CHAPTER ELEVEN

Energy metabolism in potoroine marsupials

MOST macropodoids have characteristics that may be interpreted as energy conserving. For example, they tend to have low resting metabolic rates (Dawson and Hulbert 1970); generally they display a bipedal hop (Strahan 1983); the usual litter size is one (Eisenberg 1978) and many of them are seasonal breeders (Tyndale-Biscoe and Renfree 1987). Macropodoids have the ability to terminate reproductive processes in adverse conditions; in most species this is supported by embryonic quiescence — the ability to restart reproduction from a quiescent blastocyst. It can be argued that these energy-saving strategies are a direct response to unpredictable supplies of food and water in many parts of Australia.

Few studies have been made of the energetics of the Potoroinae. Exceptions are the studies of fasting metabolic rates in *P. tridactylus* by Hudson and Dawson (1975) and Nicol (1976) and of thermoregulation in *A. rufescens* at different ambient temperatures (Rübsamen *et al.* 1983). Apparently, nobody has studied the energetics of *B. penicillata.* This chapter, divided into three sections, describes some further aspects of energy metabolism in potoroine marsupials. Section 11.1 describes the measurement of fasting heat production (FHP) and maintenance heat production (MHP) in *A. rufescens*, *P. tridactylus* and *B. penicillata.* Section 11.2 deals with the energetic costs to *A. rufescens* of raising pouch young. Section 11.3 discusses the energetics, in winter and summer, of free-living *A. rufescens.*

11.1 The fasting heat production and maintenance requirement of *A. rufescens*, *P. tridactylus* and *B. penicillata*

11.1.1 Introduction

THE discovery that metatherians exhibited lower body-temperatures than is general in eutherians (Sutherland 1897) may have prompted Martin (1903) to investigate their basal metabolism. He measured carbon-dioxide production in several resting metatherians, which included a dasyurid (either *Dasyurus maculatus* or *D. viverrinus*) and the diprotodonts *Trichosurus vulpecula* and *Bettongia gaimardi*. His measurements indicated that the minimum metabolic rate of at least some metatherians is only 30% of that of eutherian mammals.

Six decades later, Bartholomew and Hudson (1962) reported that the minimum oxygen consumption of the pygmy possum, *Cercartetus nanus*, was approximately 70% of that expected for a eutherian of similar size. They explained this low value by assuming that the animal had large fat reserves. Shortly afterwards, Dawson and Hulbert (1969, 1970) showed that a low rate of fasting metabolism was probably a physiological characteristic of metatherians in general. The researchers measured the oxygen consumption of eight metatherian species (9-54000g) over 30-minute periods in open-circuit calorimetry chambers. They calculated a mass-metabolism curve that had a similar exponent to Kleiber's (1932) but, when compared statistically with the data for eutherians published by Kleiber (1961), the standard metabolism of the metatherians was significantly lower ($P<0.001$). In contrast to Martin's (1903) finding, Dawson and Hulbert found that metatherians had a standard metabolic rate only 30% lower than that of eutherians. MacMillen and Nelson (1969) confirmed the findings of Dawson and Hulbert (1969). In experiments with 12 species of dasyurid marsupials, they reported a rate of metabolism 32% less than that reported for eutherian mammals. Other correlates of basal metabolism also are low in marsupials — for example, heart rate (Kinnear and Brown 1967), body temperature (Martin 1903; Dawson and Hulbert 1970) and creatinine excretion (Mitchell 1962).

Because few calorimetric studies have been made of potoroine marsupials, FHP and MHP were measured in *A. rufescens*, *P. tridactylus* and *B. penicillata* in a series of experiments as follows:

11.1.2 Materials and methods

Measurements of oxygen consumption by captive animals

Measurements were made at 23-25°C on individual, mature animals which were acclimatised to the respiration chambers for at least three days. Three closed-circuit respirometry chambers, based on indirect measurement of heat production, were used for all calorimetric measurements of captive animals. The construction of the chambers is shown in Figure 11.1.1. Their design and operation were described in detail by Farrell (1972) with modifications (Pym and Farrell 1977). The glass chambers were sealed in a galvanized iron trough containing water. The system consisted of a pump (Thomas 107-CD; capacty 280 $ml.s^{-1}$), which circulated air through a flask with an excess of potassium hydroxide (ca $250g.l^{-1}$) to remove carbon dioxide, and through a cylinder of silica gel (to remove water), before returning the air back to the chamber. The drop in chamber pressure, through removal of carbon dioxide, was registered by a manometer. The manometer contained sodium bicarbonate and functioned as an electrolyte switch. It was adjusted so that a drop in chamber pressure (ca 6 mm electrolyte solution) caused activation of a solenoid valve; this released about 0.5 g of oxygen into the chamber. At the start and finish of each run, the oxygen cylinder was weighed, the wet- and dry-bulb chamber temperatures were taken, the barometric pressure was recorded, and the chamber air was sampled for later analysis of oxygen and carbon dioxide. All temperature and pressure values were converted to standard temperature and pressure (STP). Shortly after the measurement period, the carbon dioxide trapped by the potassium hydroxide was precipitated as barium carbonate, after the excess hydroxyl ions were removed with ammonium ions (Chapter 4). Heat production (kJ) was calculated according to Brouwer's formula (Blaxter 1965), without correction for methane or urinary nitrogen.

$$HP = (16.175 \times O_2) + (5.022 \times CO_2)$$

where O_2 = oxygen consumed (L) and CO_2 = carbon dioxide expired (L).

All measurements were made on individual, mature animals confined to standard metabolism cages (Fig 4.1) within the chamber. This facilitated the accurate measurement of feed intake, and separated urine and faeces. Nesting boxes were provided within each cage. The temperatures of the chambers were kept between 23 and 25°C. This range is within the thermoneutral zone (20 to 30°C) reported for *P. tridactylus* (Hudson and Dawson 1975; Nicol 1976) and at the lower end of that determined (25-35°C) for *A. rufescens* Rübsamen *et al.* (1983). To my knowledge there are no published calorimetric studies of *B. penicillata*. Therefore, its thermoneutral

zone was assumed to be similar to those of *A. rufescens* and *P. tridactylus*. Illumination was between 0630 and 2030 h.

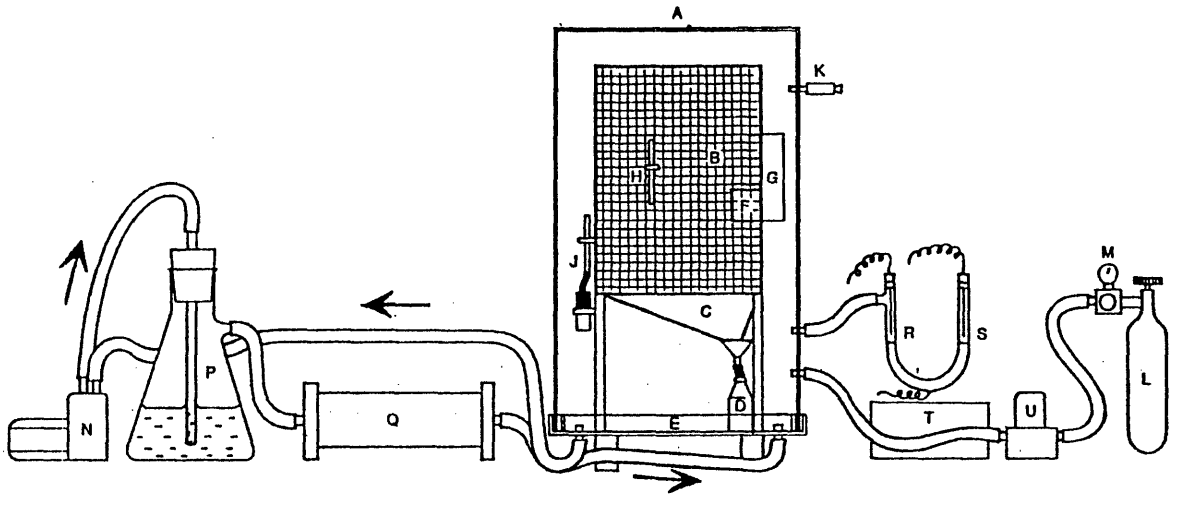

Figure 11.1.1 Diagrammatic representation of a closed-circuit respirometer used for the measurement of heat production of potoroine marsupials (redrawn from Foley 1985)

A Glass chamber
B Wire metabolism cage (Fig 4.1)
C Faeces and urine collection tray (Fig 4.1)
D Urine collection bottle
E Trough containing water
F Water/feed container
H One of three dry-bulb mercury thermometers
J Wet-bulb mercury thermometer
K Gas-sample outlet
L Oxygen cylinder
M Oxygen regulator and pressure-reducing valve
N Pump
P CO_2 trap containing KOH solution
Q Drying train containing silica gel
R Glass manometer containing $NaHCO_3$
S Platinum contact in manometer open arm
T Relay system
U Solenoid valve

Measurements of fasting heat production

FHP was measured in two series of experiments. In each, animals were placed in the respiration chambers three days before measurements started. During this adaptation period, the chambers were opened daily to remove urine and faeces and to replenish food and water, which were available *ad libitum*; during the measurement period, only water was available. In both, the duration of fasting was calculated by assuming that the animals stopped feeding at 0200 h.

In the first series, three animals of each species were used. Measurements lasted 24 h and were repeated two weeks later. Animals were fasted for 30 h before each run which started at 0800 h. Body mass was determined before and after the run, the mean value being used for all calculations.

In the second series, six animals from each species were used in four experimental runs, each lasting 12 h. The animals were not removed from the chambers between runs. Thus, body mass was determined before the first run and after the last. It was assumed that the loss of body mass was linear over time. At 0800 h the first FHP run started and the second at 0800 h next day. The third, a night-time run, followed immediately after the second — at 2000 h. The fourth came immediately after that — at 0800 h on the third day. On the assumption that each animal stopped feeding at 0200 h, at the start of the first run the animals had fasted for 6 h, at the second for 30 h, at the third for 42 h and the fourth for 54 h.

Measurements of heat production by fed animals

In calorimetric measurements lasting four days, seven non-reproductive female *A. rufescens*, three male *P. tridactylus* and three male *B. penicillata* were used. They were offered the basal ration (Table 4.1) and water *ad libitum*. The following procedures were used for each measurement. Between 1500 h and 1700 h on Day One the animals were placed in the chambers. The pumps were turned on to circulate air through the chambers, but the carbon dioxide and water traps were left disconnected. On Days Two and Three, the chambers were reopened to remove faeces and urine and to replenish the food. Measurements began on Day Four. The chambers were opened at 0700 h and thoroughly cleaned. Known amounts of food and water were placed in the appropriate containers and the carbon dioxide and water traps were put in place. At about 0800 h, the chamber was sealed and the measurement started. This procedure was repeated for another three days. On Day Eight the faeces and urine were collected, but the food was not replenished. The animals were starved until 0800 h on Day Nine, when a 24 h measurement of FHP began. The animals were weighed at the start and finish of both the feeding and fasting measurements.

11.1.3 Results

Fasting heat production

The body mass, FHP and RQ of the three species measured over 24 h are shown in Table 11.1.1. The RQ was similar in all species (O.75-0.80). FHP, expressed both per kg body mass and per kg metabolic body mass, differed significantly ($P<0.001$) between species: *A. rufescens*, all females, had a substantially lower FHP ($P<0.001$) than the other two species. Values were similar to those reported for mature eutherian animals (Brody 1945; Kleiber 1961).

The night-time activity of *P. tridactylus* and *B. penicillata* increased their FHP by 98% and 96% respectively; the corresponding increase for *A. rufescens* was only 25% (Table 11.1.2). At the end of the fourth run — that is, after 60 hours' fasting, the body mass of *A. rufescens* was 95% of its starting mass; that of *P. tridactylus* and *B. penicillata* was 92%.

Analysis of variance shows the main effects and interaction (Table 11.1.2). The starvation period affected the RQ of all species significantly ($P<0.001$). Only in *B. penicillata*, however, was there a significant effect ($P<0.05$) of starvation duration on FHP. In this species FHP was reduced by about 11% between 6 and 30 h of fasting. The lowest mean FHP for each of the three species was 11-20% less than the interspecific value of 295 $kJ.W^{-0.75}$ for basal metabolism of mature, non-reproductive eutherian animals (Kleiber 1961). *Aepyprymnus* had the lowest mean daytime FHP. In the 12 h run that started 6 h after fasting, *A. rufescens*, *P. tridactylus* and *B. penicillata* all showed lower metabolic rates than the mean value for eutherians. These were *A. rufescens* (about 8% lower), *P. tridactylus* (18%) and *B. penicillata* (3%).

Maintenance energy requirement

In the calorimetric measurements of fed animals the species showed no differences in digestible energy (DE) or metabolisable energy (ME) of the diet. Mean DE was 13.68 ± 0.147 (sem) $MJ.kg^{-1}$ DM. and ME was 12.98 ± 0.170 (sem) $MJ.kg^{-1}$ DM. Also, they showed few differences with respect to the other parameters (Table 11.1.3). Nevertheless, *P. tridactylus* and *B. penicillata* produced significantly more heat per kg body mass and per metabolic mass than did *A. rufescens* ($P<0.001$). There was a tendency, albeit non-significant ($0.05<P<0.10$), towards higher ME intakes by *P. tridactylus* and *B. penicillata* relative to those of *A. rufescens*. These ME intakes by *P. tridactylus* and *B. penicillata* balanced their higher heat production. Therefore, there were no interspecific differences in energy balance.

Table 11.1.1 Calorimetric measurements made over 24 hours on three species of potoroine marsupials fasted for 30 hours prior to the start of the run

	Aepyprymnus		*Potorous*		*Bettongia*			significance	
	Run 1	**Run 2**	**Run 1**	**Run 2**	**Run 1**	**Run2**	**sed**	**Species**	**Run**
Body mass	3.0 ± 0.23	3.2 ± 0.28	0.93 ± 0.086	0.87 ± 0.056	1.03 ± 0.027	1.00 ± 0.027			
number	**3**	**3**	**3**	**3**	**3**	**3**			
Heat production									
(kJ.kg^{-1}.d^{-1})	229	233	450	440	374	346	9.0	***	ns
(kJ.kg$^{-0.75}$.d^{-1})	301	310	440	426	377	347	10.8	***	ns
RQ	0.75	0.77	0.75	0.79	0.77	0.80	0.019	ns	ns

Table 11.1.2 Twelve hour calorimetric measurements made during the day and night (n=6) on three species of potoroine marsupials fasted for 30 hours prior to the sta of the run

	Aepyprymnus				*Potorous*				*Bettongia*					significance		
	Hours of starvation [a]				Hours of starvation				Hours of starvation				sed	spp	t[b]	spp x t
	6	30	42	54	6	30	42	54	6	30	42	54				
	D[c]	D	N	D	D	D	N	D	D	D	N	D				
Body mass	3000	2920	2870	2870	950	950	890	870	1070	1.04	1.01	0.99				
(sem)	160	160	160	180	30	30	30	30	30	0.03	0.03	0.03				
Heat production																
($kJ.kg^{-1}.d^{-1}$)	205	202	253	205	245	250	494	264	279	247	461	238	19.4	***	***	***
($kJ.kg^{-0.75}.d^{-1}$)	270	264	328	263	241	244	477	255	283	250	462	236	16.3	***	***	***
RQ	0.91	0.79	0.83	0.82	0.94	0.84	0.77	0.80	0.98	0.81	0.78	0.85	0.025	ns	**	***

[a] estimated hours of starvation at the start of the run.
[b] **t** time; **spp** - species.
[c] **D** - daytime measurement; **N** - nighttime measurement.

Table 11.1.3 Mean calorimetric data of fed A. rufescens, P. tridactylus and B. penicillata.

	Aepyprymnus	*Potorous*	*Bettongia*	ems*	sig
number	7	4	3		
Body mass (g) (sem)	2741 (93.3)	998 (66.6)	133 (31.5)		
Dry matter intake ($g.kg^{-0.75}.d^{-1}$)	30.4	31.4	37.6	63.78	ns
MEI ($kJ.kg^{-0.75}.d^{-1}$)	394	403	553	10.1	ns
DE (%)	77.7	76.4	76.6	7.51	ns
ME (%)	73.1	72.7	73.8	11.63	ns
Heat production ($MJ.kg^{-0.75}.d^{-1}$)	354	463	516	7.0	***
Energy balance ($kJ.kg^{-0.75}.d^{-1}$)	35	-65	46	9.2	ns
Heat increment (%)	21	15	26	0.03	ns
RQ	1.016	0.950	0.979	0.0052	ns
N balance ($g.kg^{-0.75}.d^{-1}$)	0.01	0.14	0.15	0.033	ns

* - error mean square

The relationship between ME intake (X, $kJ.kg^{-0.75}.d^{-1}$) and energy balance (EB, Y, $kJ.kg^{-0.75}.d^{-1}$) for seven female *A. rufescens* was:

$$Y = -278 + 0.805X,\ r^2 = 98\%,\ s = 21.1,\ (P<0.001),\ n = 14.$$

for the four male *P. tridactylus* was:

$$Y = -402 + 0.849X,\ r^2 = 97\%,\ s = 36.0,\ (P<0.001),\ n = 8$$

and for the three male *B. penicillata* was:

$$Y = -370 + 0.749X,\ r^2 = 97\%,\ s = 16.0,\ (P<0.001),\ n = 6$$

It can be calculated from the regression equations that maintenance energy (m, $kJ.kg^{-0.75}.d^{-1}$) was 345 for *A. rufescens*, 479 for *P. tridactylus* and 494 for *B. penicillata*. Corresponding values for the net-availability of ME (NAME) were 0.81, 0.85, and 0.75. These data include one measurement of FHP per animal within species. Measurements on fed animals were either marginally below or above EB, as expected in studies with

animals (Table 11.1.3). Heat increment, calculated as the increase in heat production of fed animals above their corresponding FHP value and expressed as a percentage of their ME intake, was (x ± sem) 21.3 ± 2.72; 15.1 ± 5.35; 26.0 ± 4.38 for *A. rufescens*, *P. tridactylus* and *B. penicillata* respectively. These are similar to the corresponding estimates derived by subtracting the NAME values (%) from 100 — that is 19, 15 and 25, respectively.

The RQ values in fed animals usually approached unity in all three species.

11.1.4 Discussion

Fasting heat production

There is much disagreement about the use of a single equation (Kleiber 1932, 1961; Brody *et al.* 1934) to describe the relationship between basal metabolic rate and body size of mature animals (Hayssen and Lacy 1985). The results from the three potoroine marsupials used in the present study confirm the earlier observations of Dawson and Hulbert (1970) and MacMillen and Nelson (1969) that the basal heat production of marsupials is substantially less than would be predicted from standard equations for eutherians. The present results indicate that the level of FHP in potoroines is 11-20% lower than that of eutherians. To some degree, this contrasts with earlier studies which indicate that the depression in basal metabolism is about 30%. How do we explain this apparent difference?

The measurement of FHP must satisfy four criteria:

1. *The animal must be mature and non-growing.* The animals used in the measurements of FHP had all attained adult body-mass, were sexually mature, and had stable body-masses for several weeks before the study.

