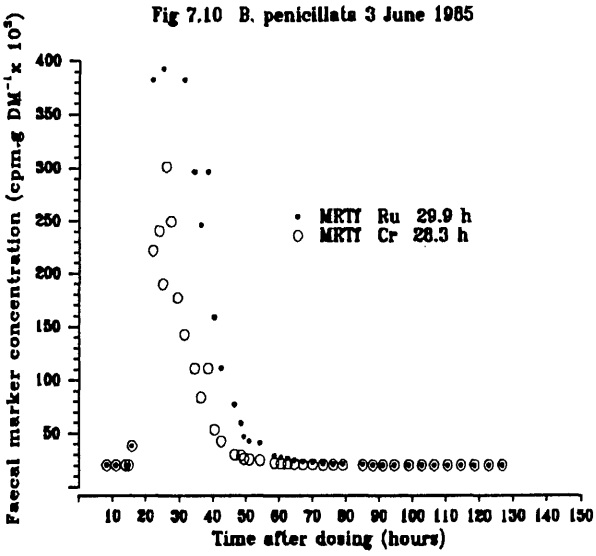
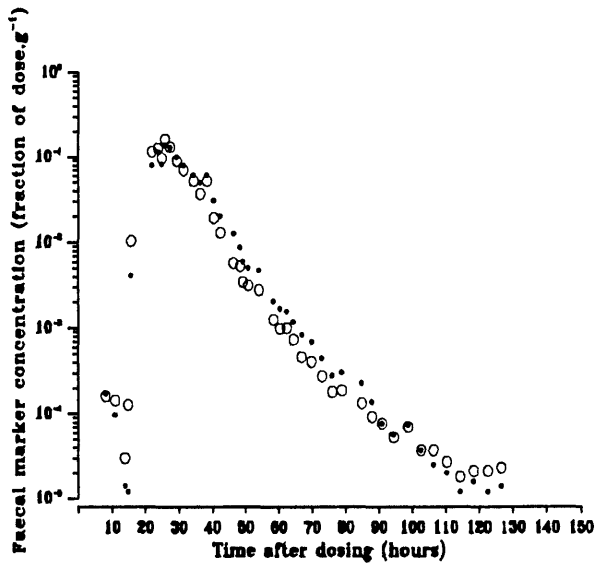
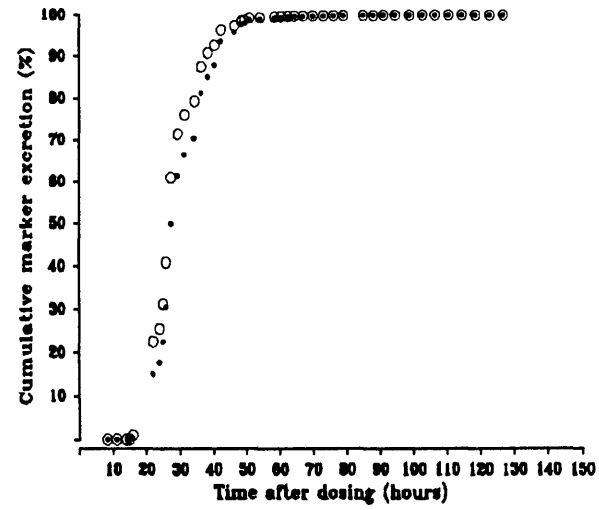


Fig 7.10 B. penicillata 3 June 1985







Marker excretion patterns in the faeces of two B. penicillata following a pulse dose.

Fig 7.11 P. tridactylus 2 October 1985

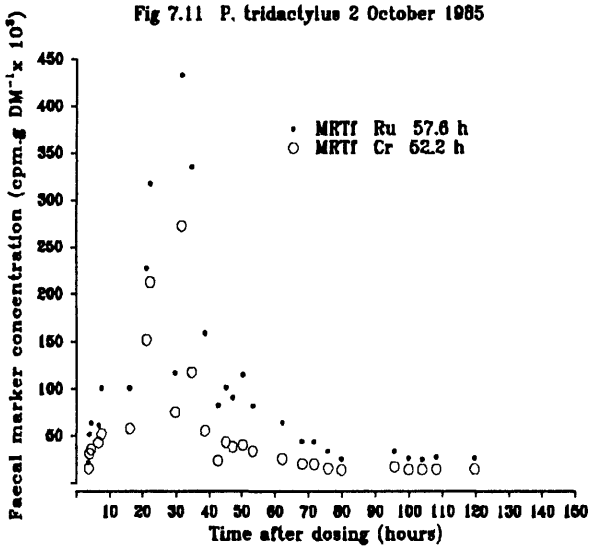
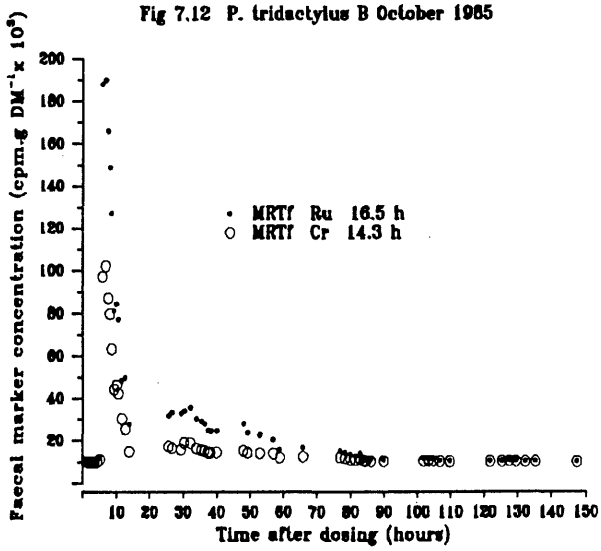
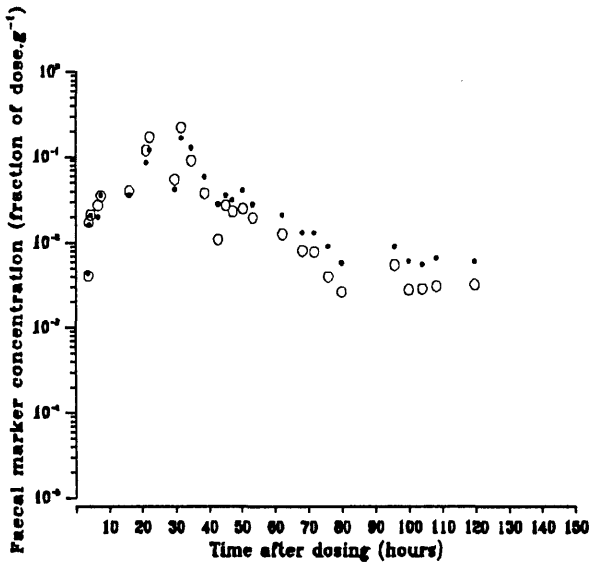
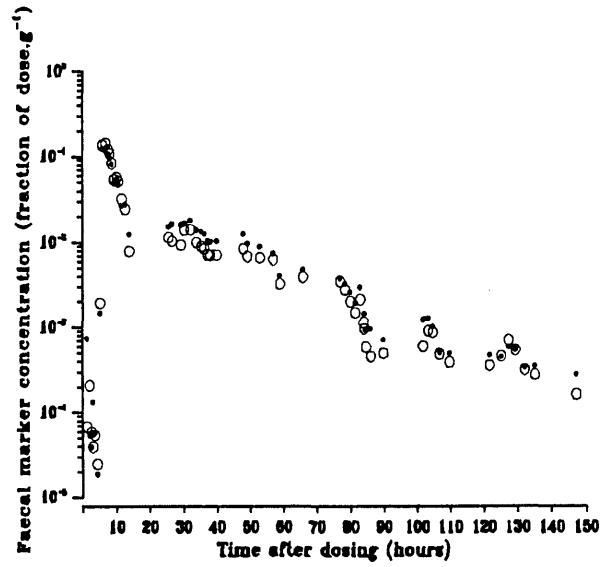
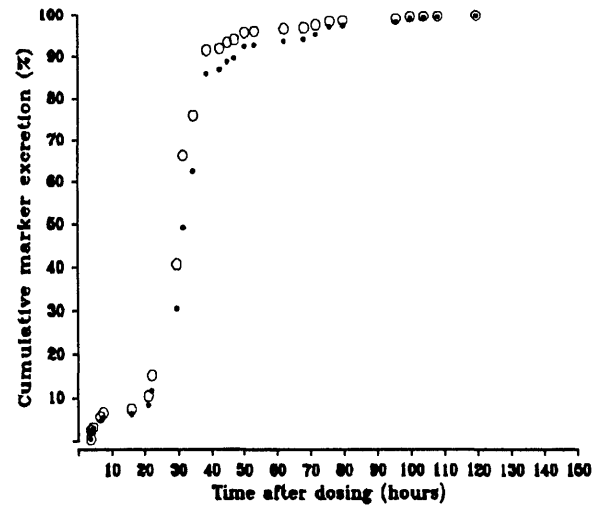


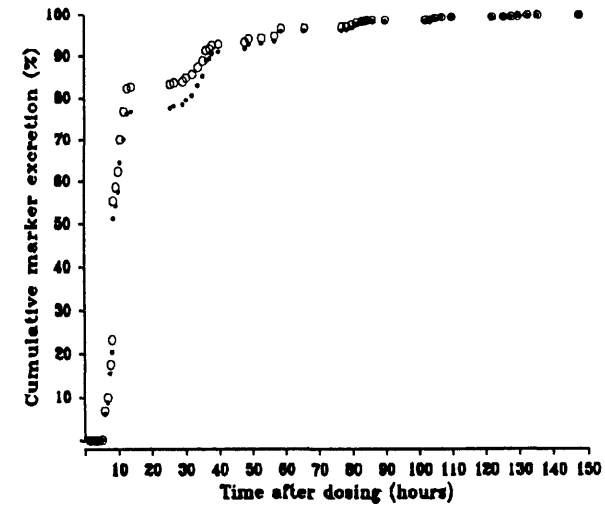
Fig 7.12 P. tridactylus B October 1985











Marker excretion patterns in the faeces of two P. tridactylus following a pulse dose.