2. *The animal must be in its thermoneutral zone (TNZ).* Measurements of FHP in *A. rufescens* and *P. tridactylus* were made at temperatures close to the TNZ reported by Rübsamen *et al.* (1983), Hudson and Dawson (1975) and Nicol (1976). It was assumed that the TNZ of *B. penicillata* is similar to those of the other species.

3. *The animal must be in a post-absorptive state.* The animals produced few faeces during the measurement of FHP. Only in *B. penicillata* did daytime heat production decrease (ca 15%) with starvation. In all three species, however, the respiratory quotient dropped from a value near unity in fed animals to about 0.8 in animals starved for 30-54 h. This showed that animals reached a post-absorptive state after about 24 h and, from this time onwards, were metabolising mainly protein and fat. Less than 1% of the mass of the carcass of *Macropus giganteus* and *M. rufus* is fat (Hopwood and

Griffiths 1984). Presumably, fat reserves are small also in potoroines; thus protein was probably the major substrate catabolized.

4. *The animals must be at rest.* The animals were very still during the light phase but extremely active during the dark phase (Table 11.1.2). Therefore, the value for basal heat production was that measured during the light phase. Nevertheless, it is difficult to assess activity. In preliminary experiments in which an event recorder monitored oxygen release into the chamber (in effect oxygen consumption), the time between events during the light phase varied by up to 20%. Simultaneous visual assessment of activity showed that the animals were always very still.

It is probable that the higher FHP values in the present study compared to those of earlier studies are explained by the different techniques. Both Hudson and Dawson (1975), and Nicol (1976) used open-circuit calorimetry systems to measure continuously the consumption of oxygen; they did not measure carbon dioxide production. Because an animal's activity is strongly correlated with its oxygen consumption, open-circuit calorimetry techniques have the advantage of giving instantaneous measurements of oxygen consumption. Thus, the "true" minimum can be obtained. However, the practical value of this true FHP is questionable. On the other hand, FHP measured over 12 h might better reflect that of the free-living animal during the resting phase.

It is clear that, for nocturnal species, measurements of FHP must be made during the day. Their night-time activity almost doubled the heat production of *P. tridactylus* and *B. penicillata*. The much larger *A. rufescens* showed a night-time increase of only 25%. This may be related to the confined space of the metabolism cage which restricted the activity of *Aepyprymnus* more than it did that of the smaller potoroines. A difference in activity is the most plausible explanation for the lower maintenance requirement of *A. rufescens*, compared with those of *P. tridactylus* and *B. penicillata*.

The calorimetric measurement made after 6 h of fasting is probably the best estimate of the resting-energy metabolism of potoroines. This is because it simulates the natural behaviour of both captive and free-living potoroines — that is, daytime fasting (Chapter 4). Measurements made during the feeding period are confounded by the higher night-time activity increments.

Measurements of HP made during the day, after a night in which food was available *ad libitum*, show that the metabolic rates of *A. rufescens*, *P. tridactylus* and *B. penicillata* were respectively 8, 18 and 3% lower than the mean FMR of eutherians. By comparison, the corresponding metabolic rates of *Thylogale thetis*, *Macropus parma* and *M. eugenii* exceeded the eutherian value by 25, 20 and 1% respectively (White *et*

al. 1988). These values for macropodids suggest HI of 80, 70 and 30% for the three species. This is unlikely. More plausible is the suggestion that the macropodids were more active when fed than when starved. In contrast, both fed and fasted potoroines remain very still during the day; thus, any increase in HP over fasted values is due largely to HI.

There are numerous examples of deviations in fasting metabolic rates from both the eutherian and metatherian means. This tends to contradict the conclusions of Tyndale-Biscoe (1973) that a low FHP is a phylogenetic trait. Nevertheless, it is widely accepted that metatherians usually have lower rates of fasting metabolism than do eutherians. Although McNab (1978, 1986) contended that FHP was primarily related to food habits and that climate has a moderating effect, terrestrial herbivorous marsupials — for example, macropodoids — have lower rates of FHP than do their eutherian counterparts. The comparison between the two mammalian groups is made more confusing because studies such as Kleiber's and Brody's are based largely on domestic species. These species have been selected for high productivity and would be expected to have high rates of metabolism. It is generally accepted that the two major contributors to FHP are the energy needed for the maintenance of membrane transport functions and that for protein synthesis (Buttery and Boorman 1976). If the rates of all metabolic processes contributing to FHP are equally depressed in metatherians, it might be expected that protein turnover would be 30% lower. However, Harris *et al.* (1985) showed that protein-synthesis rates are almost identical in *Trichosurus vulpecula* and rabbits. Surprisingly, their protein-synthesis rate was well below those for six eutherians (Waterlow 1984) and accounted for only 5% of FHP. In contrast, White *et al.* (1988) showed that the protein turnover rates in three macropodid species were 23-47% lower than Waterlow's (1984) value for eutherians. The situation is by no means clear, because the three macropodids had similar rates of FHP but markedly different maintenance nitrogen requirements, a parameter thought to be correlated with FHP. In a different approach, Setchell (1974) attempted to explain FHP in terms of thyroid function, but concluded that any differences in FHP between eutherians and metatherians were independent of thyroid function. In conclusion, there seems to be little understanding of the differences between metatherians and eutherians with respect to the metabolic processes that contribute to FHP. There is an even poorer understanding of why some species have a low FHP in the first place. Perhaps their lower FHP is an indication of evolution in an unpredictable environment.

Maintenance heat production

There do not seem to be any published long-term calorimetric measurements on macropodoid marsupials. However, the estimates of NAME for the three species in the

present study are within the range of values reported for mature, domestic, non-ruminant animals. This is not surprising because the diet was cereal-based and, apart from the oaten chaff, similar to diets normally fed to pigs and poultry. Furthermore, in potoroines fed cereal-based diets, a low pH appears to limit microbial activity in the forestomach (Chapter 9), and may protect readily fermentable substances from fermentation. The maintenance requirement of pigs (Thorbek *et al.* 1984) and laying hens (Johnson 1983) is about 440 $kJ.kg^{-0.75}.d^{-1}$. This can be compared with 340 $kJ.kg^{-0.75}.d^{-1}$ for *A. rufescens*, 476 $kJ.kg^{-0.75}.d^{-1}$ for *P. tridactylus* and 503 $kJ.kg^{-0.75}.d^{-1}$ for *B penicillata*. Thus, it would seem that maintenance-energy requirements may vary considerably among this marsupial sub-family. This variation would not necessarily be predicted from minimum FHP values, which were similar between species. FHP measurements made over 24 h correspond closely to maintenance metabolism. Therefore, the variation within potoroines is a consequence of the different levels of activity. It should be treated with caution, or verified with measurements of metabolism in free-living animals. Further caution should be exercised because of the few feeding measurements on *P. tridactylus* and *B. penicillata*, and because of the large influence of the 24h FHP values on the regression equations. On the other hand, there is close agreement between heat-increment values calculated from the regression equations or calculated as the increase in heat production of fed animals above their corresponding FHP. The maintenance-energy requirements, heat increment and net availability of metabolisable energy of *Aepyprymnus* are discussed in more detail in Section 11.2.

The RQ values for fed animals were usually greater than unity and, in some instances, as high as 1.2. There seems no reason to question these high values, because they dropped as soon as the animals were fasted; also, after 30 h of fasting, they stabilized at about 0.8 — the value expected for a starved animal that is metabolizing mainly protein tissue. Instead, the high RQ values in fed animals support the statement of Brody (1945): "...RQ does not always have the rigorous significance given it in the above consideration. Thus, cattle and other ruminants produce hugh quantities of CO_2 in the digestive tract by anaerobic bacterial fermentation and by liberation of CO_2 from bicarbonate." This latter aspect is of particular relevance to the present study. The rapid fermentation of the grain-based diets causes low forestomach pH (ca 4; Chapter 9) and probably significant release of CO_2 from bicarbonate. This extra-metabolic CO_2 cannot be distinguished from respiratory CO_2. Furthermore, its production undoubtedly fluctuates diurnally. Therefore, it is inappropriate to extrapolate to daily production rates of extra-metabolic CO_2.

The changes in RQ in fasted potoroines provides information on the way in which metabolism is regulated in this physiological state. A few hours after feeding

ended, RQ values dropped below 1.0. However, they rarely dropped below 0.80. This suggests that protein was the major substrate catabolized and that the contribution from fat was minor. This conclusion is based on the observation that both free-living and captive *A. rufescens* maintain a relatively stable body mass and probably store little fat. This is confirmed from observations of carcasses that appear very lean. Also, total body-water content is high (Chapter 10).

11.1.5 Summary

Daytime and night-time measurements of FHP were made on *A. rufescens*, *P. tridactylus* and *B. penicillata* starved for 6-54 hours. RQ was similar in all species (0.75-0.80). The night-time activity of *P. tridactylus* and *B. penicillata* doubled their FHP; that of *A. rufescens* was increased by only 25%. RQ was not affected by the time of the measurement. Starvation reduced RQ but did not affect heat production. The minimum mean FHP for each species was 11-20% lower than the mean value for eutherians.

The maintenance requirement for *A. rufescens* (340 $kJ.kg^{-0.75}.d^{-1}$) was about 25% lower than values reported normally for eutherian stock. The corresponding maintenance requirements for *P. tridactylus* and *B. penicillata* were similar to the energy needs of eutherians. The differences between potoroine species were explained by the higher activity of the smaller species. However, this hypothesis can only be tested in studies of field metabolic rates

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11.2 The energy cost to A. rufescens of raising pouch young

11.2.1 Introduction

BECAUSE of the demands it makes for extra nutrients, reproduction is a critical period for survival. How expensive is reproduction? Scientists in many disciplines have asked the question. The answer, said Harvey (1986), depends on how we measure costs (see also Loudon and Racey 1986). Many evolutionary biologists and behavioural ecologists evaluate costs in terms of demographic aspects — reproductive turnover in a population. In contrast, ecologists and physiologists tend to focus on energetic costs, which can be measured during pregnancy and lactation. These include direct costs, such as increases in metabolism and such indirect costs as losses of body reserves by the female. Calow (1979) suggested that reproductive effort, defined as the energy invested in reproduction as a proportion of energy taken in, is the most satisfactory measure of the cost of reproduction, because it expresses reproductive output in terms of nutrient input.

Metatherians and eutherians clearly utilise different strategies to partition reproductive costs. Metatherians invest minimal time and energy in gestation, and employ a prolonged lactation (Tyndale-Biscoe and Renfree 1987). Often, this reliance on lactation is assumed to be energetically more costly than an equivalent time of *in utero* development. The synthesis of milk products presumably entails a greater cost than that required for placental transport of nutrients (McNab 1978) However, it has recently been postulated (Kirsch 1977, Low 1978) that metatherian reproduction involves low initial energy investment and, if conditions prove unsuitable for continued development of the pouch young, may in fact minimize overall energy loss.

Although the comparative ecological costs of reproduction in the two major groups of mammals is the subject of continuing debate, there have been few studies of the energy costs of marsupial reproduction. Instead, within Australia, most research is still directed towards obtaining basic reproductive data (Rose 1986). This situation is changing in the Potoroinae following the recent studies of reproduction in *Bettongia gaimardi* and *P. tridactylus* by Rose (1986) and of *B. penicillata* (Green pers. comm.). However, I know of no detailed long-term calorimetric studies of reproduction in Australian marsupials. The purpose of the study reported in this section was to document energy expenditure by female *A. rufescens* at specific times during development of pouch young. Simultaneous measurements were made also of the mass

and dimensions of pouch young, milk composition and milk intake although the latter parameter is not reported in this thesis.

11.2.2 Materials and methods

Animals and diet

Calorimetric measurements were made of eight non-gestating *A. rufescens* and five *A. rufescens* at various times during their raising of pouch young. A single batch of the basal ration (Table 4.1) was made at the start of the experiment and was fed *ad libitum* throughout.

Experimental design

Because equipment was limited and the few animals could not be kept continuously in metabolism cages, the timing of calorimetric measurements was planned with the growth curve of the pouch-young *Aepyprymnus* in mind (Johnson 1978). The young *Aepyprymnus* is furred and detaches from the teat 85-90 days after birth at a body mass of about 110 g; it leaves the pouch after 113-115 days, weighing 500 g. Accordingly, plans were made for baseline calorimetric measurements of FHP and MHP when the animals were non-pregnant and non-lactating; later measurements (MHP only) were planned for when the females had unfurred young (one measurement), a young just getting fur (one measurement) and during the phase of maximum growth of the pouch young — the last 30 days of pouch life (two-three measurements). Other factors determined the number of actual measurements: three animals (009, 010, 014) failed to get pouch young in a reasonable time; two animals (112, 206) were placed in outdoor enclosures to give them a break from cage life when they had large pouch young. Unfortunately, both lost their young.

Animal husbandry and measurements

After the initial measurements, the animals were moved to individual outdoor enclosures, which already contained a male *Aepyprymnus*. The females were left undisturbed for 25 days. Their pouches were then checked for young every seven days (Chapter 4). Two weeks before they were placed in the respirometers, animals with pouch young were moved indoors to metabolism cages.

The baseline measurements of FHP and MHP were mainly those reported for the seven female *A. rufescens* in Section 11.1. The calorimetric measurements of females with pouch young followed the same procedures described in Section 11.1.2, but with one exception: Because of the possible risk to the young, FHP was not measured. Feed

intake and faecal and urinary output were measured simultaneously with gaseous exchange, so that metabolisable energy intakes (MEI) and energy balance (EB) could be determined for all measurement periods.

On four occasions (Ar011, Ar012, Ar203, Ar135), a calorimetric measurement was made on the young after it emerged from the pouch. For these measurements, the young was wrapped loosely in an open-weave hessian sack and placed in the chamber. The chamber was then sealed and heat production was determined from the accumulation of CO_2 and loss of O_2 in a six-hour period. A pump continuously circulated air in the chamber, which was maintained at 25°C.

Table 11.2.1 Details of the age and corresponding mass of pouch young carried by female A. rufescens when calorimetric measurements were made.

Animal number	**Pouch young age (days)**	**Pouch young mass (g)**
Ar009	0	0
Ar010	0	0
Ar014	0	0
Ar203	0, 46, 88, 116	0, 27, 151, 526
Ar112	0, 36, 65, 85, 98	0, 16, 52, 111, 206
Ar012	0, 13, 40, 63, 76, 89, 101, 109, 115	0, ?, 19, 46, 82, 140, 265, 365, 476
Ar206	0*, 53, 65, 80, 94, 106	0, 34, 61, 96, 190, 355
Ar135	0*, 75, 89, 102, 108, 116, 121	0 85, 150, 275, 371, 480, 590
Ar011	0, 52, 82, 97, 109, 118	0, 33, 140, 222, 390, 618
Ar013	0, 36, 65	0, ?, 53

0 - indicates a four-day determination of heat production by a fed animal and a 24 h measurement of FHP.

0* - measurement of fasting heat production only.

? - the mass of the pouch young was not measured.

Statistical

On the advice of a biometrician (Dr Ian Davies), statistical methods were used only sparingly to analyse the data from this experiment. There were two main reasons for this. First, most regression techniques to analyse such relationships as that between heat production (HP) or energy balance (EB) and pouch-young age are of little value here, because there are insufficient data points for each animal. Secondly, analysis of variance of HP and EB data, divided into various pouch-young age-classes, is confounded by repeated measures, the number of which varies between animals. Accordingly, most data for MEI, HP and EB were pooled into pouch-young age-classes and presented graphically as means and their standard errors. Although statistical procedures were used sparingly, the relationship between EB and MEI was examined for each animal by least squares regression analysis. Significant linear relationships were found for each animal. Furthermore, all regression lines had similar slopes and Y-intercepts and, therefore, the data for each animal were pooled. Two regression lines — with and without fasting values — were then calculated to describe the relationship between EB and MEI. A complete data set has been included in Appendix 3 to allow readers to make their own analyses.

Measurement of milk composition

The procedures for the removal of pouch young, measurement of the young, collection of milk and the determinations milk composition are described in detail in Chapter 4.

11.2.3 Results

Body-mass changes

Mean body mass of female *A. rufescens* remained more or less constant from the pre-partum stage through to the time that the young left the pouch (Fig 11.2.1). The corresponding growth curve for the pouch young is shown in Fig 11.2.2.

Respiratory quotient (RQ)

The RQ estimates plotted in Fig 11.2.3 were not corrected for nitrogen excretion. The RQ of the female was consistently greater than 1.0 and did not change after parturition or during the growth of the young.