Fig 7.13 P. tridactylus J October 1985

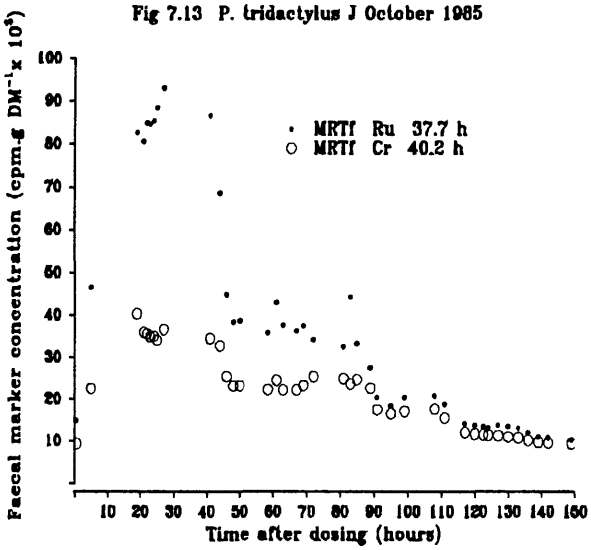
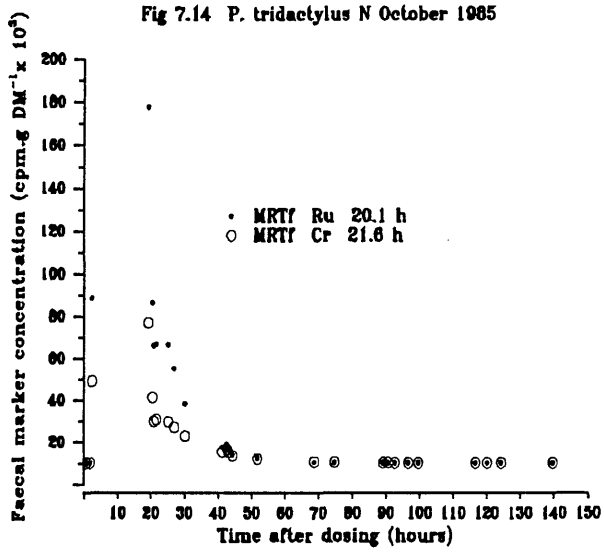
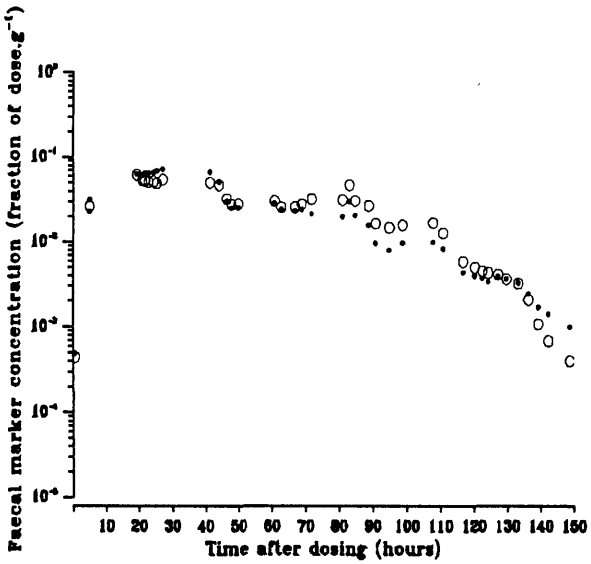
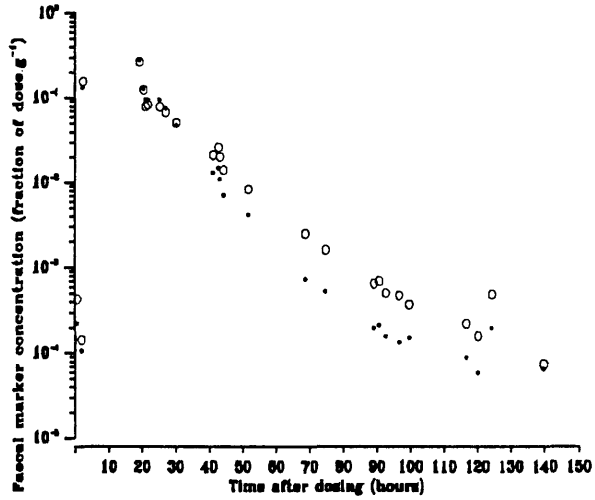
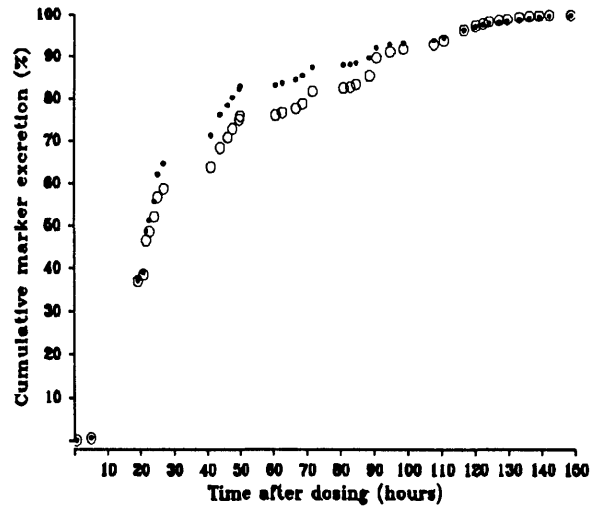


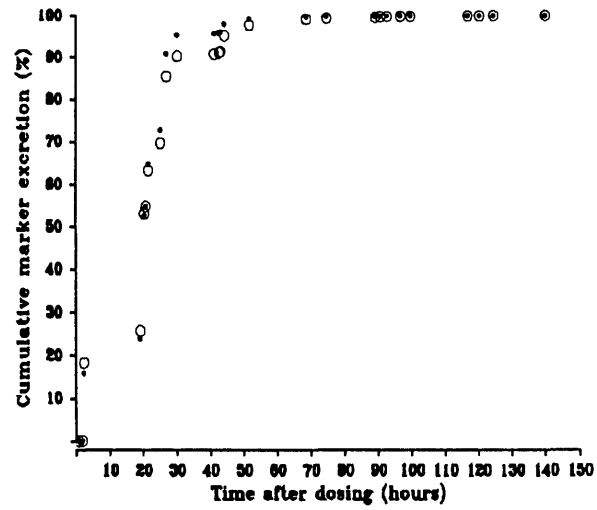
Fig 7.14 P. tridactylus N October 1985











Marker excretion patterns in the faeces of two P. tridactylus following a pulse dose.

accounts for about 60% of total marker excretion, and was associated with a very steep peak of marker concentration against time.