Heat production (HP)

The absolute HP ($MJ.d^{-1}$) of the female *A. rufescens* did not change at parturition (Fig 11.2.4). Indeed, the combined HP of the female and her pouch young stayed

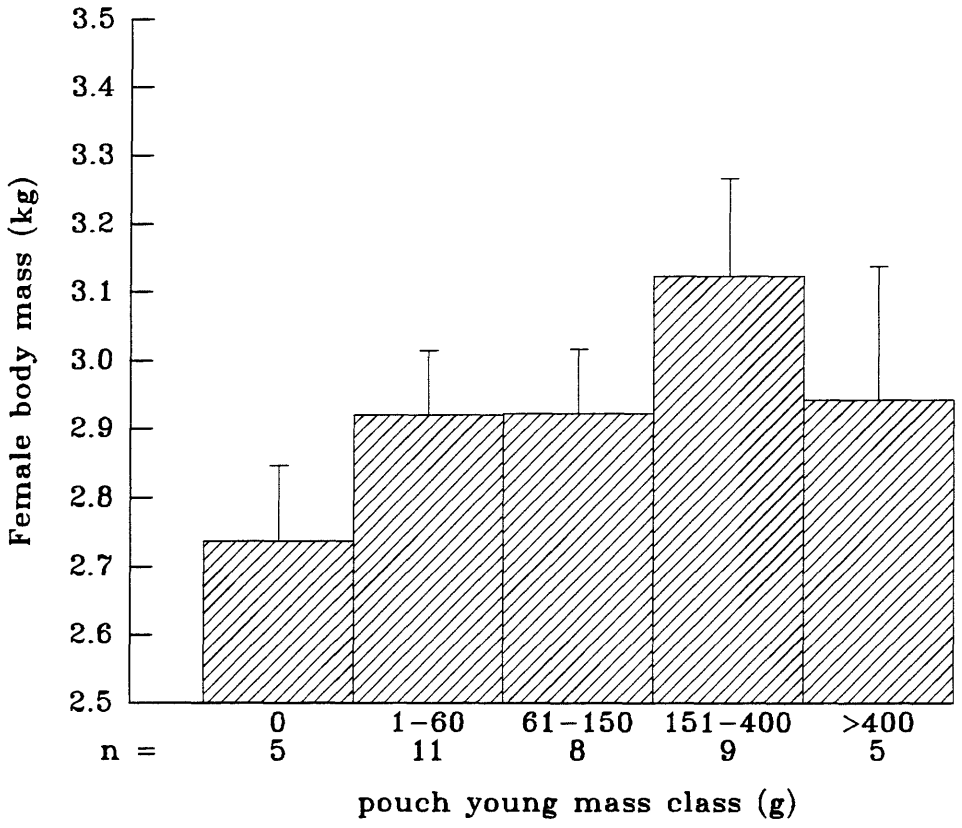


Fig 11.2.1 Changes in female body mass with the growth of pouch young

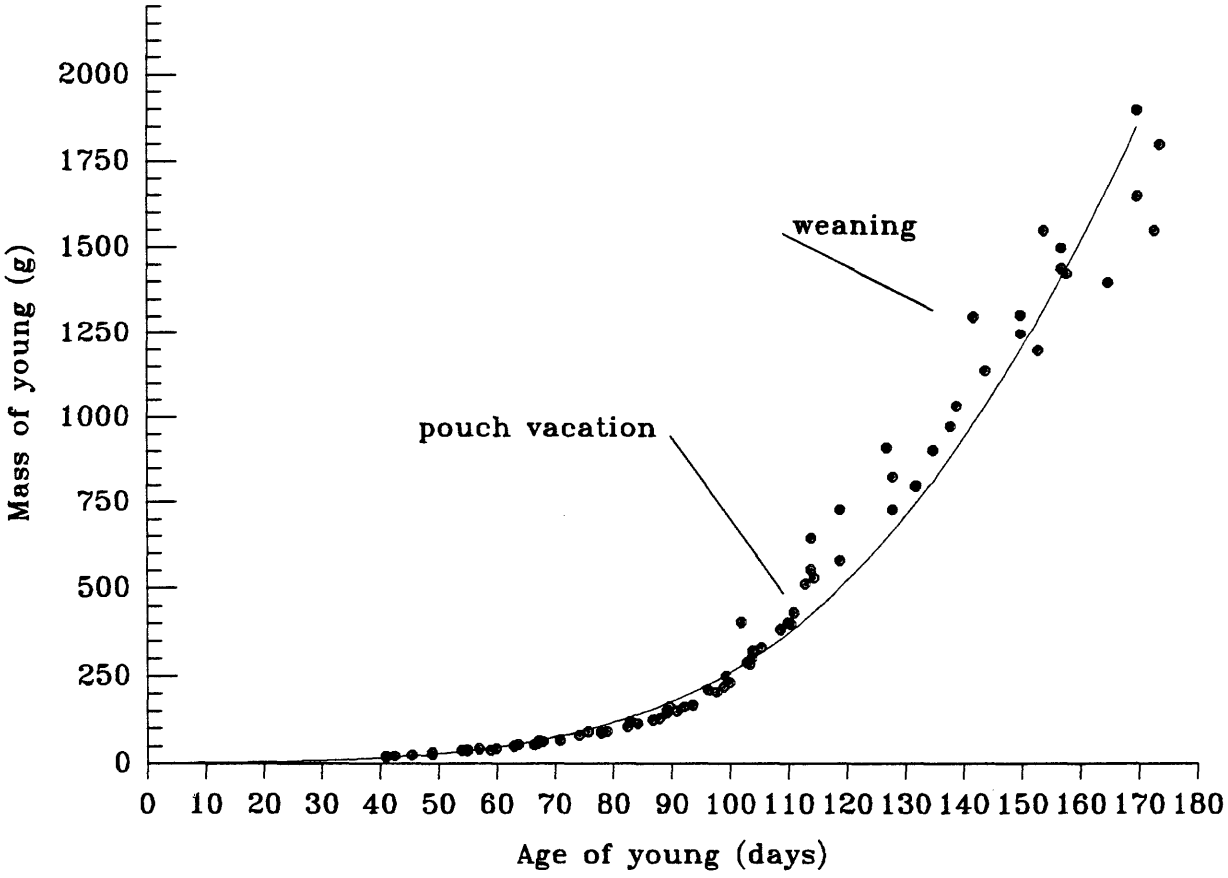


Fig 11.2.2 The growth of A. rufescens from birth until weaning

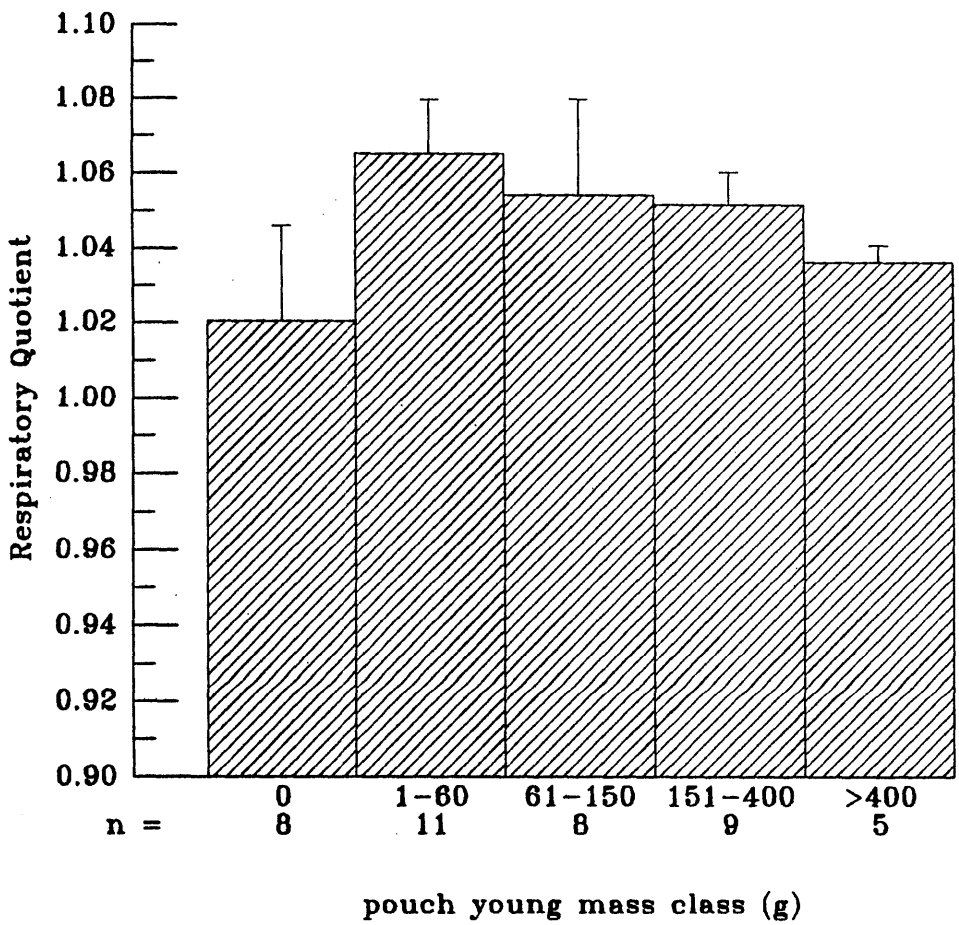


Figure 11.2.3 Respiratory quotient versus pouch-young age class

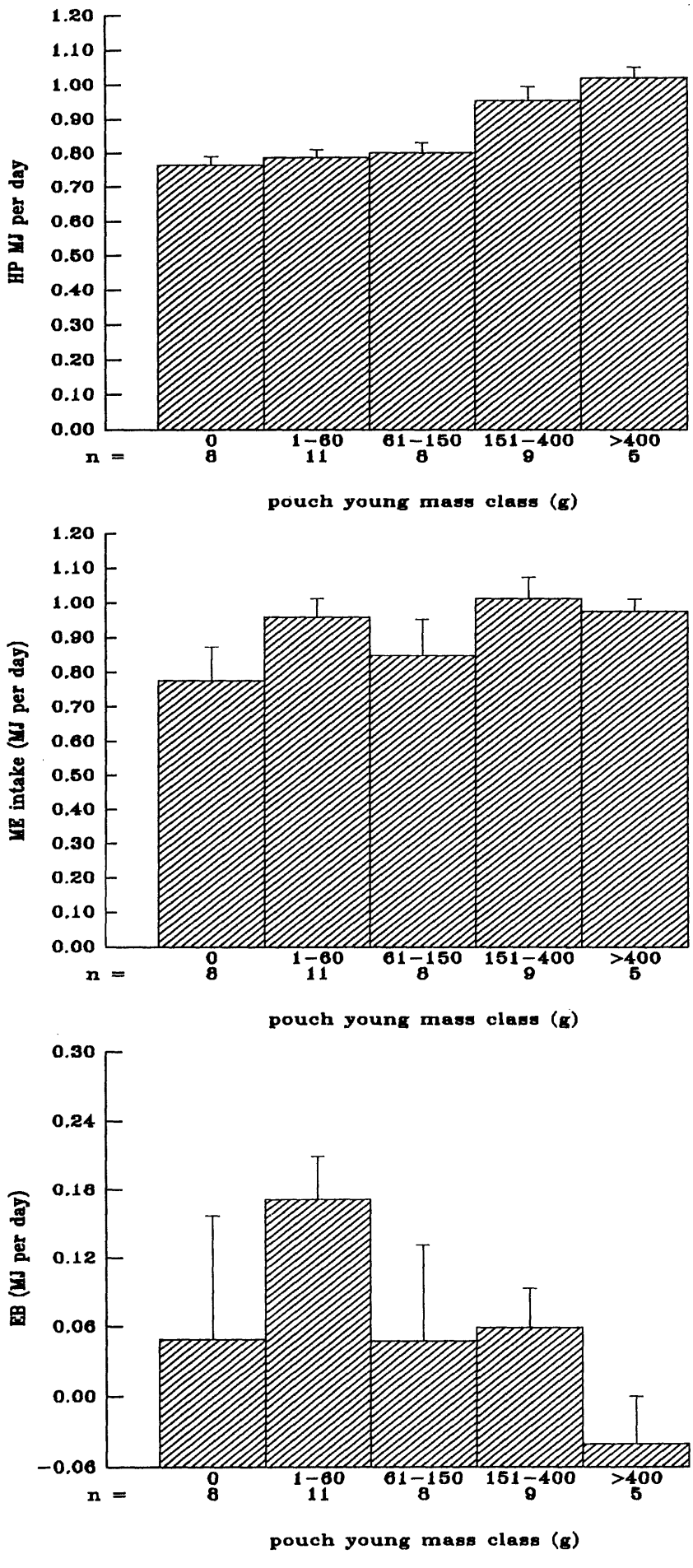


Fig 11.2.4 Changes in heat production (HP) metabolisable energy intake (MEI) and energy balance (EB) with the growth of pouch young.

relatively constant until the last third of pouch life: the time of most rapid growth of the young. During this time, when the mass of the young was between 150 and 550 grams, absolute HP rose by about 20%. A similar result was observed when the data were expressed per unit metabolic mass (Fig 11.2.5).

Metabolisable-energy intake (MEI)

Although the animals were allowed considerable time to adapt to the experimental conditions, the MEI varied widely between individuals (Fig 11.2.4, 11.2.5). This variation is mainly due to differences in food intake rather than to differences in digestibility or excretion of urinary energy. Food intake was measured both gravimetrically and by recovery of the marker, acid-insoluble ash, in the faeces. A paired t-test found no differences between the two methods; thus, the mean value was used to measure food intake. Regardless of whether MEI is expressed as $MJ.d^{-1}$ or as $MJ.kg^{-0.75}.d^{-1}$, it appears to rise as soon as a young is born and to stay high until the mass of the young is about 60g. MEI then declines until the last third of pouch life, when it again increases sharply.

Energy balance

Energy balance is the difference between MEI and HP and thus reflects the trends of these parameters (Fig 11.2.4, 11.2.5). Because MEI rises immediately after parturition, and because HP at this time is less than MEI, EB also is positive. In other words, energy is being retained as tissue and as milk. After the young reaches about 60 g and until it weighs 400 g, the mother's EB falls but remains positive. From this time on, HP exceeds MEI and the female has a slight net loss of energy.

The efficiency of utilisation of metabolisable energy

EB was regressed against MEI for all individual animals which had at least five calorimetric measurements, including one of FHP. For all these animals the relationship expressed as $MJ.d^{-1}$ or as $MJ.kg^{-0.75}.d^{-1}$ was highly significant ($P<0.001$) (Fig 11.2.6). This approach should be treated with some caution, because the FHP value has a large effect on the slope of the regression equation. Because there were no differences ($P>0.10$) between the slopes or the intercepts of the six regression lines, the data for all animals were pooled. The data were examined in two ways — with or without the FHP data. An examination of the two regression lines found no differences in their slopes or intercepts ($P>0.10$). This is true for data expressed as $MJ.d^{-1}$ (Fig 11.2.7) and as $MJ.kg^{-0.75}.d^{-1}$ (Fig 11.2.8). Thus, further discussion is based on the relationship between EB and MEI when the FHP data were included. It is interesting to note that the net availability of metabolisable energy (NAME) was about 70% when

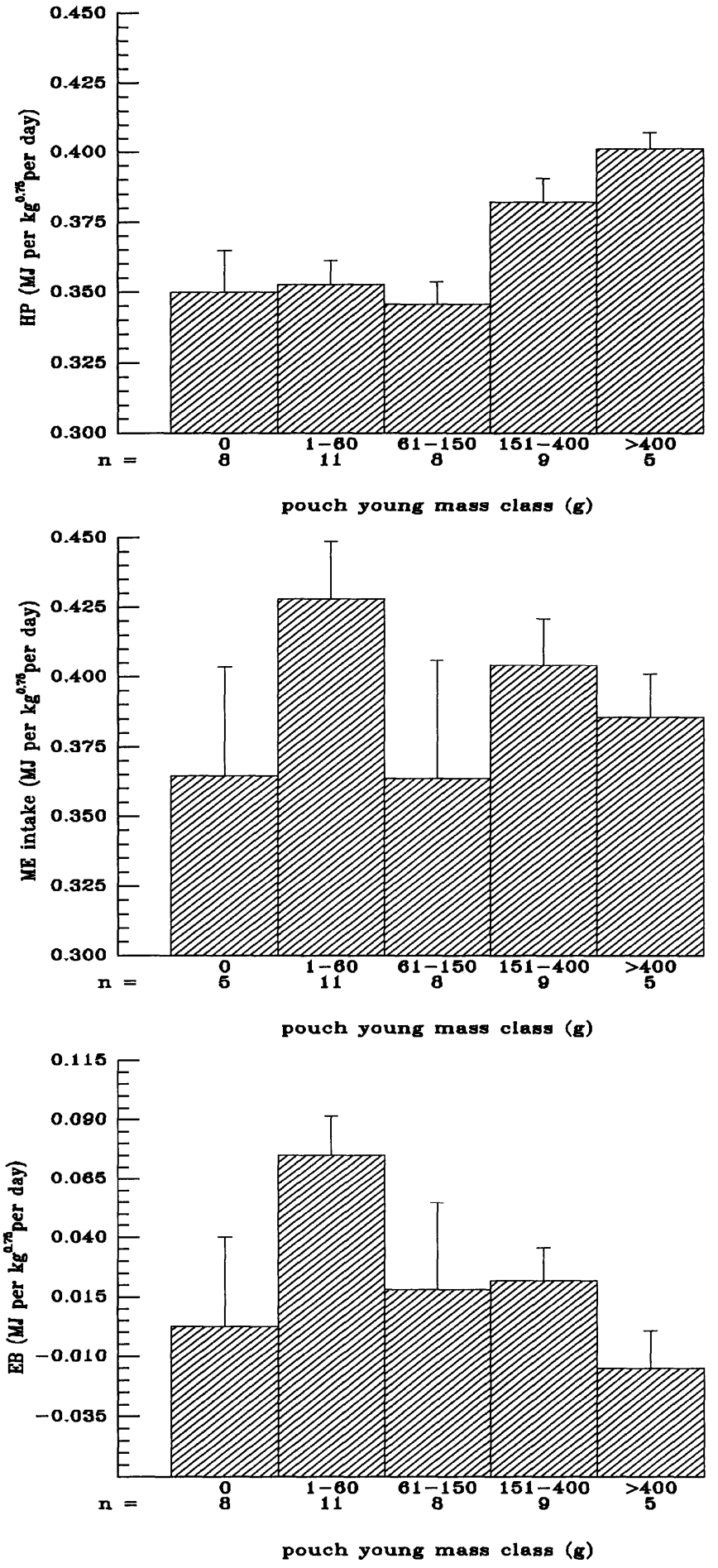
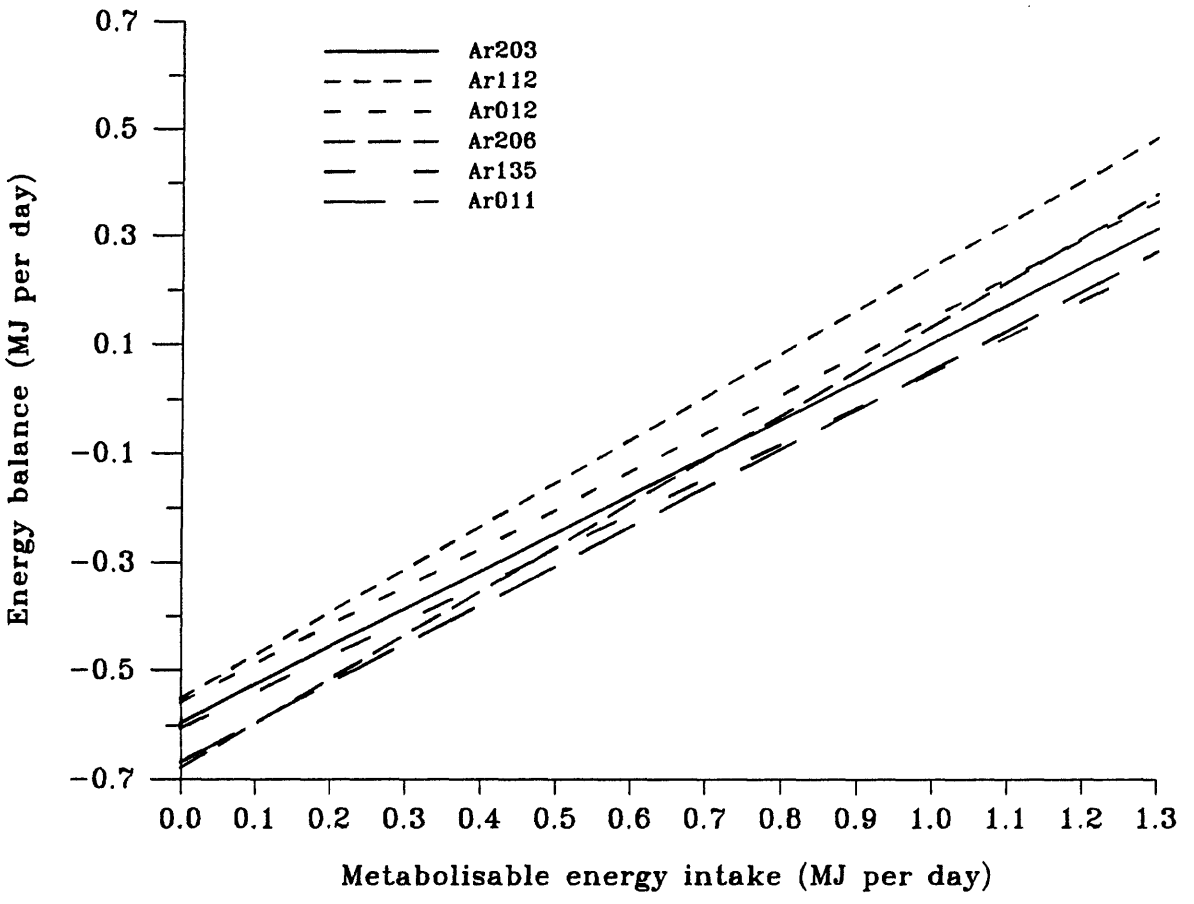


fig 11.2.5 Changes in heat production (HP) metabolisable energy intake (MEI) and energy balance (EB) with the growth of pouch young.



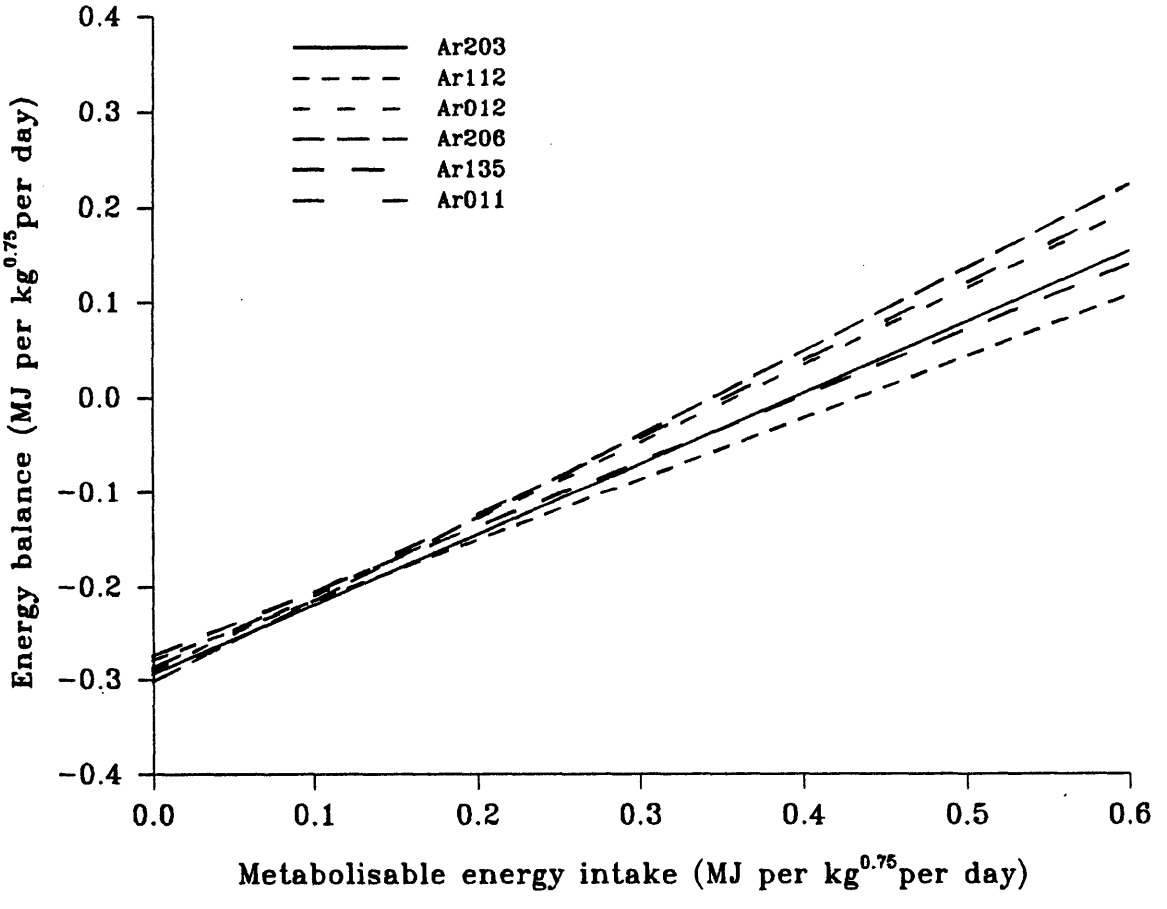
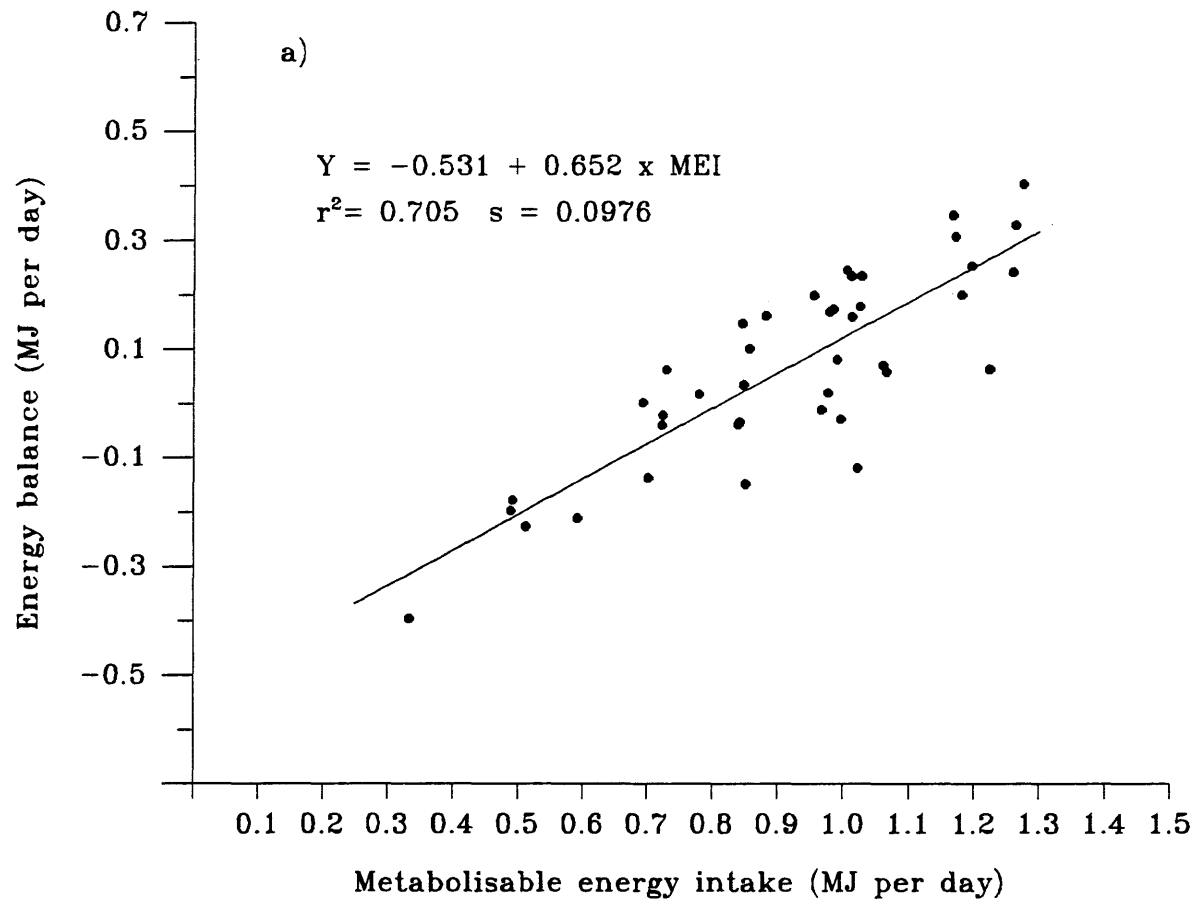


Fig 11.2.6 The relationship between energy balance and metabolisable energy intake of six female A. rufescens. The data include one measurement of fasting heat production for each animal



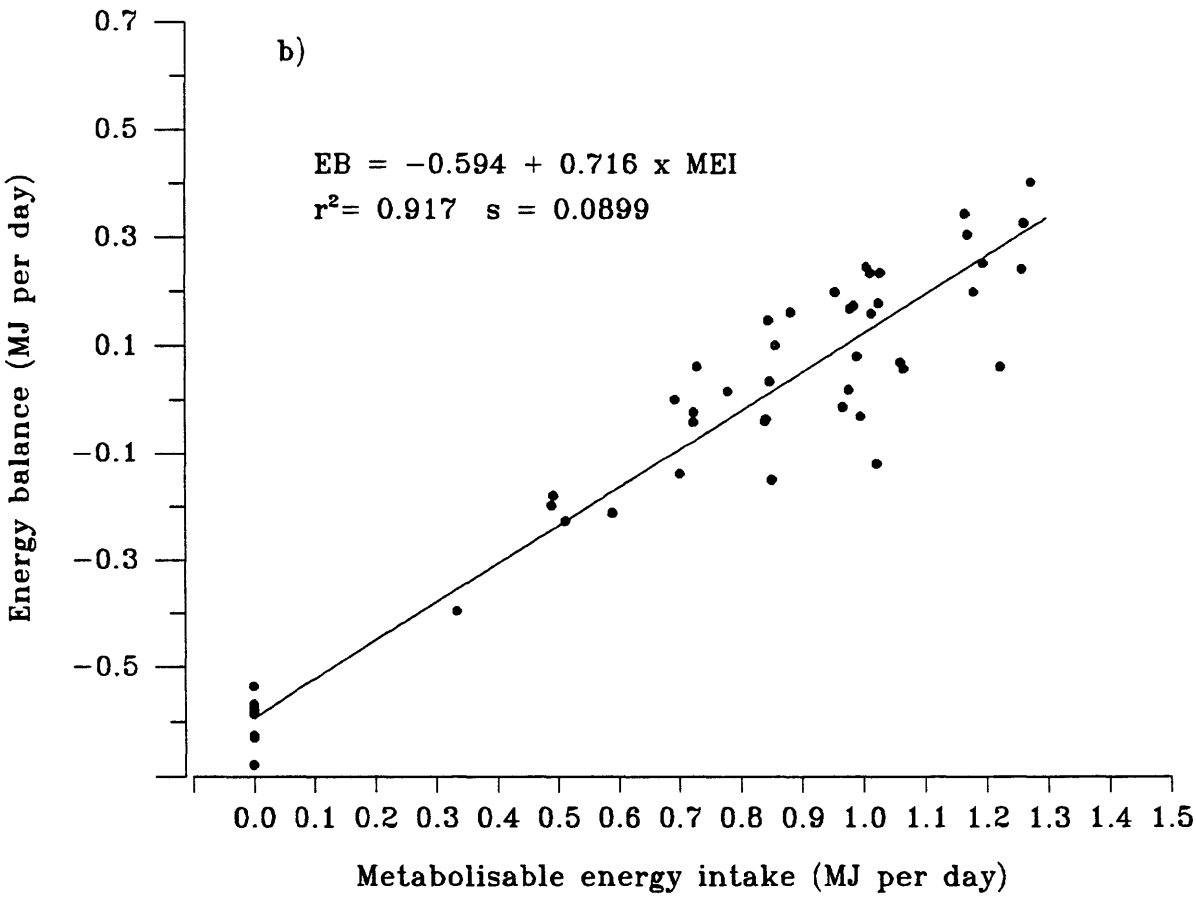
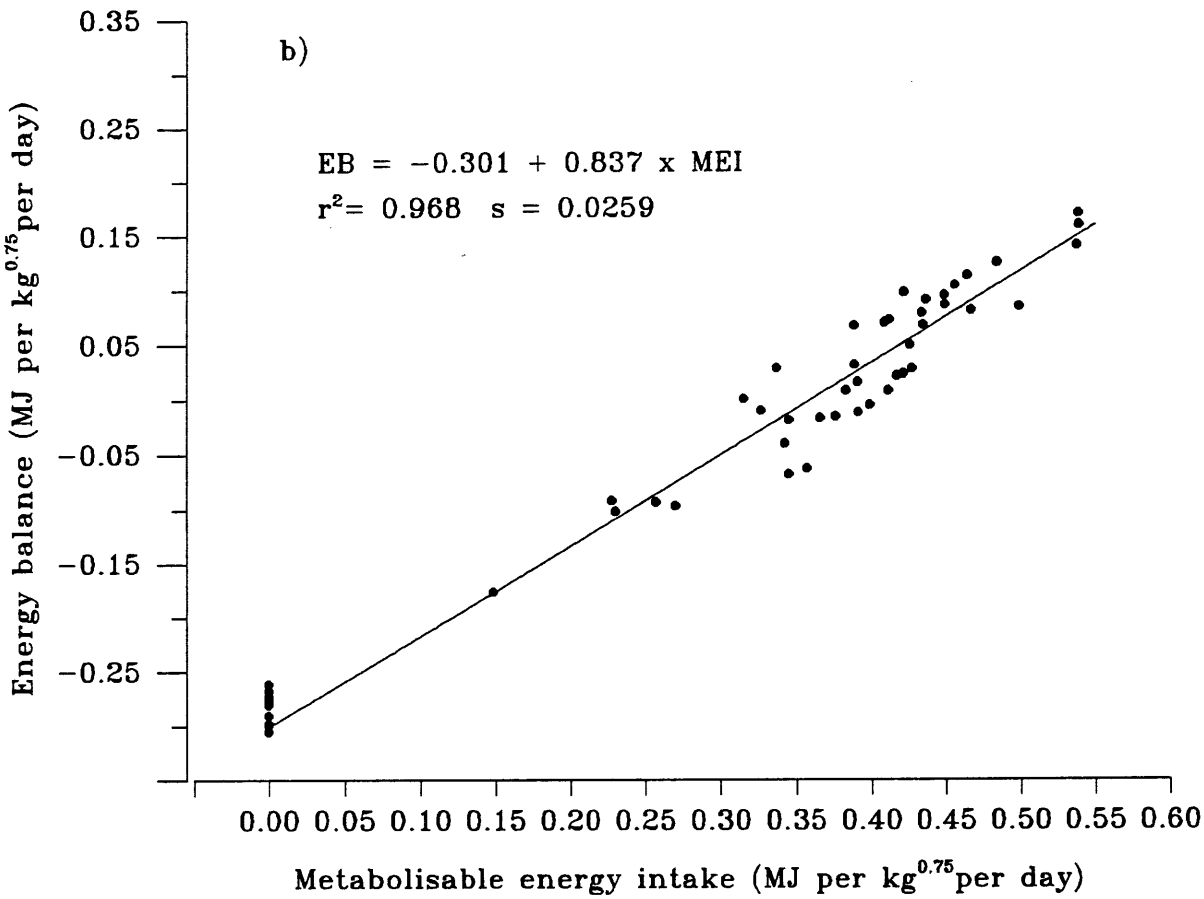


Fig 11.2.7 The energy balance of female A. rufescens compared to their intake of metabolisable energy.
a) fasting heat production data excluded.
b) fasting heat production data included.



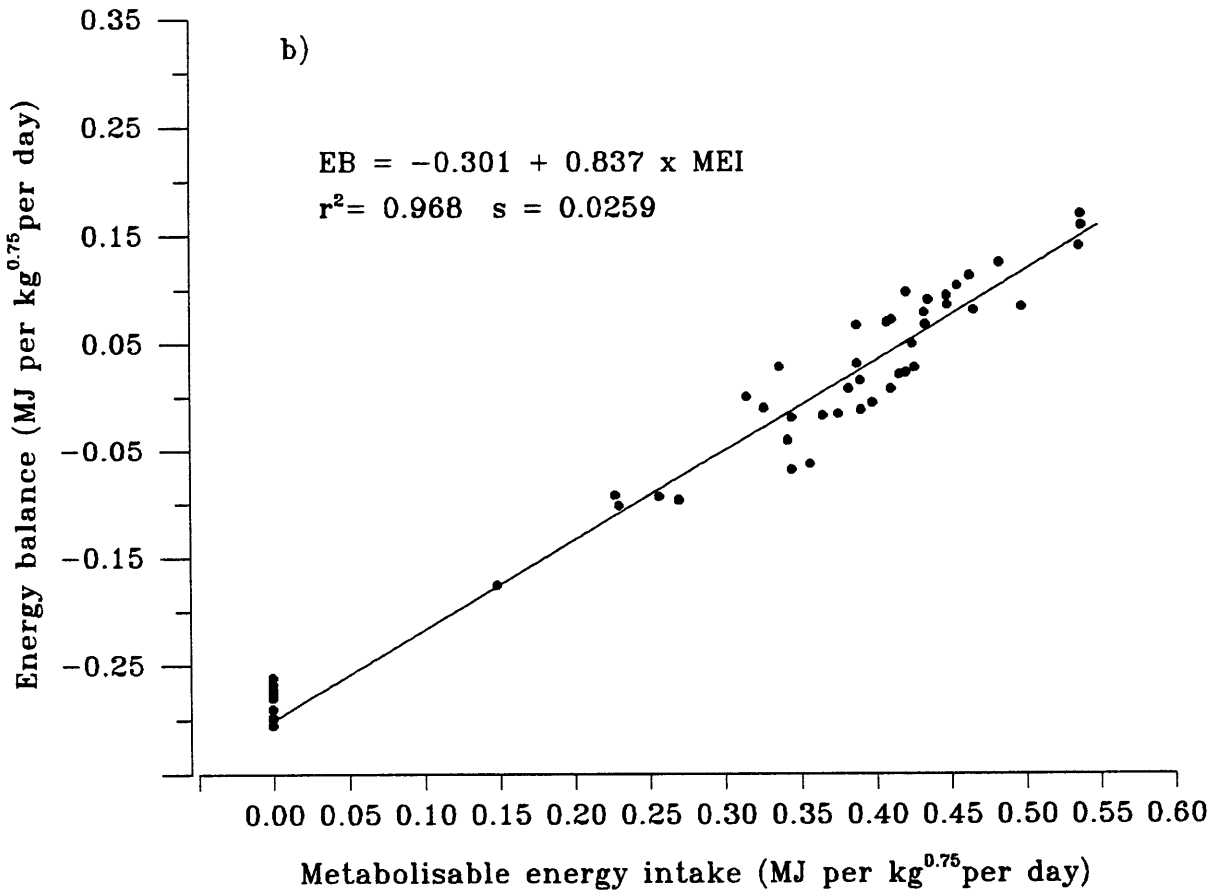


Fig 11.2.8 The energy balance of female A. rufescens compared to their intake of metabolisable energy.
a) fasting heat production data excluded.
b) fasting heat production data included.

calculated as $MJ.d^{-1}$ but more than 80% when data are transformed to units of metabolic body mass. The daily energy requirements for maintenance — the MEI at zero EB — was 0.82 $MJ.d^{-1}$ or 0.36 $MJ.kg^{-0.75}.d^{-1}$.

Nitrogen balance

There was a highly significant relationship ($P<0.001$) between nitrogen balance and nitrogen intake (Fig 11.2.9) as described by the equation:

$$Y = 0.956\ X - 0.346;\ r^2 = 80.3\%,\ s = 0.1381$$

Also, a significant relationship ($P<0.001$) was found between nitrogen balance and metabolisable energy intake (Fig 11.2.10). The regression equation was:

$$Y = 1.25\ X - 0.299;\ r^2 = 60.2,\ s = 0.0839$$

Calorimetric measurements of emergent young

The FHP of four juvenile *A. rufescens* was measured at about the time of permanent pouch vacation (Table 11.2.2)

Table 11.2.2 Calorimetric measurements of juvenile A. rufescens

	Identification of mother			
	Ar011	**Ar012**	**Ar203**	**Ar135**
Body mass of young (g)	690	518	570	613
Age of young (d)	121	118	119	124
RQ	0.65	0.64	0.70	0.67
Heat production				
($kJ.d^{-1}$)	140	191	209	162
($kJ.kg^{-0.75}.d^{-1}$)	184	313	319	268

The rates of heat production ($kJ.kg^{-0.75}.d^{-1}$) of the progeny of Ar012, Ar203 and Ar135 were, respectively, 20%, 22% and 3% higher than the mean minimum FHP of mature *A. rufescens*. However, the progeny of Ar011 produced heat at a rate 30% below the minimum FHP measured in *A. rufescens*. The RQ was similar in all animals.