Defaecations with anomalous concentrations of both markers

More than half the marker excretion curves had one or more defaecations in which marker concentrations deviated from those expected from the preceding and succeeding samples. The most striking examples are: Figures 7.4 - 35h, 100h, 130h; 7.5 - 100h; 7.6 - 70h; 7.11 - 35h and 48h; 7.13 - 85h and 7.14 - 125h. The procedure for handling samples by weighing them into pre-labelled tubes and the system of data recording, makes it highly unlikely that samples with anomalous concentrations were incorrectly labelled.

Grovum and Williams (1973) used compartmental analysis to describe the marker excretion curves of sheep. They concluded that the patterns of excretion were best described by two exponential terms and a delay, which translates, in the animal, to two mixing compartments joined by a tube. The potoroine gut also contains two organs — the SFS and the hindgut — which are potential mixing compartments (Chapter 3). Ignoring the anomalies discussed above, the potoroine marker-excretion curves show a basic resemblance to those of sheep (Grovum and Williams 1973). These factors suggest that compartmental analysis might help to describe the pattern of digesta flow through the potoroine gut. Nevertheless, this was not attempted because first, compartmental analysis assumes steady-state conditions — that is a constant rate of dry matter excretion — which does not occur in potoroines; and secondly, radiographic analyses show that a mechanism allows some digesta to bypass the SFS completely (Hume and Carlisle 1985).

7.4 Discussion

The markers used in studies of gut function encompass a wide range of physical and chemical properties. These range from those with seemingly no resemblance to the traced material (for example gold and glass), through to others, such as rare earths or chromium mordants, that bind tenaciously to the particulate matter. The materials, their applications and the techniques specific to their use have been reviewed by Kotb and Luckey (1972), Engelhardt (1974), Faichney (1975a) and Warner (1981a). These

authors discussed in detail the attributes of an ideal marker. The four most important attributes are:

1. It must not be absorbed from the gastrointestinal tract.

2. It must not affect, nor be affected by, the gastrointestinal tract or its microbes.

3. It must be physically similar to, or intimately associated with, the material it is to mark.

4. The method of estimation of the marker in digesta samples must be specific and sensitive, and must not interfere with other intended analyses.

Both ^{103}Ru-P and ^{51}Cr-EDTA satisfy most of these criteria. For example, in studies with *P. cinereus*, Cork and Warner (1983) showed that most of the ^{103}Ru-P associated with the particulate phase at the pH found in the gut; that repeated extraction of faeces with water removed most of the ^{51}Cr-EDTA but not the ^{103}Ru-P; that less than 1% of the ^{51}Cr-EDTA dose and less than 0.1% of the ^{103}Ru-P appeared in the urine; and that only negligible quantities of the absorbed markers are recycled to the gut. Dellow (1982) confirmed these findings in macropodids.

We know of no ideal marker. The major detriment of ^{103}Ru-P is its tendency to exchange rapidly between binding sites (Faichney and Griffiths 1978; Dixon *et al.* 1983) with a net movement to smaller particles including micro-organisms (Faichney 1986). The retention time of ^{103}Ru-P may better reflect the flow of small particles — possibly of endogenous origin — rather than large particles. The fact that fine particles are known to associate with the fluid phase (Björnhag 1987) suggests that this situation may at least partially mask any separation of digesta phases. ^{51}Cr-EDTA has been criticised because it does not always associate with the solute phase. Faichney (1975b) reported that only 70% of ^{51}Cr-EDTA was associated with the fluid phase of rectal contents from sheep fed a pelleted diet of 50% lucerne and 50% concentrates.

Despite these shortcomings, ^{103}Ru-P and ^{51}Cr-EDTA were deemed suitable for use in many recent studies of digesta transit in marsupials (Table 7.7) and sheep (Faichney 1975b; Grovum and Williams 1973; Faichney and Griffiths 1978; Faichney and Boston 1983; Faichney and Barry 1986; Faichney and White 1988; Fadlalla *et al.* 1987). This was a decisive factor in the choice of both markers for the present study, allowing comparison between derived and published data.

It was not the aim of this study to evaluate the suitability of ^{103}Ru-P and ^{51}Cr-EDTA in potoroines, fed concentrate diets, although measurements were made of the excretion of both markers in the urine. In the case of ^{51}Cr-EDTA, this accounted on average for about 10% of the total excreted. Warner (1981b) reported that *M. eugenii*, fed once daily, excreted about 5% of the ^{51}Cr-EDTA dose in the urine. He also cited

unpublished work in which sheep, fed once daily, excreted up to 13% of the ^{51}Cr-EDTA in the urine.

In sheep there is evidence that absorption of ^{51}Cr-EDTA from the rumen is enhanced by hypertonic conditions (Dobson *et al.* 1976). Several factors affected the intensity and duration of the rise in osmotic pressure. Diets with rapidly fermentable carbohydrates accentuated the rise. A similar response occurred in animals that ate their ration quickly and in those with limited access to water. In the present study, a number of factors which may cause hypertonic conditions in the gut can be identified. One of these is the highly digestible nature of the diet. Another is the potoroines' nocturnal habits which encompass, in both captive and wild animals (pers. obs.), a spate of feeding just after dark. It was thought that the absorption of ^{51}Cr-EDTA might be used to advantage in that low urinary levels might indicate those animals in which the dose bypassed the SFS. However, there was no correlation between MRT and urinary ^{51}Cr-EDTA excretion.