Milk composition (Fig 11.2.11)

Because of the risk of losing pouch young, few milk samples were obtained during the first third of pouch life. Total solids (dry matter) represented about 23% (w/w) of milk after 40 days of lactation and gradually increased to 30% at the time of pouch vacation (115 days). Over the same period protein increased from 5 to 9% (w/v); fat increased from 3 to 12% (w/v); and carbohydrate decreased from 14 to 6% (w/v).

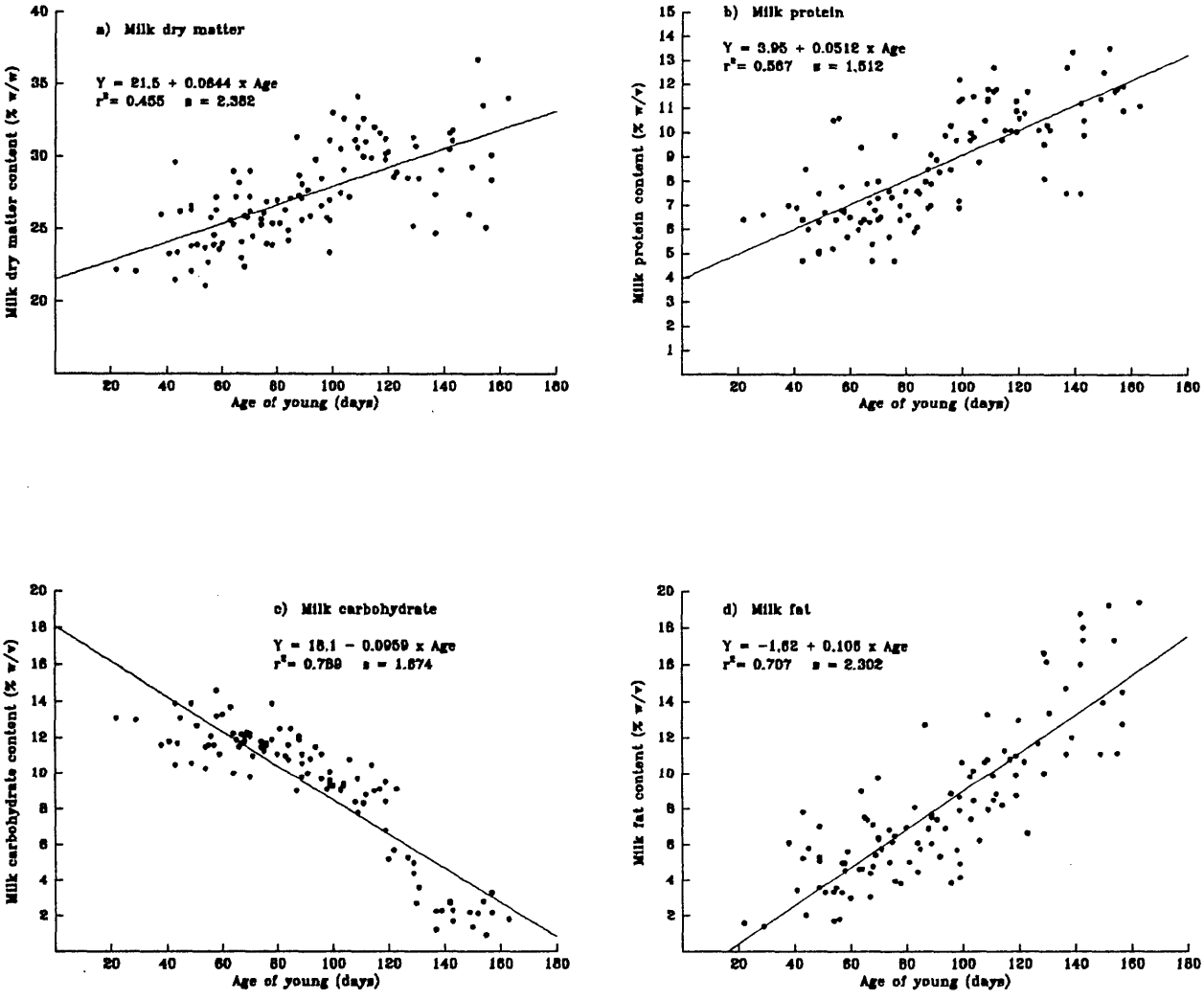


Fig 11.2.11 Milk composition in captive A. rufescens

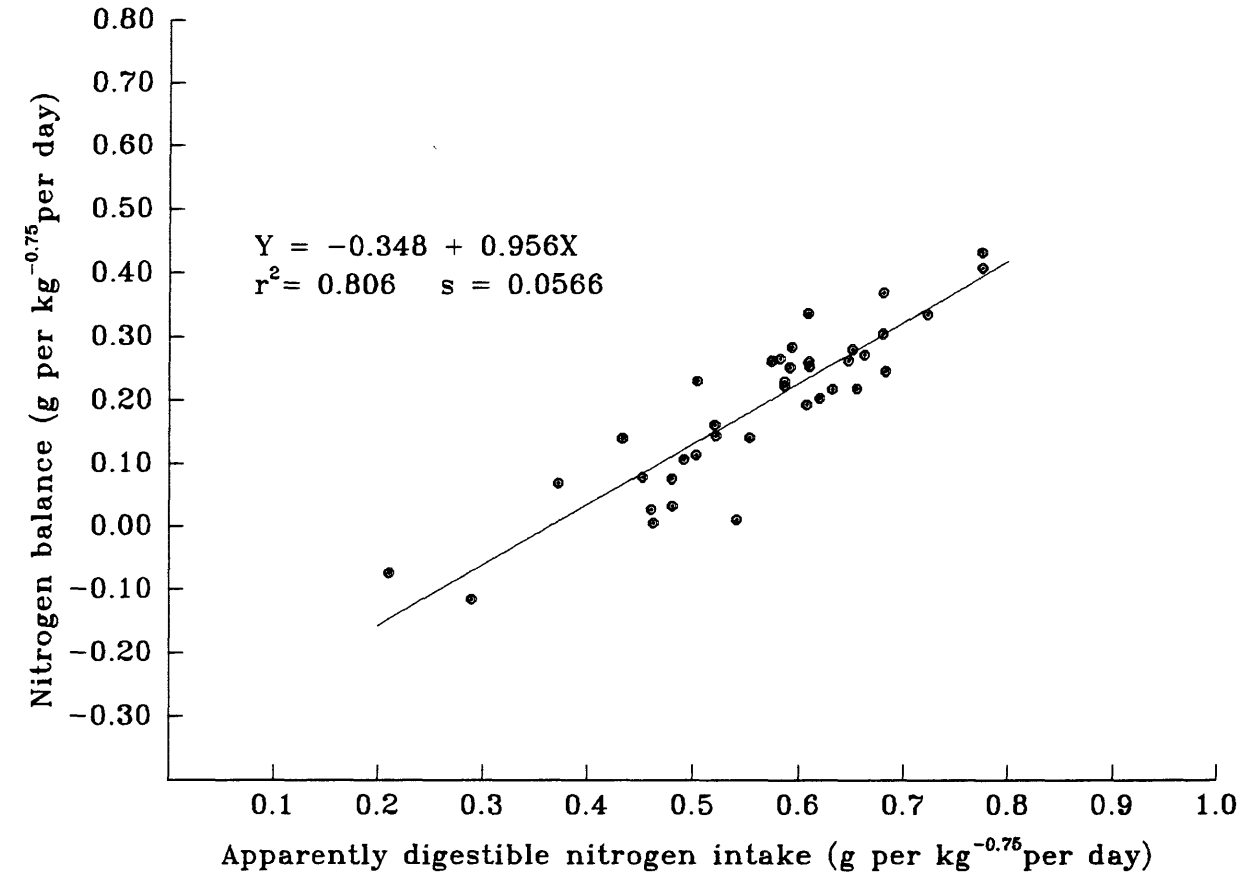


Fig 11.2.9 Relationship between nitrogen balance and digestible nitrogen intake in female A. rufescens

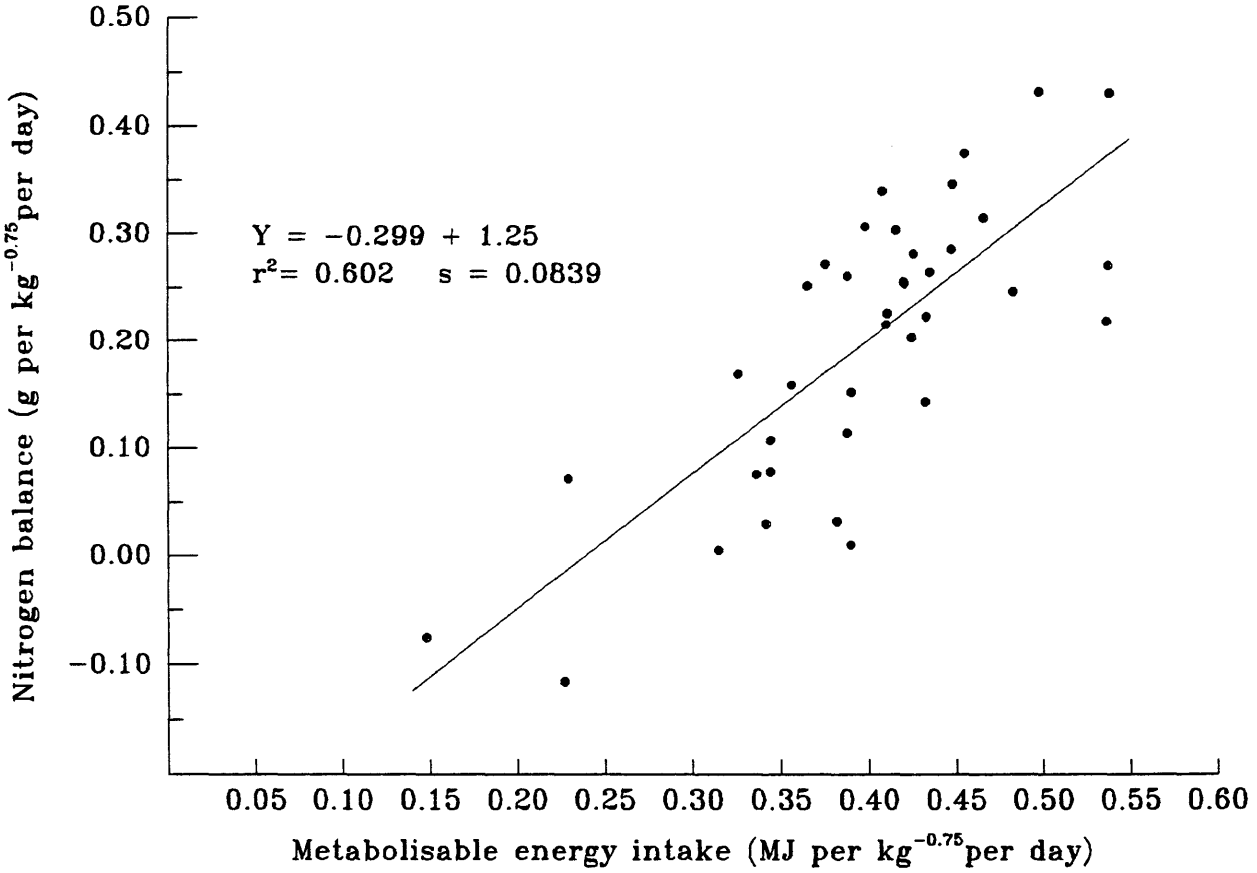


Fig 11.2.10 Relationship between nitrogen balance and metabolisable energy intake in female A. rufescens

11.2.4 Discussion

The stable body mass of female *Aepyprymnus*, from pre-pregnancy until the young left the pouch, suggests that they met the nutrient requirements of lactation mainly from an increased nutrient intake during pouch life, rather than from body tissue stored before or during gestation. A stable body mass was noted also in the free-living females during their rearing of pouch young (Section 11.3). This, together with the fact that *Aepyprymnus* breed continuously, supports the notion that *A. rufescens* is rarely exposed to nutritional deficiencies large enough to disrupt reproduction. This is explained by their lactational strategy which makes allowance for unpredictable conditions:

During the course of lactation the milk of *A. rufescens* shows a pattern of change in composition similar to that reported for other metatherians (Green 1984; Green *et al.* 1987; Crowley *et al.* 1988; Merchant and Libke 1988). The progressive increase in milk solids is associated with increases in fat and protein and a gradual decrease in milk carbohydrates. Likewise, *Aepyprymnus* milk from different stages of lactation, has levels of proximate nutrients that resemble those found in other macropodoids, including *Macropus eugenii*, *B. gaimardi* and *P. tridactylus* (Green *et al.* 1980; Crowley *et al.* 1988; Smolenski and Rose 1988); and other metatherians — for example, *Dasyurus viverrinus* (Green *et al.* 1987) and *Trichosurus vulpecula* (Gross and Bolliger 1959). Presumably, milk composition in early lactation, and milk intake throughout lactation, is similar to that in other macropodoids.

Green (1984) presented data which showed that metatherians convert milk to body mass as efficiently as do eutherians. However, eutherians grow faster and, therefore, have a shorter lactation than do metatherians. Green concluded that a "lack" of milk restricts growth in the latter group. This theory is supported by the high growth-rates recorded when pouch-young are transferred to mothers with larger mammary glands (Green 1984). Thus, most metatherians, including potoroines, have evolved a prolonged but low intensity lactation by restricting milk production. The Peramelidae (bandicoots), which are omnivorous and generally seasonal breeders, provide an exception. The milk of *Isoodon macrourus* exhibits the typical metatherian pattern. However, *Isoodon macrourus* and other bandicoots (Tyndale-Biscoe and Renfree 1987), accomplish these changes in about half the time that it takes metatherians of comparable size. It is unlikely that the more concentrated milk produced by *Isoodon macrourus* explains the difference; increased milk output is a more plausible explanation. At least some bandicoots store fat and then mobilize it during lactation (Hulbert and Gordon 1972). *Aepyprymnus* does not do this. The comparison between macropodoids and

peramelids suggests that food habits may influence lactational strategies in much the same way that they are proposed to determine metabolic rates (McNab 1986).

As expected from the foregoing discussion of body mass and lactational strategy, MEI in the present experiment rose sufficiently after parturition to support positive energy balance until the pouch young reached 400 g. The negative energy balance during the last week of pouch life also is expected. Recent evidence (Rose 1989) suggests that pouch vacation occurs when the mother can no longer tolerate the added heat burden of the young. The four measurements made of young that had just vacated the pouch indicated a heat production of about 270 $kJ.kg^{-0.75}.d^{-1}$. The mother's response to this heat burden is to eat less. It is interesting to note the apparent rise in MEI immediately after parturition which tends to offset the low MEI in the days just before pouch vacation. For two reasons caution is needed in interpreting the MEI data. First, the data are somewhat confounded by the effects of repeated measurements. Measurements made late in the development of pouch young are of females that had already spent considerable time in the chambers; those made of females without pouch young were of animals with less exposure to the chambers. Secondly, MEI was probably overestimated because no allowance was made for losses of combustible gases or of heat formed in fermentation. Methane production has been estimated as 12% of DE in *Taurotragus oryx* and *Connochaetes taurinus* fed hay and concentrates (Rogerson 1968), but less than 1% in *Macropus giganteus* and *M. eugenii* fed lucerne hay (Kempton 1976; Engelhardt *et al.* 1978). The *Aepyprymnus* in the present experiment were fed concentrates, which would tend to decrease methane production. More important, however, is the minimal activity of cellulolytic microbes in the forestomach of *Aepyprymnus* fed concentrates (Chapter 9). Thus, the losses of DE as methane energy were probably negligible.

The high RQ values in the present study, caused by the probable excretion of extra-metabolic CO_2, almost certainly led to slightly elevated estimates of heat production. As discussed in Section 11.2, it was impossible to measure the extra-metabolic CO_2 and to make the necessary correction. However, it can be calculated from the equations of Brouwer (1965) that overestimating RQ by 20% results in only a 4.5% overestimation of heat production. Because RQ values remained constant during the growth of the pouch young and because RQ was probably overestimated by less than 10% (assuming that the correct value on a high carbohydrate diet is about 1.0), it is unlikely that the data will be interpreted incorrectly. Other researchers — for example White *et al.* (1988) — also have reported RQ values in excess of 1.0. They explained these values in terms of the availability of carbohydrate for fat synthesis. Nevertheless, they did not acknowledge high RQ values in their estimation of energy expenditure;

instead they modified Brouwer's formula so that, if CO_2 output exceeded O_2 consumption, the RQ was assumed to be 1.000.

The relationship between MEI and EB was represented by a single linear function in Fig 11.2.6 and 11.2.7. In theory (Blaxter 1962; Reid *et al.* 1980; Webster 1980) the relationship should be curvilinear. Both above and below maintenance, the efficiency with which energy is retained depends on the composition of the absorbed nutrients and of the tissue that is anabolized or catabolized. Thus, fat is used more efficiently as an energy substrate than is carbohydrate which, in turn, is used more efficiently than protein. For example, among growing animals the efficiency with which MEI is used is high in those gaining a lot of fat but not much protein. Other factors also affect the efficiency of utilisation of ME. In order of efficiency are maintenance, lactation, body gain, reproduction (Reid *et al.* 1980). Fermentation end-products decrease NAME (Armstrong *et al.* 1961). Thus, monogastric species and specialized hindgut fermenters tend to have higher NAME than do foregut fermenters. Moreover, NAME decreases as the ME content of the diet decreases (Reid *et al.* 1980). In view of these factors, the high NAME in *Aepyprymnus* in the present study is to be expected for the following reasons: the diet was a concentrated source of ME; it probably caused low forestomach pH that restricted microbial growth but probably allowed normal enzymatic digestion (Chapter 9); the animals were not growing and for most measurements were lactating. Furthermore, the rapid growth of the young in the last third of pouch life corresponds with increasing milk solids, in particular fat (Fig 11.2.11). Theoretically, a high-fat milk is synthesized with high efficiency. There are perhaps insufficient data of both non-reproductive animals and those with pouch young to identify any changes in NAME as lactation proceeds. Also, there are insufficient data points between maintenance (EB = 0 $MJ.kg^{-0.75}.d^{-1}$) and the energy balance of fasting animals (ca -0.30 $MJ.kg^{-0.75}.d^{-1}$) to interpret confidently the shape of the regression line below maintenance. Nevertheless, incorporation of FHP data (Fig 11.2.7, 11.2.8) had no effect on either the slope or the intercept of the regression equation. This agrees with the findings of Blaxter and Wainman (1961) that no serious error is involved if the relationship between EB and MEI is described by straight lines — one above maintenance, the other below. Because the current data are described by a single line, the efficiency of utilisation of ME (the slope of the line) is similar both above and below maintenance. Does this indicate that the efficiency of utilisation of ME for lactation is similar to the efficiency for maintenance? The fact that the slope of the regression of EB against MEI for non-reproductive animals (Section 11.1) was unchanged by the inclusion of data for lactating animals tends to suggest similar efficiencies for both physiological states. Thus, net energy values obtained in the

present study may be compared with those published for other species for both maintenance and gain.

The estimates of net availability of metabolisable energy — about 70% when calculated as $MJ.d^{-1}$ but more than 80% when data are transformed to units of metabolic body-mass — are high for species with a foregut fermention. High values have been reported also for other foregut fermenters, especially those under maintenance conditions and those fed diets that enhance gluconeogenesis (Table 11.2.3).

Table 11.2.3 Net energy coefficients as a proportion of apparent metabolisable energy in wildlife. Adapted from Robbins (1983).

Species	**Diet**	**Metabolic process**	**Net energy coefficient (%)**
Monogastrics			
Halichoerus grypus	fish	maint.	91
Lepus timidus	browse, conc.	gain	79
Microtus sp	nuts, grain	gain	89
Sciurus carolinensis	conc.	gain	51
Ruminants			
Alces alces	browse	maint.	68
Connochaetes taurinus	hay, conc.	maint.	71
Odocoileus virginianus	browse	maint.	64
Odocoileus virginianus	browse	maint.	48
Odocoileus virginianus	pelleted diets	maint. gain	81
Taurotragus oryx	hay, conc.	maint.	73
Cervus elaphus	hay, conc.	gain	47
Connochaetes taurinus	hay, conc.	gain	59
Taurotragus oryx	hay, conc.	gain	52

conc. - concentrates; maint. - maintenence

The gaseous exchange by juvenile *A. rufescens* was measured in a rather crude manner. Nevertheless, although the RQ values were low, they were consistent, implying a reasonable level of accuracy. In his discussion of "abnormal" RQ values, Kleiber (1961) cites the work of Soskin (1941), who pointed out that the theoretical RQ for gluconeogenesis from fat ranges from 0.65 to zero, depending on the number of Beta-hydroxy-butyric-acid molecules produced. This may explain the "low" RQ values in the juvenile *A. rufescens* obtaining their nourishment from milk with a fat content of 20%. The relatively low heat production by the progeny of Ar011 has no obvious explanation. Indeed, this animal's rate of metabolism was similar to the mean basal

metabolic rate of metatherians (Dawson and Hulbert 1970). It is generally accepted, however, that the metabolic rate of a growing animal is considerably higher than that of a mature animal (Brody 1945).

At least four explanations may be given for the lower heat production by the offspring of Ar011:

1. *The value is in error.* This seems unlikely because the RQ value for this animal is similar to those of the others.

2. *The progeny of Ar011, which had permanently vacated the pouch four days before the calorimetric measurement was made had a higher mass.* The energy metabolism of the other animals was measured on the day of permanent pouch vacation. It is unknown whether metabolism changes soon after pouch vacation.

3. *Animals showed differences in activity.*

4. *The animals were starved to a different degree.* Similar RQ values make this less likely.

11.2.5 Summary

The energy expenditure by female *A. rufescens* was measured before lactation and at specific times during the development of pouch young. Simultaneous analyses of milk composition were made also. In the week preceding pouch vacation, the combined heat production of the female *A. rufescens* and her young were about 20% higher than that of the barren female. The changes in milk composition were similar to those reported in other metatherians. It was concluded that the long lactation of *A. rufescens* serves to minimize nutrient output at any one time. Therefore, the lactational strategy is ideally suited to an unpredictable environment.

11.3 The energy costs of free existence

11.3.1 Introduction

ANIMALS need energy to sustain their major life processes. These may be broadly classified as maintenance, thermoregulation, growth, activity and reproduction. Compared with captive animals, free-living organisms need more energy, principally to furnish the additional demands of thermoregulation and activity. Before the advent of techniques for measuring the energy needs of free-existence — that is, field metabolic rates (FMR) — animals were studied in the laboratory and the results were extrapolated to those in the field. A value of three times basal metabolism was often used as an estimate of FMR (Gessaman 1973). However, such arbitrary figures are usually inappropriate, because free-living animals respond to factors that captive animals may not experience — predation, climate, reproductive status, disease status and social interactions, and particularly variations in the quantity and quality of food. Furthermore, estimating the energy costs of free-existence as a simple function of basal metabolism has the underlying assumption that animals with low basal metabolism also have low FMR. This assumption has never been adequately tested. It will be answered only when sufficient FMR data are collected to examine the possible allometric equations in different mammalian groups.

In the southern part of its range *A. rufescens* experience climatic extremes. Maximum temperatures in summer sometimes exceed 35°C; winter minima approach zero. Because *A. rufescens* is strictly nocturnal, it avoids the high temperatures of summer, but is exposed to very low winter temperatures. Indeed, *Aepyprymnus* actively forages on cold winter nights (pers. obs.). Because the lower critical temperature of *Aepyprymnus* is relatively high (25°C; Rübsamen *et al.* 1983), the energy cost of thermoregulation may raise significantly the FMR in winter. In particular, females may have very high levels of metabolism in winter, because they breed continuously and presumably experience simultaneously the high energy costs from thermoregulation and lactation.

The following study was conducted to measure the energy expenditure of male and female *A. rufescens* in winter and summer.

11.3.2 Materials and Methods

The Study Area

Aepyprymnus rufescens was studied on the property Cheviot Hills, located 7km north of the Bruxner Highway at Drake in north-eastern New South Wales (28° 53'S, 152° 23'E), within the Clarence River catchment area. This study area is approaching the southern limit of *Aepyprymnus*'s current range. Because the area is about 500 m above sea-level the minimum temperature in winter often approaches zero. In contrast summer nights are often mild (ca 20°C). A full description of the study area was given by Wallis *et al.* (1989) (Appendix 5).

Isotope Turnover

FMR were measured using the doubly labelled water (DLW) technique of Lifson and McClintock (1966). Animals were first caught on a moonless night by a vehicle-borne catching team. When "held" in the spotlight beam, animals often allowed vehicular approach to within 2 metres, enabling the catcher, seated on the bonnet of the vehicle, to drop a long-handled net (3 m) with an opening diameter (60 cm) over the animal. The animal was immediately placed in a hessian sack and taken to the laboratory. Each *A. rufescens* was weighed, sexed, and fitted with a reflective collar equipped with a 151 Mhz radio-transmitter weighing 47 g (AVM Instruments, Illinois). An initial blood sample (ca 4 ml) was taken from a lateral tail vein using a pre-dried heparinised syringe and winged infusion kit (Chapter 4). This blood was used to estimate isotope background levels. Bleeding was facilitated by threading the animal's tail through a small hole in the sack. Preweighed doses of 37 Mbq ^{3}HOH in 1 ml and 0.9 ml of 98 atom% ^{18}O (YEDA-Stable Isotopes, Israel) were then injected into a hind-limb muscle. The exact mass of the injection solutions was determined by weighing (to the nearest 0.01g) the syringe(s) before and after injection. In the January study a combined dose was given in a single injection; in July, the isotopes were administered separately. The volume of ^{18}O injected was sufficient to raise the total body ^{18}O by about 0.045 atom percent — more than double the initial enrichment needed for analysis by isotope-ratio mass spectrometry. After allowing four hours for isotope equilibrium with the body water pool, a second blood sample was taken. The animal was then returned to the site of capture and released.

Recaptures were made by day about 5 and 10 days later (in some cases a third recapture was made after 15 days). A radio-receiver (AVM LA12-DS) was used to locate the animal. The method was accurate enough to pinpoint an area within a 3 m radius of the animal's nest. By experience, the precise location could then be guessed

(for example, under a tussock or other cover). The suspected site was encircled by a net (mesh size 10 cm) about 6 m in radius and 3 m high. Closer approach tended to flush animals prematurely. The netting was suspended from saplings, trees or other convenient objects (stakes and vehicles were used in open country). The top of the net was 1-1.2 m above the ground. About 80 cm of netting was left on the ground, and this was weighted with logs and rocks, leaving a belly at the bottom of the net. When the circle was complete, the animal was flushed from the nest and captured upon entanglement. The *A. rufescens* was weighed, bled, the collar was checked and the animal released. Nest details were recorded (Wallis *et al.* 1989). Evidence gathered by radio-tracking animals upon release showed they had settled into another nest within 20 minutes.

Analysis

The packed-cell volume was determined in the blood soon after sampling (Chapter 4). The remaining blood was centrifuged, and the cells and plasma were separated and then frozen (ca -5°C) for later analysis. Water was extracted from the cell fraction by vacuum sublimation (Vaughan and Boling 1961; Chapter 4). The ^{3}H content of this distilled water was determined at the University of New England, Armidale, by liquid scintillation counting (Chapter 4). Another fraction of the distilled water was analysed for ^{18}O concentration by Dr Brian Green (CSIRO Division of Wildlife and Ecology, A.C.T) using isotope-ratio mass spectrometry as follows: About 50 µl of the extracted water were placed in Urey tubes along with standard charges of CO_2 gas. The tubes were incubated overnight at 80°C after which the equilibrated CO_2 charge was removed and the ^{18}O:^{16}O ratio was determined with a VG Isogas 903 isotope-ratio mass spectrometer. Body-water pools were derived from the level of dilution of the injected isotopes compared to standard dilutions. Using the equations of Lifson and McClintock (1966) and Nagy (1980) CO_2 production rates and water fluxes were derived from the declines in the levels of isotopes in the body water during the release period. It was assumed that mass-specific pool sizes did not change during the release period and that any changes in body mass and absolute body-water pools were linear.

To the best of our knowledge, *A. rufescens* is predominantly a rhizophagous herbivore. Flower buds, grass seeds, acacia gum, insects and hypogeous fungi also are consumed but to a much lesser extent (Chapter 3). Such a diet provides carbohydrate as the predominant metabolic substrate, and presumably, the RQ was 1.0. Thus, heat production was estimated by using the thermal equivalent of 21.8 $kJ.l^{-1}$ CO_2 (Nagy and Martin 1985).

The blood plasma was analysed for a number of compounds including protein, lactate, creatinine, urea and glucose. These results are given in Appendix 4.

Observations of animals

For two reasons, the animals were not observed at close quarters during their activity period. First, the nocturnal habits of *Aepyprymnus* call for observations by spotlight, which would obviously disturb the animal and possibly affect its energy metabolism. Secondly, close observation would have been almost impossible, because *Aepyprymnus* often moves up to one kilometre soon after leaving its nest (Wallis *et al.* 1989). Also, long grass during both study periods made it difficult to locate animals at night. Nevertheless, it was established that animals wait for darkness before leaving their nests and that they are frequently seen near the feeding site where they were originally captured.

Statistical analyses

The loss of two animals (1 and 2) between the summer and winter study periods unbalanced the experimental design. Although not strictly correct, the results were analysed by two-way analysis of variance (ANOVA) assuming that all observations were independent. This rather conservative approach was taken after the results were analysed in two other ways: first the males were compared between study periods using a paired t-test; secondly, the sexes were compared in each study period by one-way ANOVA.

11.3.3 Results

Climate

There was almost no rain during either the winter or the summer study period (Fig 11.). The average maximum air temperatures during 26 days in January-February and 20 days in July were 30.5°C (range 25 to 37) and 20°C (15 to 23); the corresponding minimum temperatures were 20°C (16 to 23) and 2.5°C (-2 to 11).

Diet

Aepyprymnus were often observed feeding on the tubers of three plants — *Hypochoeris radica*, *Murdannia graminea* and *Trachymere incisa*. The nutritional analysis of these is given in Table 10.8.c. It was assumed that these formed a major part of the diet. On occasions, *Aepyprymnus* were also seen eating acacia gum, the succulent growing tips of *Paspalum dilatatum* and, in the summer, scarab beetles.

Body mass and field metabolic rates

Because it was not practicable to carry out a straightforward statistical analysis, a complete data set, with results for individual animals, is shown in Table 11.3.1

The mean body-mass and the range of body masses of the animals captured at Drake was similar to that reported at the same study site by Schlager (1981). There was, however, a tendency for males to be heavier than females in winter ($P<0.06$; one-way ANOVA), probably because males themselves tended to be heavier in winter than summer ($P<0.06$; paired t-test). This observation is based on few animals and must be treated with caution. Within study periods the mass of individuals fluctuated little.

Field production of carbon dioxide

The ratio of TBW estimated by ^3HOH to TBW estimated by $H^{18}OH$ was 1.046 ± 0.061; $n = 14$.

The means and standard errors of differences between means for the various parameters computed by two-way ANOVA are shown in Table 11.3.2 This procedure, although not strictly correct because of the repeated measures effect from some animals used in both seasons, shows that little of the variation in energy metabolism is explained by the sex of the animal or by the season. The two-way analysis was done after a one-way ANOVA showed that body mass, water flux and heat production were similar in males and females in winter and summer. Similarly, within males, a paired t-test showed that season had no detectable effect on energy metabolism.

Relationships between parameters

Under the dry conditions prevailing during the present study, *A. rufescens* obtained most of their water from food both as free and as metabolic water. Thus, if the diet of all animals is similar, a significant correlation between CO_2 production and water flux would be expected. However, the least-squares regression was not significant ($0.05>P>0.10$). If all animals have similar time-energy balances, then production rates of CO_2 might be correlated with body mass. Again, the regression was insignificant.

Table 11.3.1 Details of body mass (g), total body water (TBW; %), pouch young mass (PY; g), CO_2 production rates (ml.g^{-1}.h^{-1}), *metabolic rates* (kJ.kg$^{-0.75}$.d^{-1}) *and wate fluxes* (g.kg$^{-0.80}$.d^{-1}) *of free-living* A. rufescens *at Drake in summer and winter*

	Summer						Winter					
Animal	**Mass (kg)**	**TBW (%)**	**PY mass (g)**	**Water flux**	**Metabolic rate**	**CO_2 production**	**Mass**	**TBW**	**PY mass**	**Water flux**	**Metabolic rate**	**CO_2 production**
Females												
1	2700	77.9	110	172	731 (3)*	1.09						
2	3100	80.1	40	134	632 (3)	0.91						
6	2800	71.3	120	176	622 (3)	0.92	2880	73.3	5; 680[1]	178	709 (4)	1.04
8	2800	73.2	25; 950[1]	127	521 (3)	0.77	2650	72.8	40; 1100[1]	137	527 (3)	0.79
12							2900	74.1	630[1]	198	921 (4)	1.35
15							2750	80.9	40	180		
Males												
3	2900	75.3	-	177	752 (3)	1.10	2980	83.3	-	172	625 (3)	0.91
7	2850	74.8	-	215	598 (3)	0.88	3030	77.1	-	144	448 (3)	0.65
9	2930	82.8	-	176	574 (3)	0.84	3300	77.7	-	159	571 (3)	0.81
10	2780	72.8	-	139	560 (4)	0.83	3000	73.0	-	163	909 (3)	1.32
14							2600	83.6	-	117	-	

* - the number of data points for the measurement = an equilibrium time sample + recaptures.
[1] - mass of young at foot

Table 11.3.2 Mean water flux and heat production in free-living A. rufescens measured in winter and summer at Drake.

	Summer		Winter			significance	
	male	**female**	**male**	**female**	**sed**	**sex**	**season**
number	4	4	4	5			
Body mass(g)	2850	2870	2800	2980	123	ns	ns
Total body water (%)	75.6	76.4	75.3	78.9	2.98	ns	ns
Water influx ($g.kg^{-1}.d^{-1}$)	123	143	141	122	14.4	ns	ns
CO_2 production ($ml.g^{-1}.h^{-1}$)	0.91	0.92	0.92	1.06	0.140	ns	ns
Heat production number	4	4	3	4			
($kJ.d^{-1}$)	1332	1331	1526	1439	231.4	ns	ns
($kJ.kg^{-1}.d^{-1}$)	469	464	539	469	76.9	ns	ns
($kJ.kg^{-0.75}.d^{-1}$)	609	604	699	621	101.0	ns	ns

11.3.4 Discussion

The water flux in free-living *A. rufescens* has been discussed elsewhere (Chapter 10) and will be referred to here only in the context of energy metabolism. Haematocrit data also were included in Chapter 10.

The DLW technique is based on the knowledge that the oxygen in the body's water and bicarbonate pools are in rapid equilibrium through the carbonic anhydrase reaction (Lifson and McClintock 1966). The ^{3}H rate-constant represents water turnover. The ^{18}O rate-constant traces the losses of oxygen from the body, both in water and via CO_2. Thus, the difference between the ^{3}H and ^{18}O turnovers represents CO_2 production. Energy metabolism is calculated from the equations of Brouwer (1965) by assuming an RQ based on the diet of the animal.

The DLW technique is based on six main assumptions (Lifson and McClintock 1966; Mullen 1973; Nagy 1980). These are discussed below with reference to potential errors in the present experiment:

1. *Total body water (TBW) remains constant during the experiment.* This assumption can be verified by measuring TBW at the start and the finish of the measurement period. This was not done in the present experiment, because *A. rufescens* had water intakes far in excess of their needs (Chapter 10) and changes in body mass during the measurement periods were negligible (± 1%). Thus, it was assumed that TBW remained stable, and calculations were based on a single estimation of TBW made from the equilibrium blood sample.

2. *The isotopes label only CO_2 and H_2O.* Previous studies have shown that ^3HOH may overestimate TBW by 4-15% (Carnegie and Tulloh 1968; Sheng and Huggins 1979; Nagy 1980; Fancy *et al. 1986)* because of isotopic fractionation and the incorporation of ^3H into non-aqueous components. Because ^3HOH and ^{18}O gave similar estimates of TBW in the present study period (^3HOH:^{18}O = 1.045), it was assumed that negligible labelling of non-aqueous components occurred.

3. *Water-turnover rates and CO_2 production rates are constant over time.* The results described in Section 11.1 show that in the confined space of a respirometer, the metabolic rates of *A. rufescens* are 25% higher at night. The assumption of constant rates is clearly less valid in free-living *A. rufescens.*

4. *Isotopes are lost only as H_2O and CO_2.* ^3H may be lost as exchangeable ions in the urine and faeces and as non-exchangeable ions in urea, but in both cases the effect is probably minimal (Nagy 1980). Fancy *et al.* (1986) showed that correction for losses of ^3H in methane may increase CO_2 production rates by 2-4%. On the concentrated diet selected by *A. rufescens* (Chapter 3), methane production is probably quantitatively unimportant. Fat synthesis increases the apparent water loss, and therefore decreases calculated CO_2 production rates. For this reason, CO_2 production rates were underestimated by 4.8% in rapidly growing pigs (Midwood *et al.* 1989). In the present study, CO_2 production rates were probably underestimated in the females with young at foot because, at this stage of lactation, the milk contains about 17% fat.