^{103}Ru-P was excreted also in the urine in greater quantities than has previously been reported (Grovum and Williams 1973; Warner 1981b; Dellow 1982; Cork and Warner 1983; Foley and Hume 1987b). However, this was still less than 1% of total excretion. The regular removal of faeces ensured that the ^{103}Ru-P in the urine originated from marker absorbed from the gut, and was not leached from the faeces after deposition.

Data describing the passage of digesta through the gut (MRT etc) varied considerably between animals. This is not surprising, because the patterns of defaecation were erratic. In fact, the coefficients of variation (Table 7.6) are similar or greater than those reported by Warner (1981b) in studies of *M. eugenii*, and for wild animals in general — that is, at least 20% (Warner 1981a). Less expected was the variation within individual animals. Several individuals of each species were used at least twice, in each part of an experiment incorporating a crossover design. Often, the shapes of the two marker excretion curves were different. In other cases, for example Figures 7.7 and 7.8, the marker excretion curves are a similar shape but displaced by about 30 h. None of this variation could be attributed to treatment differences or to uniform differences between experimental periods. It must be due instead to variation within individuals. This is in direct contrast to Warner's (1981b) work with *M. eugenii*, which suggests that replicated marker excretion curves contain features characteristic of an individual animal. However, Warner (1981a) mentions that within-animal variation may equal that between animals. Presumably, the numerous potential mechanisms for regulation of digesta flow in potoroine marsupials means that the variation in MRT, within an individual, may equal that between individuals.

Many of the unusual features of the marker concentration curves have been reported previously in *M. eugenii* by Warner (1981b). The tentative explanations for these phenomena rely heavily on his speculative interpretations.

The early appearance of marker occurred when faeces were being removed at the time of excretion. This, along with the fact that they contained both markers, makes it impossible that the marker in the faeces came from contamination with urine. Instead, small quantities of digesta traverse the length of the gut rapidly, perhaps overtaking other digesta. Early appearance of marker has been reported previously in *M. eugenii* by Warner (1981b) who also cited a similar occurrence in humans (Hoelzel 1930). The phenomenon was also observed radiographically in *A. rufescens* and *P. tridactylus* (Hume and Carlisle 1985; Hume *et al.* 1988) and in *P. tridactylus* by Frappell and Rose (1986). Warner (1981b) suggested that the gastric sulcus aids passage through the forestomach, and that passage through the rest of the gut must also be accelerated. This explanation fits *P. tridactylus*, which has a gastric sulcus, but excludes *A. rufescens* which does not. However, as mentioned previously, the gastric anatomy of all potoroines allows some ingesta to bypass the SFS. The absence of twin peaks in the concentration curves refutes the notion that the marker dose was split, with one part being shunted to the SFS and the other passing to a more distal part of the tract. Hence, an alternative explanation for the rapid transit of small amounts of marker is that, although the dose is destined for the SFS, the mechanism is not perfect. It allows a negligible part of the dose to continue down the tract.

Three distinct peaks were identified in the marker concentration curves. The very sharp peaks probably indicate that the dose bypasses the SFS and that there was negligible mixing of digesta in the hindgut. This view is supported by the MRT, which are usually short in these animals. Furthermore, the caecum and proximal colon are narrow organs and lack the prominent musculature seen in the foregut. This may restrict mixing in the hindgut.

The peaks that indicate TT of 10-30 h resemble those of the particle marker in *M. eugenii* fed once a day (Warner 1981b). Warner associated this phenomenon with the long periods during which the concentration of the marker in the SFS is not being diluted with fresh ingesta. The similar shape of both the solute and particle marker curves in potoroines implies that saliva flow during resting periods is negligible.

The curves with long, flat peaks — indicating long TT — are difficult to explain. They suggest a very gradual release of digesta from a mixing pool, presumably the SFS. The most pronounced example is Figure 7.9. What causes the slow release of digesta? One might surmise that this situation arises because food intake is depressed, but this was not the case. Fluid flow — induced by drinking and/or increased secretion of saliva

during the normal feeding period — may cause marker separation. However, this hypothesis suggests that, at times, particles are selectively retained.

Two wave patterns were recognized among the different marker excretion curves. The first (Figures 7.4 and 7.6) was associated with the pattern of defaecation. Dry matter output peaks soon after dark, and coincides with the onset of feeding. In contrast, defaecation rarely occurs during the day. Thus, for 12-18 h a day no faeces are deposited. However, faeces deposited just before and just after this period have similar marker concentrations. Both markers show similar waves, which probably indicates that some faeces spend many hours in the rectum before excretion.

The second type of wave, best seen in Figures 7.1 and 7.12, is similar to that reported by Warner (1981b), and is independent of the pattern of dry matter output. Warner attributed this feature to the sequestration and subsequent release of a small amount of marker in or before the main mixing compartment. The fact that both markers have similar waves is further evidence that both phases traverse the gut in unison. Warner related the occurrence of two or three secondary peaks at 24 h intervals to separate releases of sequestered material. He suggested that the impulse for release is the strong movement of digesta in animals with restricted feeding times. It is clear that for detection of secondary peaks, the sequestered material must be released when marker concentrations are much lower than those in the sequestered digesta.