5. *No water or CO_2 enters the animal via the lungs or the skin.* Nagy (1980) has shown that, in atmospheres enriched with H_2O and CO_2, cutaneous and respiratory input of CO_2 may cause errors of up to 90% in calculated CO_2 production rates. Because the nest of *A. rufescens* is a rather confined space, H_2O and CO_2 may accumulate and be recycled. The effect, however, on CO_2 production rates is probably minimal, because the H_2O and CO_2 molecules would have specific activities of ^3H and ^{18}O similar to those of the body water.

6. *The specific activities of excreted H_2O and CO_2 equal those of the body water.* Isotope fractionation was considered by James *et al.* (1988) as "the most critical factor

affecting calculated CO_2 production rates". It is also the most difficult to quantify because it relies on knowledge of evaporative water losses (EWL) and the routes of these losses. James *et al.* (1988) calculated that errors of 100% in the measurement of EWL result in errors in CO_2 production rates of -3% to +3%. In the present study, because water intakes were well in excess of the minimum water requirements determined in the laboratory (Chapter 10), losses of faecal and urinary water would have been high. Consequently, EWL was probably less than 50% of total water flux (see Chapter 10). Nagy (1980) suggested that fractionation was a major concern when EWL was a high proportion of total water flux. In this study, fractionation was assumed to be constant at all times.

Finally, the conversion of CO_2 production rates to FMR requires knowledge of the RQ. In some studies, inappropriate RQ values have been used. For example, Smith *et al.* (1982) applied the RQ of fasting animals to obtain FMR. In the studies of fed *A. rufescens* (Section 11.2), RQ was rarely less than unity except in those animals with low feed intakes. Because free-living animals maintained body mass and presumably energy balance, and because the available evidence suggests that they eat a diet rich in carbohydrates (Chapter 3), an RQ of 1.0 was used to calculate FMR in *A. rufescens*.

In each animal it is the balance of all of the deviations from ideal marker behaviour that determines the validity of the method. Despite the many potential errors surrounding the DLW technique, validation studies with simultaneous respirometry measurements usually show discrepancies of less than 10% (Nagy 1980). The differences are often much less: Westerterp *et al.* (1988) showed that energy expenditures obtained with the DLW technique and respirometry differed by less than 1.5% in humans subjected to low and high activity levels. Seale *et al.* (1988) reported that DLW estimated metabolisable energy intake to within 0.4%, while whole body room calorimetry underestimated energy expenditure by 17%. Also, wide discrepancies between DLW and respirometry have been reported. For example, in some arthropods, DLW overestimated CO_2 production by more than 30% (King and Hadley 1979; Cooper 1983). This was probably because the hydrogen in water was exchanged with non-aqueous hydrogen. Likewise, in *Rangifer tarandus* (reindeer and caribou), the DLW technique gave results 5-25% higher than those determined using calorimetry in summer (Fancy *et al.* (1986); in winter, values obtained with both methods agreed closely. The seasonal difference was attributed to net deposition of isotopes in growing tissues.

Because validation studies are usually carried out under highly controlled conditions, which approach steady state, it is questionable whether they are of much value in assessing the accuracy of the DLW technique in free-living animals. For this

reason, DLW was not validated in the present study. Instead, the field study was carried out with the assumption that any errors would be uniform across all treatments and would not mask treatment differences.

Despite its limitations, the DLW method is the most accurate way of studying the energy metabolism of animals free to interact with their natural environments. The DLW method gives an integrated value for the metabolic costs of resting, specific dynamic action, activity, thermoregulation and, particularly in females, reproductive costs. The total metabolic cost of about 3.3 times SMR for *A. rufescens* is the highest ratio of the seven FMR studies reported in macropodoids (range 1.7-3.3; Green 1989). These ratios were obtained by calculating the SMR from the equation of Dawson and Hulbert (1970). The ratio for *A. rufescens* is less (2.9) if the FHP determined in Section 11.1 is used. The only similar value (3.23) was measured in *B. penicillata* (Green, Best and Turner in prep. cited by Green 1989). Thus, when the FMR data for macropodoids are plotted on a log-log scale against body mass, the value for *A. rufescens* lies well above the regression line (Fig 11.3.1). Indeed, the FMR of *A. rufescens* is about 50% higher than the value predicted from this macropodoid regression line and 40% higher than that predicted by the allometric equation for herbivorous marsupials (Nagy 1987).

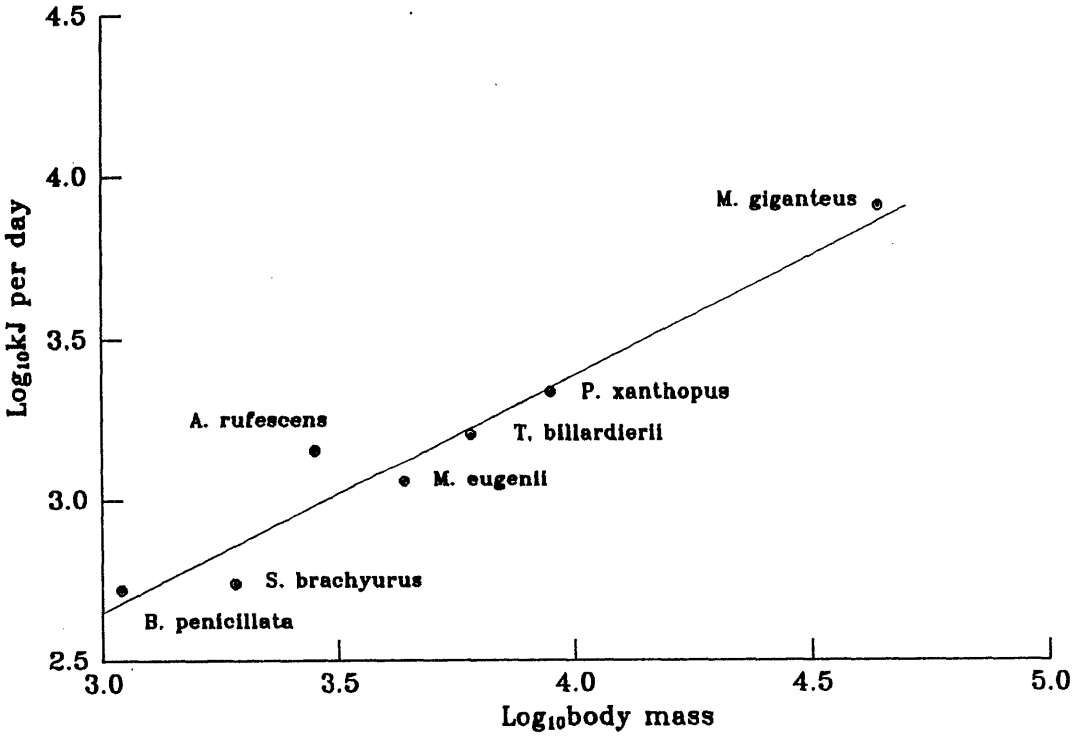


Figure 11.3.1 The relationship between body mass and FMR in macropodoids during summer. $Log_{10}kJ$ per day = 0.43 + log_{10}(body mass $g^{0.74}$); r^2 = 0.97; SEb = 0.08. (After Green 1989)

Why do *A. rufescens* and *B. penicillata* have higher FMR than other macropodoids? There is no obvious explanation for this difference, although several differences between the potoroines and macropodids are evident. The minimum FHP determined for *A. rufescens* and *B. penicillata* in Section 11.1 was 10-18% higher than that predicted by the equation of Dawson and Hulbert (1970). Nevertheless, Rübsamen *et al.* (1983) reported a SMR for *A. rufescens* similar to the predicted marsupial value. A more plausible explanation is that potoroines feed largely on hypogeous foods, which presumably require more energy to obtain than the epigeous foods eaten by macropodids. Finally, is it significant that of the seven macropodoids for which there are FMR data, the two with the highest FMR breed continuously and those with the lowest FMR are seasonal breeders? McNab (1980, 1986a,b) has repeatedly argued that eutherian mammals exhibit the highest metabolic rates that their diet will allow, because high rates permit high reproductive output. However, he has argued also that this does not stand for marsupials. The high FMR of potoroine marsupials and the capacity of some mammals with low MR to increase their resting MR during gestation and lactation (Thompson and Nicoll 1986) indicate a need for more FMR studies of a range of mammals with different reproductive stategies.

It is interesting to note that, in macropodoids, the allometric relationship for FMR has a slope of 0.74 — similar to that describing the relationship between SMR and body mass in eutherians (Brody 1945) and metatherians (Dawson and Hulbert 1970). In other words, in macropodoids, FMR is a simple multiple of SMR. However, when all FMR data for metatherians are included in an allometric equation (Nagy 1987), FMR is correlated with body mass (r^2 = 97.7%) but the slope of the line (0.576) is significantly less ($P<0.001$) than the slope (0.737) reported by Dawson and Hulbert (1970). Does this imply that close consideration should be taken as to which species to include in allometric equations? For example, should the allometry of FMR take into account an animal's habitat and feeding habits as suggested by McNab (1980) for SMR? There are too few FMR measurements presently available to evaluate the relative importance of diet and habitat correlates of FMR (Nagy 1987). Nevertheless, it is interesting to examine some arboreal species. The folivorous *Alouatta palliata* (Nagy and Milton 1979), *Bradypus variegatus* (Nagy and Montgomery 1980), *Phascolarctos cinereus* (Nagy and Martin 1985) and *Petauroides volans* (Foley *et al.* 1987) have FMR:SMR between 1.8 and 2.7; the more-omnivorous arboreal marsupials — *Gymnobelideus leadbeateri* (Smith *et al.* 1982) and *Petaurus breviceps* (Nagy and Suckling 1985) have FMR:SMR of 5.8.

Why was there no difference between the FMR of *Aepyprymnus* in winter and summer and between males and females? Without a detailed knowledge of the

behavioural ecology and diet of *Aepyprymnus* this question cannot be answered conclusively. However, by making some broad assumptions and using the data obtained in Sections 11.1 and 11.2 and the findings of Rübsamen *et al.* (1983), the question can be partially answered. First, let us consider winter and summer FMR. Rübsamen *et al.* (1983) showed that *Aepyprymnus* has a TNZ between 25°C and 35°C. The mean maximum temperatures in summer and winter were 31.5°C and 20°C respectively. Assuming that the nest is warmer than the ambient temperature in winter, and of a similar temperature in summer (Rübsamen *et al.* 1983), then in both seasons the hourly cost of nesting — that is, daytime metabolic rates — would approximate the average metabolic rates of captive animals measured after six-hours of fasting (270 $kJ.kg^{-0.75}.d^{-1}$; Section 11.1.). Some assumptions must now be made about activity. In summer and winter, *A. rufescens* left its nest at about 2100h and 1800h respectively. Often, we radio-tracked animals late at night and could ascertain that they were away from their nesting areas, and presumably active. If the animals returned to their nests about 2h before dawn, they were active for about 11 hours in winter and eight hours in summer. From this, it can be calculated that the total daytime HP in *Aepyprymnus*, in winter and summer, is 146 and 180 $kJ.kg^{-0.75}$ respectively. This leaves, respectively, 514 and 480 $kJ.kg^{-0.75}$ in winter and summer for night-time metabolism. The costs of thermoregulation in summer were probably negligible, because the mean minimum temperature was only slightly less than TNZ. Also, it is generally accepted that heat increment substitutes for thermoregulatory costs (Robbins 1983). In winter, the animals were exposed to ambient temperatures of less than 10°C for about 11 hours. At these temperatures *Aepyprymnus* has a resting metabolic rate of 414 $kJ.kg^{-0.75}.d^{-1}$ (Rübsamen *et al.* 1983). Thus, the thermoregulatory cost was about 66 $kJ.kg^{-0.75}$ or 10% of daily energy expenditure. It is known that the measurements of FMR have high standard errors. Also, we can be reasonably sure that *Aepyprymnus* conserve energy in winter by such means changes in coat characteristics, activity patterns or posture. Taking all thes factors into account, the lack of seasonal differences is not unexpected.

Only one other study of FMR in macropodoids has included both summer and winter measurements. Green *et al.* (in prep cited by Green 1989) found that the FMR of *B. penicillata* was 33% higher in winter than in summer. The area inhabited by *B. penicillata* was described by Green (1989) as having cool, wet winters. If the animals were wet at any time in winter, this would certainly explain the seasonal differences. Because *B. penicillata* is only one third the size of *Aepyprymnus*, it would be expected to have higher thermoregulatory costs per unit body-mass.

In Section 11.2 it was shown that, in the last three weeks of pouch life, the combined heat production of females and their pouch young was about 20% higher than

the heat production of females without pouch young. If all the other contributions to FMR remain constant, free-living female *A. rufescens* would have even higher FMR, because free-living animals have the added cost of transporting the young. Just before pouch vacation, the female is moving 20% of her own body mass. Why didn't the DLW method detect a higher FMR in females with large pouch young or young at foot? First, the DLW technique may be in error because significant amounts of ^{3}H are used in milk-fat synthesis and are not measuring water flux. Thus, CO_2 production rates are underestimated. This can be tested only with a validation study. The alternative hypothesis is that lactating animals really do have a lower FMR, because they conserve the energy cost of lactation by reducing their energy expenditure in other activities, or by physiological changes. Again, these mechanisms will be explained only by further studies. Presumably the heat generated by the young may be used in winter for thermoregulation. In summer, at Drake, the additional heat would be a burden and would have to be dissipated.

The ratio of FMR to maintenance requirements (Section 11.2) in *Aepyprymnus* is about 1.9. Maintenance requirements include FHP, HI and modest activity — for example, grooming, maintenance of posture and mastication. The increase in FMR above maintenance includes the added costs of thermoregulation, locomotion, activities such as detecting and acquiring food, and nest building. In the present study this amounted to about 300 $kJ.kg^{-0.75}.d^{-1}$. Because thermoregulation appears to account for relatively little of the increment above maintenance, it seems that feeding and nest building are major costs. The energy cost of procuring hypogeous foods has already been mentioned. *Aepyprymnus* used many nests (Wallis *et al.* 1989). Each contained up to one kg of nesting material, which sometimes had to be carried long distances (pers. obs.). This suggests that the energetic cost of nest-building is high.

Free-living *A. rufescens* obtain well in excess of their water requirements from free-water in their food and from metabolic water. This explains why Schlager (1981) and myself have never observed free-living *Aepyprymnus* drinking. If the water content of the diet is known, the animal's food intake can be calculated from water flux. *A. rufescens* was often observed feeding on the tubers of three dicotyledenous plant species, whose water content and nutritional composition are shown in Table 10.8.c. The mean gross energy content of the tubers was about 15 $MJ.kg^{-1}$ dry matter. If we assume an assimilation efficiency of 65%, *A. rufescens* must eat about 60 $g.kg^{-0.75}.d^{-1}$ of tubers to meet their FMR. This would involve a water flux of 300 $g.kg^{-0.75}.d^{-1}$, almost double the measured flux. It is clear from this discussion that much of the food eaten by *A. rufescens* has a lower water-to-energy ratio than do tubers. Seeds and acacia gum are

two examples. Therefore, *A. rufescens* can balance water and nutrient requirements by dietary selection.

11.3.5 Summary

The metabolic rate of free-living *A. rufescens* was found to be 650 $kJ.kg^{-0.75}.d^{-1}$ or 2.9 times the lowest metabolic rate of fasting animals determined in Section 11.1. The FMR was similar in winter and summer even though the difference in mean minimum temperatures between the two seasons was 20°C. The DLW method did not detect any differences in FMR between males and females. A poor understanding of the diet and the behavioural ecology of *Aepyprymnus* makes it difficult to explain the similarities between sexes and seasons.

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CHAPTER TWELVE

The nutrition, digestive physiology and metabolism of potoroine marsupials

General Discussion

THE Macropodoidea, along with the Artiodactyla (which includes the Ruminantia) and the Perissodactyla have been on earth for a long time — more than 30 million years (Janis 1976; Flannery 1989). Their ancestors were probably organisms with simple dentition and simple gastrointestinal tracts, and inhabited a planet dominated by forest (Janis 1976). The three groups have many other similarities indicative of a parallel evolution. The common sight in Australia of horses, ruminants and macropodids grazing together emphasizes their adaptational similarities.

At the start of the Miocene (26 million years ago), the earth became cooler and drier. New niches appeared, dominated by grasslands. Among the Ruminantia, Perissodactyla and Macropodoidea, distinct digestive strategies emerged. The Macropodoidea and the Ruminantia responded by developing the cardiac region of their stomachs, albeit in different ways; the Perissodactyla expanded their hindguts. In both cases the benefit was a symbiosis with microbes capable of releasing the potential energy stored in the ß-1,4 glycosidic bonds of cellulose (Moir *et al.* 1956; Janis 1976; Hume 1978). Cleavage of the plant-cell wall releases also the nutrients within the cell. Unfortunately, fermentative digestion is a slow process and digesta must be retained for many hours (Hungate *et al.* 1959). Thus, most herbivores are burdened by large gastrointestinal tracts.

Fluctuations in climate since the Early Miocene have resulted in the evolution of a wide diversity of herbivores. For example, among some 150 ruminant species are morphophysiological variations and adaptations relating to foraging behaviour, digestive physiology, to interactions between plant and animal and to geographic and climatic diversity of ecological niches (Hofmann 1989). Hofmann (1973, 1989) recognized three distinct but overlapping feeding types, each with its own forestomach anatomy: concentrate selector (40% of species), grass and roughage eaters (25%), and intermediate, opportunistic, mixed feeders (35%). Despite the richness of forms among the Ruminantia, the conventional "ruminant model" is based on thousands of research projects on just two species from the grazing grade — sheep and cattle.

Nutritional research of macropodoids started in much the same way when Owen

(1834 cited by Hume 1982) remarked on the "resemblance to the ruminating tribes, to which the kangaroos make so near an approach in the complexity of the stomach, and the simplicity of the caecum and colon". Similarly, in more recent times, Moir *et al.* (1956) described *Setonix brachyurus as* a "ruminant-like" marsupial. The forestomach anatomy of macropodoids is quite different from the various forms found within the Ruminantia. But, in common with ruminants, a transition in feeding type and digestive anatomy is recognized also within the macropodoids (Hume 1978; Langer 1980; Hume and Dellow 1980; Dellow and Hume 1982c; Langer *et al.* 1980). Like the digestive systems of the Ruminantia, the morphology of those in the Macropodoidea are loosely determined by body size. The forestomach of concentrate-selecting potoroines, the smallest macropodoids, has a volume that is about 80% sacciform; the forestomach of a large grazing species, for example *Macropus giganteus, is* about 70% tubiform; species with intermediate diets, such as *Thylogale thetis,* have forestomachs more equally divided between the tubiform and sacciform regions. Concomitant with these differences in gastrointestinal anatomy are morphological adaptations of teeth to diets and feeding. From their studies of dentition, Raven and Gregory (1946) concluded that the extant macropodids show an evolutionary transition from the ancestral forest inhabitants, represented now by the genus *Thylogale,* through to a grazing grade, essentially *Macropus.* In recent times, Sanson (for example, 1989) has elaborated these views on dental morphology. He recognized four dental grades: potoroid and basal macropodoid, browser, intermediate browser/grazer and grazer. Finally, preliminary observations of the intestine and caecum of macropodoids by Osawa (1986) indicate both inter- and intra-specific differences. For example, grazers have a more developed caecum and colon than do browsing macropodids; and within a species there are morphological changes (eg the dimensions of villi) in response to diet, season and physiological state, such as lactation. Clearly, the comment by Hofmann (1989) that ruminant evolution is "a bush not a ladder" (Gould 1986) applies equally well to macropodoid evolution.