The two distinct components of the negative portion of the marker excretion curve (Figures 7.1 and 7.8) suggest two digesta pools turning over at different rates. In this animal, the tmax of both markers occurred within 5 h of dosing. Perhaps the most plausible explanation for the change in slope is that the dose was swallowed as several boli. Some of these entered the SFS and were excreted gradually; others passed to more distal parts of the tract and appeared in the faeces soon after dosing.

The anomalous marker peaks are similar also to those described in *M. eugenii* by Warner (1981b). There are two situations. In the first, the faecal marker concentration corresponds with that from a collection several hours later (for example, in Figures 7.4 the marker concentration at 30h is similar to that at 50h; and in Figure 7.6 — 50h corresponds with 70h, and 70h with 90h). This feature may arise if a bolus overtakes others of higher marker concentration. The mechanism may be the same as that causing marker excretion soon after dosing. The reverse also occurs — a defaecation with high marker concentration is excreted later than expected. An example is shown in Figure 7.10 (40h=35h). Apparently, a bolus has been sequestered caudal to the main mixing compartment and, after a delay, has re-entered the sequence.

Previously, there have been only radiographic studies of gut function in potoroine marsupials (Hume and Carlisle 1985; Frappell and Rose 1986; Hume *et al.* 1988).

Their results, in contrast to those from the present studies, show by two methods that particles and solutes behave differently in the gut, and possibly have different retention times. Frappell and Rose (1986), in studies with *P. tridactylus*, showed that barium sulphate incorporated in a food pellet passed to the SFS. However, when administered in suspension, the barium sulphate flowed, via the gastric sulcus, to the hindstomach. In contrast, Hume and Carlisle (1985) found no separation of phases at the cardia of either *A. rufescens* or *P. tridactylus*. In both species, a proportion of both 3-5 mm radio-opaque surgical gauze-threads and barium sulphate suspension bypassed the SFS. Instead, their evidence for phase separation is based on the observation that particles reaching the SFS, stayed there for longer than the suspension. This was most pronounced in *A. rufescens*. Some particles remained 95 hours after dosing, when all traces of the suspension had passed from the SFS.

Both approaches have limitations. The long retention of a few gauze threads suggests that they may have been trapped by the rugose gut wall (Frappell and Rose 1986). Hume and Carlisle (1985) do not give the specific gravity of the gauze threads. It is likely that they are not physically similar to, or intimately associated with, the food particles ingested. Thus, they do not satisfy one of the fundamental requirements of a marker. Nevertheless, in a more recent study, Richardson (1989) identified phase-separation in *B. penicillata*.

The only obvious difference between the studies of Hume and Carlisle (1985) and Frappell and Rose (1986) was that the latter starved their animals for 12-15 h before dosing. Nevertheless the results from the two groups differed markedly. Apart from reporting the presence of a functional gastric sulcus and a phase separation mechanism, the results of Frappell and Rose included shorter retention times throughout the gastrointestinal tract. Although not mentioned by Frappell and Rose (1986), it was reported by Frappell (1984) that all animals studied were sedated with ketamine hydrochloride to assist dosing.

This finding prompted the additional radiographical studies reported by Hume *et al.* (1988). Under sedation, there is phase separation in the forestomach and rapid movement of contrast medium through the gut. The results of Frappell and Rose are clearly an artefact of ketamine sedation. They do little to enhance our understanding of potoroine gut function.

There is still no explanation, in the present work with *A. rufescens*, for the failure of ketamine sedation to reduce the retention times of ^{103}Ru-P and ^{51}Cr-EDTA. Apart from using a different marker, the studies of Frappell and Rose (1986) and Hume *et al.* (1988) involved frequent animal handling and restraint of animals in an upright position during radiography. Neither of these procedures was used in the present studies, which suggests that an increase in gut motility may involve several factors.

Hume *et al.* (1988) reported that, in fed *A. rufescens*, the initial dispersion of a contrast medium suspension is predominantly into the SFS. In fasted animals, the marker dispersed into the hindstomach. Barium-marked pellets usually dispersed into the hindstomach. When the hindstomach and tubiform forestomach fill, digesta overflow into the SFS. *Potorous tridactylus* reacted quite differently. In both fed and fasted animals, suspension and pellets dispersed to the SFS.

The above results suggest that in studies with ^{103}Ru-P and ^{51}Cr-EDTA there might be marked differences between MRT measured in fed and fasted *A. rufescens*, but not in *P. tridactylus*. However, in the present study radioactive markers traversed the gut at similar rates in both *A. rufescens* and *P. tridactylus* regardless of whether the markers were given before feeding, or when animals had consumed about 40% of their normal daily intake.

Hume *et al.* (1988) found that, in both *A. rufescens* and *P. tridactylus*, fasting delayed the transit of contrast medium to the hindgut. If we keep in mind that radioactive markers traversed the gut at the same rate and that the initial dispersion of contrast medium is into the hindstomach of fasted *A. rufescens*, the finding of Hume *et al.* was unexpected. The delay, they suggest, "is consistent with the observation in kangaroos and other herbivores that a reduction in level of feeding leads to longer retention times of digesta markers (Warner 1981a)". In other words, passage from the hindstomach is slowed because fasting reduces gut motility (I.D. Hume pers comm). A normal potoroine does not have a reduction in feeding level, but maybe less time available for feeding than diurnally active animals. Unfortunately, Hume *et al.* (1988) fasted their animals from 2100 h until the commencement of radiography the next day — between 0900 and 1200 h. This may well have restricted food intake and altered digesta passage. The study would have been more conclusive, and comparable to the present study, had it provided for the normal behaviour of potoroines. This would have entailed dosing the animals in the evening, and then radiographing at night.

Some data of particular relevance to the present study are shown in Table 7.7. Both hindgut- and foregut-fermenting eutherian and metatherian species are listed. To simplify comparisons, data obtained with ^{103}Ru-P and ^{51}Cr-EDTA have been chosen where possible. This, together with the comprehensive inventory of rate-of-passage data compiled by Warner (1981a), is used in the ensuing discussion.

Different experimental conditions impel caution when making generalisations from rate-of-passage data. However, some are possible. Herbivores have the longest retention times. This is most pronounced in arboreal species — for example, *P. cinereus* — a hindgut fermenter, and in *Bradypus spp* and *Choleopus spp* sloths, which are foregut fermenters. Body mass alone is not necessarily a good indicator of retention times. *Loxodonta africana*, a hindgut fermenting herbivore, has an MRT similar to that

Table 7.7 The rate of passage of digesta through the gastrointestinal tracts of various herbivores.

Species	Diet	Excretion (%)	Time (h)	MRT	Marker	Phase	Reference
Foregut-fermenters							
Eutheria							
sheep	lucerne hay	50	25	—	^{103}Ru-P	particles	Dellow (1979)
		50	21	—	^{51}Cr-EDTA	solute	
sheep	2 mm hay	—	—	40	^{103}Ru-P	particles	Fadlalla *et al.* (1987)
	5 mm hay	—	—	41	^{103}Ru-P	particles	
	20 mm hay	—	—	43	^{103}Ru-P	particles	
	chopped hay	—	—	45	^{103}Ru-P	particles	
sheep	concentrates	—	—	73	^{103}Ru-P	particles	Faichney (1975b)
		—	—	58	^{51}Cr-EDTA	solutes	
L. guanacoe	hay, concentrates	—	—	52	^{139}Ce	small particles	Heller *et al.* (1986)
		—	—	60	^{155}Sm	large particles	
		—	—	36	PEG	solutes	
Alouatta palliata	fruit, leaves	95	24-72	—	plastic	particles	Milton (1981)
Metatheria							
M. rufus	lucerne hay	95	58-89	41-57	stained feed	particles	McIntosh (1966)
S. brachyurus	lucerne hay	90	38-60	—	stained feed	particles	Calaby (1958)
M. giganteus and *M. rufus*	hay	90	46-56	35-44	stained feed	particles	Forbes and Tribe (1970)
M. giganteus	lucerne hay	50	30	—	^{103}Ru-P	particles	Dellow (1982)
M. giganteus		50	14	—	^{51}Cr-EDTA	solutes	
M. r. robustus	lucerne hay	50	33	—	^{103}Ru-P	particles	
		50	19	—	^{51}Cr-EDTA	solutes	
T. thetis	lucerne hay	50	23	—	^{103}Ru-P	particles	
		50	12	—	^{51}Cr-EDTA	solutes	
M. eugenii	lucerne hay	50	24	—	^{103}Ru-P	particles	
		50	15	—	^{51}Cr-EDTA	solutes	

Table 7.7 continued

M. eugenii	lucerne pellets	90	47	29	^{103}Ru-P	particles	Warner (1981b)
	one meal/day	90	26	16	^{51}Cr-EDTA	solutes	
	lucerne pellets	90	29	19	^{103}Ru-P	particles	
	continous feed	90	17	11	^{51}Cr-EDTA	solutes	
A. rufescens	pelleted	50	28	37	^{103}Ru-P	particles	Experiment 7.2
	concentrates	50	20	31	^{51}Cr-EDTA	solutes	
P. tridactylus	pelleted	50	20	30	^{103}Ru-P	particles	
	concentrates	50	19	28	^{51}Cr-EDTA	solutes	
B. penicillata	pelleted	50	25	35	^{103}Ru-P	particles	
	concentrates	50	20	27	^{51}Cr-EDTA	solutes	
Hindgut fermenters							
Eutheria							
Horse	oaten chaff	—	—	24	^{103}Ru-P	particles	Orton *et al.* (1985)
		—	—	22	^{51}Cr-EDTA	solutes	
O. cuniculus	hay/grain pellets	—	—	14-21	^{141}Ce	particles	Laplace and Lebas (1975)
	alfalfa pellets	85	79	—	PEG	fluid	Murphy *et al.* (1982)
	hay/grain pellets	95	61	—	Cr_2O_3	particles	Clemens and Stevens (1980)
Metatheria							
T. vulpecula	semi-purified	90	264-336	69-74	^{103}Ru-P	particles	Wellard and Hume (1981b)
		90	259-346	64-65	^{51}Cr-EDTA	solutes	
T. vulpecula	Eucalyptus leaves	95	107	49	^{103}Ru-P	particles	Foley and Hume (1987b)
		95	109	51	^{51}Cr-EDTA	solutes	
P. volans	Eucalyptus leaves	95	105	46	^{103}Ru-P	particles	Foley and Hume (1987b)
		95	128	50	^{51}Cr-EDTA	solutes	
P. cinereus	Eucalyptus leaves	95	266	81-140	^{103}Ru-P	particles	Cork and Warner (1983)
		95	633	115-443	^{51}Cr-EDTA	solutes	

of the horse but shorter than that of the sheep. However, among the ruminants, cattle seem to retain digesta for longer than sheep, goats and small deer, which in turn retain it for longer than *Nesotragus moschatus*, a 7-8 kg antelope. Whether this is a consequence of body mass alone is debatable. Perhaps a more important parameter is gut structure. Unfortunately there is insufficient data to compare the grazing, concentrate selecting and intermediate-grade ruminants.

It is also uncertain whether body size affects rate of digesta-passage in macropodoids. Dellow (1982) measured retention times in various macropodid species and sheep. Although the larger species — *M. giganteus* and *M. r. robustus* — had longer retention times than the smaller *T. thetis* and *M. eugenii*, the differences were small. However, the 50% and 90% retention times of ^{103}Ru-P and ^{51}Cr-EDTA were much shorter than those obtained with the same markers in potoroines. This does not necessarily indicate differences between species. Grain-based and semi-purified diets, similar to those fed in the present study, often have relatively long rates of passage. Two examples are shown in Table 7.7. Sheep fed a concentrate diet had a 78 h MRT for ^{103}Ru-P; those fed hay had MRT of 40-45 h. Similarly, when fed semi-purified diets, *T. vulpecula* had much longer MRT and 90% excretion times for both particle and solute markers (Wellard and Hume 1981) than when fed *Eucalyptus* foliage (Foley and Hume 1987b). Other examples of long retention times in animals fed concentrate diets are given by Warner (1981a).

Apart from the data on arboreal folivores, there is little published information on the transit of ^{103}Ru-P and ^{51}Cr-EDTA through the gastrointestinal tracts of strictly nocturnal herbivores. In the present study, the preponderance of MRT of about 24-30 hours suggests that the nocturnal habits of potoroine marsupials might be an important regulator of digesta flow. In other words, MRT might be synchronised diurnally so that the animal does not defaecate in its nest, and thus increase the chance of detection by predators.

The striking difference in Dellow's (1982) work was the extent of phase separation. His work showed that ^{103}Ru-P and ^{51}Cr-EDTA had similar MRT in sheep but, in the macropodids, 90% of the solute marker passed in the time taken for 10% of the particle marker. Dellow suggested that separation is accomplished by the forestomach musculature, which expresses the fluid through the particles. Is this a process for minimising the microbial degradation of the nitrogen-rich fine particles which might be washed along the tract in the fluid? In other words a mechanism for protecting soluble protein from microbial degradation.

Forestomach morphology of macropodoids varies markedly with body size. The forestomachs of the largest species are predominantly tubiform; those of the smallest species — the potoroines — mainly sacciform. The wallabies, which are intermediate

in body size, tend to have morphologies midway between the sacciform and tubiform types. The extent of phase separation does not show a similar gradation. In all the macropodid species, the MRT of the solute marker is always about half that of the particle marker. Indeed, it is not until we examine the potoroines, in which the foregut has lost all resemblance of a tube that phase separation disappears.

The apparent lack of marker separation in potoroines suggests that their digestive strategy differs markedly from those of the larger kangaroos and wallabies. In fact it may have a closer affinity with that of the concentrate-selecting ruminants discussed by Hofmann (1989).

Although the rate and patterns of digesta flow in potoroines have been studied with both radiographic and radio-isotopic tracers, the movement of ingesta within the foregut is a subject of contention. The mechanisms controlling digesta flow to the SFS are not understood, nor is the importance of secondary digesta pools. Thus, it seems premature to construct a model to describe the sequence of events from ingestion to excretion. Instead, the following section integrates the present findings with the published radiographic work.

Hume *et al.* (1988) propose that, in *A. rufescens*, ingesta flows into the SFS when the TFS is full. SFS distension peaks early in the morning and declines gradually during the day. The sequence of events differs slightly in *P. tridactylus*, because all food passes to the SFS. As the volume of the potoroine TFS is small, most of the ingesta probably passes to the SFS. What, therefore, is the role of the SFS?

Hume *et al.* (1988) argued that the diets consumed by potoroines are best utilised in the small intestine, without any microbial intervention. Thus, the SFS is primarily a storage vessel, designed to accommodate the ingesta from short, intensive feeding bouts. However, several factors underline the importance of the microbial metabolism — long MRT; the presence of substantial short-chain fatty-acids (SCFA) in the SFS of *B. penicillata* (Kinnear *et al.* 1979) and *P. tridactylus* (Carr 1970 cited by Frappell and Rose 1986); and the fact that the SFS environment is suited to microbial growth rather than to acid hydrolysis (Langer 1988).

Assuming that potoroine diets are highly digestible, the main contribution of the microbial synergism is possibly not SCFA, but amino acids. Many of the foods eaten by *A. rufescens* contain about 1% nitrogen — adequate to meet the the maintenance nitrogen requirement (Chapter 5). It is unknown whether these foods can supply the correct balance of essential amino acids without microbial intervention. The same may apply to *P. tridactylus*. Although fungi are rich in protein, the quality is sometimes poor (Cork and Kenagy 1989). Thus, if amino-acid balance cannot be corrected from

complementary dietary sources, microbial protein synthesis, using dietary and endogenously-recycled nitrogen, is necessary.

7.5 Summary

The similar mean retention times (MRT) (ca 25-30 hours) of ^{103}Ru-P and ^{51}Cr-EDTA in the gastrointestinal tracts of potoroine marsupials contrasts with the marked separation of digesta phases in macropodids. The different patterns of digesta flow, in the two groups, were explained by differences in foregut anatomy. No significant differences were found between *A. rufescens*, *P. tridactylus* or *B. penicillata* for any parameter of digesta passage. Because MRT were often between 24 and 30 hours, it was suggested that the nocturnal habit of potoroine marsupials might be an important regulator of digesta flow. Digesta passage was not affected by the level of dietary plant-cell wall constituents, or the level of gut fill when the markers were administered. It is suggested that future studies examine the effects of particle size, the transit of low-concentrate diets and the possibility that, although solutes and particles have similar MRT, they flow through the gut independently.

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