To what extent are the behavioural and anatomical differences between potoroine marsupials and macropodid marsupials reflected in their nutrition, digestive physiology and metabolism? The consensus is that potoroines are concentrate selectors (Hume 1982; Seebeck *et al.* 1989). Several parts of the present study support this thesis. First, although the number of dietary studies of potoroines are few (Chapter 3), they indicate that potoroines select foods that are low in plant-cell walls and often hypogeous. To detect these food items, the olfactory bulb in the potoroine brain is well developed (Beckmann 1986; Jackson pers comm). To obtain hypogeous foods, potoroines have well-developed claws. Of course, these claws can equally well hold grass stems, enabling the animal to remove seeds, scrape gum from acacia trunks, or make a

depression for a nest (Wallis *et al.* 1989; Appendix 5). Other circumstantial evidence that potoroines are concentrate selectors relates to their body size (Chapter 2) and dentition and habitat (Chapter 3). Second, the captive potoroines used in the study, after a relatively long adaptation period, ate a lucerne-based diet high in plant-cell walls. However, they reverted to a cereal-based diet immediately, when it was offered. This occurred suddenly and without ill-effect, even though their forestomach pH probably fell from neutral to about 4.5 (Chapter 9), and implies that potoroines have flexible feeding habits. In the wild they might readily exploit a sudden flush of starch-rich food — for example, seeds. The low digestibility of cell walls in the second lucerne experiment (Section 8.3) was explained by a drop in forestomach pH caused by the relatively high level of soluble carbohydrate in the lucerne. From this it might be inferred that potoroines have a labile forestomach pH. This provides an environment that is better suited to metabolising a concentrated diet than one needing fermentation. Finally, free-living *A. rufescens* were often seen eating tubers, acacia gum and young grass shoots. The low cell-wall content and significant concentration of soluble carbohydrates in the potoroine diet was indicated in the *in vitro* digestibility studies of free-living *A. rufescens* (Chapter 9); the SCFA produced had a high molar proportion of propionate (27-43%).

Because of their concentrated diet, potoroine marsupials might be expected to have a relatively simple stomach, possibly resembling that of *Hypsiprymnodon moschatus*. Instead, the potoroine forestomach is more complex and more closely resembles those of the macropodids, in particular that of the forest-dwelling *Thylogale spp*. How is this explained? The several roles of a "complex" forestomach were discussed in Chapter 2. These include the fermentation of plant-cell walls, the microbial improvement of dietary protein and the microbial synthesis of B-vitamins, the detoxification of plant allelochemicals and the storage of ingested food. The results from the lucerne experiments (Chapter 8) and from the *in vitro* study of free-living *A. rufescens* (Chapter 9) both show that potoroine marsupials can digest significant amounts of plant-cell walls. In the first lucerne experiment (Section 8.2) *Aepyprymnus*, *Potorous* and *Bettongia* digested a greater proportion of ingested NDF than did *M. robustus spp* fed a similar diet (Freudenberger *et al.* 1989). Indeed, this might be expected, because the large blind sacciform region in the potoroine forestomach is better suited to digesta retention than is the TFS of *M. robustus spp*. It was calculated (Chapter 9) that the potoroines fed lucerne obtained between 30 and 40% of their digestible energy intake from hydrolysis of plant-cell-wall constituents. Because the animals maintained body mass and were, therefore, presumably at about zero energy balance, 30-40% of maintenance energy requirements came from fermentation end-products. However, the energy requirements of free existence are about double maintenance (Chapter 11).

Prince (1976 cited by Dawson 1989) showed that digestibility in macropodoids decreases with increasing food intake. Thus, free-living animals eating a diet of similar composition to the lucerne-based diet (Section 8.2) and obtaining 30-40% of their digestible energy from fermentation, would have to eat 80-100 g dry matter per kilogram metabolic body mass per day. If this diet has a digestibility of 50% and a mean retention time of 24 hours, the gut of a three-kilogram *A. rufescens*, at the end of the peak feeding period, would contain about 200 g of dry matter. This level of gut fill far exceeds the maximum gut capacity measured in free-living *A. rufescens* (Chapter 9) which in turn exceeded that predicted from the equation of Parra (1978). The thesis developed here supports the arguments above: that free-living potoroines select a concentrated diet — one digested mainly enzymatically and in which fermentation products probably contribute less than 30% of the digestible energy intake. Thus, the primary role of the forestomach is probably not fermentation. However, the supplementary value of SCFA to the energy balance of the animal, and the importance of fermentation when concentrated food items are scarce, cannot be overlooked.

It is generally accepted that, as plants age, the proportion of cell-wall constituents increases and that of cell contents decreases (for example, Mattson 1980). Most of a plant's nitrogen is found in the cell contents. Accordingly, concentrate-selecting herbivores/omnivores, such as potoroines, probably have high nitrogen intakes relative to those of strict grazers (Prins and Beekman 1989). Furthermore, omnivores and herbivores eating a varied diet would be expected to have a balanced intake of essential amino acids, and especially so if invertebrates also are eaten as is often reported about potoroines (Chapter 3). If the ingested amino acids escape microbial degradation in the forestomach, they would be of greater value to the animal. The foregoing discussion questions the thesis of Kinnear et al. (1979) that the primary function of the potoroine forestomach is to improve the biological value of ingested protein. Also, it suggests that the potoroine forestomach contains a mechanism to protect ingested protein from microbial degradation.

Is the primary role of the potoroine forestomach to supply a balanced mixture of essential amino acids to the duodenum? For several reasons this role is disputed. Kinnear *et al.* (1979) based their hypothesis on the low lysine concentration of a small sample of hypogeous fungi. Doubts about their analyses have been raised already in Chapter 9. The finding that potoroines fed diets containing 16% crude protein (similar to hypogeous fungi) recycle up to 55% of the urea synthesized, suggests that microbial protein synthesis would contribute significantly to the nitrogen economy of the animal from endogenous sources alone. This aside, Kinnear *et al.* (1979) disregard the possibility that *B. penicillata* balances its amino-acid intake from other dietary items. They assume also that all the amino acids in the fungi are available. Recent studies of

ground squirrels by Cork and Kenagy (1989) indicate that hypogeous fungi are poorly digested. It follows that the animal may have to obtain much of its amino-acid needs from sources other than fungi. Alternatively, in those potoroines that depend on fungi — for example, *B. gaimardi* and *Potorous spp* — the forestomach may be important in the fermentation of fungal carbohydrates (Dawson 1989).

It was proposed earlier that animals selecting concentrated diets have relatively high nitrogen intakes. Does this infer also that these species should have high nitrogen requirements because there is less selection pressure for nitrogen conservation? In Chapter 5 it was argued that a species' nitrogen requirements are not explained simply by phylogenetic or environmental factors. Instead, they are probably related to a combination of these factors, in particular the predictability of a species' environment throughout evolution. This may explain why potoroine marsupials have low nitrogen requirements, which in turn demonstrates their adaptation to an unpredictable environment. In much the same way, their capacity for fermentative digestion was interpreted earlier as being important when concentrated foods are becoming scarce.

Now let us consider whether potoroine marsupials have a mechanism for protecting ingested protein and, presumably, some soluble carbohydrates from microbial degradation. This would, it seems, involve a separation of the solute and particulate phases of digesta, enabling the solute to bypass the SFS. In the potoroine forestomach, the oesophagus opens into the distal TFS; in macropodids, the cardia is near the SFS-TFS border (Langer 1980). Thus, the distance between the cardia and the hindstomach in potoroines is small and it is conceivable that some ingesta could bypass the SFS. This explained the occasional rapid passage of ^{103}Ru-P and ^{51}Cr-EDTA through the gut of *A. rufescens*, *P. tridactylus* and *B. penicillata*. This bypass was confirmed by radiographic studies of *B. penicillata* (Richardson 1989) and of *A. rufescens* and *P. tridactylus* (Hume and Carlisle 1985; Hume *et al.* 1988) in which barium sulphate occasionally passed directly into the TFS. In the present study, regardless of mean retention time, the faecal excretion curves for ^{103}Ru-P and ^{51}Cr-EDTA were very similar. This indicates that the solute and particulate phases of the digesta are traversing the whole gut at similar rates. Thus, if particles are selectively retained in the forestomach, then the solute must be selectively retained elsewhere — for example, in the caecum or colon. Because it was not possible to fistulate animals (see Chapter 4), differential transit of solutes and particles through the potoroine gut could not be identified with ^{103}Ru-P and ^{51}Cr-EDTA. However, selective retention mechanisms are apparent in the findings of Richardson (1989). He observed that barium sulphate passed from the stomach in about 8 hours, and from the entire tract in 38 hours; large particles passed from the stomach in 15 hours and from the entire tract in 35 hours. Although there is no obvious physical barrier preventing the passage of ingesta to the

hindstomach, Richardson (1989) proposed that the pyloric ostium may function in this way. Thus, in potoroine marsupials, the peculiar forestomach anatomy may protect ingested protein from microbial degradation by allowing it to pass directly to the pyloric region of the stomach. This may be analogous with separation processes in the TFS of macropodids. In this organ, the fluid is squeezed through the particulate matter (Dellow 1982).

Does the potoroine forestomach partake also in detoxification of plant allelochemicals and in food storage? Plant secondary compounds evolved as a defence against leaf-eating insects (Cooper and Owen-Smith 1985) long before the evolution of mammalian herbivores. Thus, detoxification of these compounds, possibly by microbial processes in the foregut (Barry and Blaney 1987), must have been a critical step in the evolution of all pregastric-fermenting mammalian herbivores. Some macropodoids — for example, *Wallabia bicolor* — are known to eat plants toxic to domestic stock (Edwards and Ealey 1975). This suggests that detoxification mechanisms persist in macropodoids as they do in some ruminants (Hofmann 1989).

Because potoroine diets are best utilised in the small intestine, Hume *et al.* (1988) argued that the forestomach functions primarily to store ingesta, thus enabling rapid feeding bouts. Parts of the present study support this thesis. In response to increasing dietary fibre, potoroines ate more and digested proportionately less, but the rate of passage of digesta was unchanged (Chapter 7). This contrasts with results from macropodids (Calaby 1958; McIntosh 1966; Richardson and Wyburn 1980) and eutherians (Tyrell and Moe 1975; Moe 1981) that an inverse relationship exists between digestibility and rate of passage. Indeed, regardless of whether the digesta markers were given before or after feeding, or to animals fed high- or low- fibre diets, the mean retention times were usually less than 30 h (Chapter 7). Both captive and free-living potoroines have a peak feeding period just after nightfall. Thus, in wild animals captured 4-5 hours after darkness, up to 12% of the body mass was gut contents. Similarly, Hume and Carlisle (1985) reported that forestomach fill was minimal at the start of feeding and maximal just after the peak feeding period. In captive animals a distinct peak in faecal excretion coincides with the peak feeding period (Chapter 7). This probably occurs also in wild *Aepyprymnus*, because they never defaecated when captured; presumably they defaecate just after leaving the nest. The fact that dung beetles were never found on nesting *A. rufescens*, but were common on those captured away from the nest soon after nightfall supports this hypothesis. From the lack of treatment effects in the rate-of-passage studies, and from the patterns of feeding and defaecation, it might be inferred that digesta passage is diurnally synchronized and regulated by retention and release of digesta in the foregut and hindgut.

From the foregoing discussion it would be unwise to rank, in order of importance,

the various roles of the potoroine forestomach. Instead, the forestomach is best viewed in the context of the flexibility that it gives the animal — as a storage organ, as a provider of microbial protein, SCFA and B-vitamins, in detoxification of plant allelochemicals and as a regulator of digesta flow.

What is the role of the potoroine hindgut? It was concluded from the *in vitro* studies (Chapter 9) that the forestomach is the dominant gastrointestinal organ. After the peak feeding period it contained 70% of the total digesta and produced 10 times more SCFA than did the hindgut (15% of digesta). The proportion of digesta found in the foregut and hindgut changes with the cycle of feeding and defaecation. Just before feeding, the forestomach contains relatively less digesta and the hindgut relatively more (Hume and Carlisle 1985). This explains the view of Hume and Carlisle (1985) and Richardson (1989) that the hindgut may be more important in fermentative digestion in potoroines than it is in macropodids. But, this contrasts with Osawa's (1986) finding that the caecum and large intestine become relatively less important in the transition from grazer to browser. The relative size of the hindgut may be another feature distiguishing potoroines and macropodids. The retention of fluid and presumably small particles in the hindgut of *B. penicillata* (Richardson 1989) is particularly interesting. As discussed in Chapter 2, selective retention mechanisms in the hindgut may aid in nitrogen conservation by retaining a portion of the bacteria that would otherwise be lost in the faeces (Sperber 1968). Separation mechanisms are particularly important in caecotrophic animals (Björnhag 1987). Although potoroines fall within the typical size-range for caecotrophic animals, many hours of continuous observation failed to detect this practice in any one of the three species. Thus, the fluid and fine particles are probably retained because they are rich in protein and will supply the hindgut with readily fermentable substrate. This was supported by the high fermentation rates in the hindgut (Chapter 9). The benefit to the animal is presumably a supply of SCFA.

In the present study, water turnover was related to food intake, dietary ingredients, the nutritional composition of the diet, the availability of water, rainfall and to an animal's activity. Of greatest interest, however, was the apparent positive correlation between water turnover and the digestibility of plant-cell walls (Chapter 8). This has important ramifications with respect to comparative studies of water metabolism and potoroine feeding ecology. First, these results suggest that omnivores or herbivores with a mixed diet may have variable water requirements depending on their current diet. Thus, close consideration should be given to diet when measuring standard water requirements (Nicol 1978). Secondly, the minimal water requirements (Maloiy *et al.* 1978) of potoroines may be affected by diet. If the animals fed lucerne-based diets had their water intakes restricted by 50% (the restriction used in Experiment 6.1), they would still have higher intakes than those fed grain-based diets and given free-access to

water. Does this suggest that fermentative digestion is an option only when there is unlimited water? Unfortunately, the effect of water-restriction on potoroines fed lucerne-based diets was not studied.

Before widespread white colonisation, potoroine species inhabited many semi-arid and arid regions (Chapter 3). They accomplished this with a variety of physiological and behavioural adaptations. In the current study, under conditions of limited water, potoroines fed grain-based diets conserved water efficiently (Chapter 10). They ate less and produced concentrated urine and very dry faeces. These strategies enabled the animals to maintain other water pools, such as plasma volume. Animals of all three species withstood large losses of body mass. Finally, both *A. rufescens* and *P. tridactylus* rehydrated quickly and without ill-effect. In wild animals, behavioural strategies — for example, nesting behaviour and night-time activity — would complement physiological processes to conserve water. Surprisingly, in view of their different habitats, all three potoroine species showed a similar response to water restriction. However, as with nitrogen requirements, water needs are probably determined by the predictability of a species' environment throughout evolution. The capacity for water conservation in *A. rufescens*, *P. tridactylus* and *B. penicillata* may reflect a basal potoroine level. Two potoroine species — *Caloprymnus campestris* and *B. leseur* — were inhabitants of extrememly arid regions (Chapter 3). This was particularly true of *C. campestris*, which reportedly spent its day in a flimsy nest affording little protection. Presumably physiological mechanisms and perhaps skin and fur characteristics protected the animal against dehydration. The burrowing, nocturnal *B. leseur* largely avoid solar radiation. In addition, the high (8.4) relative medullary thickness of their kidney (Yadav 1979) suggests that they may excrete also a highly concentrated urine.

The energy metabolism of potoroine marsupials seems finely tuned to a nocturnal existence. The daytime metabolic rate was low — less than the FHP of eutherian mammals. At night the situation was reversed. Even within the confined space of a metabolism cage the night-time FHP of *P. tridactylus* and *B. penicillata* was more than double the daytime rate (Section 11.1); that of *A. rufescens* was raised by just 30%. It was argued, that because *A. rufescens* are larger than the other species, their activity was restricted in the small metabolism cages. This view is supported, albeit indirectly, by measurements of FMR. Both *A. rufescens* (Section 11.3) and *B. penicillata* (Green *et al.* in prep., cited by Green 1989) have FMR about three times the standard metabolic rate of metatherians. Furthermore, these FMR are high compared to those of other macropodoids (Green 1989). Whether this is a function of their small body size or some other parameter — for example, feeding habits — will be determined only from further FMR studies of a wide range of macropodoids.

Above basal metabolism, thermoregulation and reproduction are recognized as major components of an animal's energy needs. Thus, the similar FMR in female *A. rufescens*, with or without large pouch young or young-at-foot, were unexpected. Likewise, contrary to expectations, FMR were no higher in winter than in summer. Because the DLW technique estimates total energy expenditure, but provides no measure of its individual components, these findings will be explained only with studies of the diet, activity budgets and behavioural ecology of *A. rufescens*.

How do potoroine marsupials differ from their close relatives, the macropodid marsupials? First, they are usually smaller and one genus — *Potorous*, is more or less quadripedal. It is generally accepted that potoroine marsupials select more concentrated foods than do macropodids. Perhaps of greater significance is that potoroine marsupials seek mainly hypogeous foods. Coincidentally, the forepaws of potoroines are highly developed digging implements. Among potoroine marsupials there is a gradation in dentition from *Potorous* to *Aepyprymnus* (Chapter 3), but all dental forms differ from those in macropodids. The digestive tract, from the oesophagus to the rectum, is essentially similar in *Aepyprymnus*, *Potorous* and *Bettongia*, but this is quite different from the various forms in macropodids. For example, noticeable differences are found in the relative proportions of the sacciform and TFS regions and of the forestomach mucosa. Also, in contrast to the browsing macropodids, in which the hindgut is relatively small (Osawa 1986), that in potoroines is often large. The different digestive anatomies of potoroines and macropodids have probably resulted in different patterns of digesta flow. Thus, faecal marker excretion curves indicate that mean retention times are longer, but separation of digesta phases less, in potoroine marsupials than in macropodids. Because potoroines and macropodids have not been compared directly, it is impossible to speculate on digestive efficiency. This aside, one may argue that a direct comparison is irrelevant because both groups have different diets. Based on a few published reports and data from the present experiments it seems that the forestomach pH in macropodids is less labile than that in potoroines.

It is important to note that many of the characters found in potoroine marsupials are found also in some if not all macropodid species. Examples include: low FHP and FMR; similar requirements for nitrogen and the capacity to recycle urea; the mode of reproduction, and milk composition; the mechanisms for water conservation; and relatively high packed-cell-volumes.

In conclusion, although there are few potoroine species and their variety has changed little since the Miocene era, the diversity of the group, as indicated by the different habitats they once occupied, is remarkable. The present study found very few differences in digestive physiology or metabolism between *A. rufescens*, *P. tridactylus* or *B. penicillata*. Nothing suggested that their small body size disadvantaged them

before European influence. However, since European settlement, the status of many small (<5 kg) marsupials has become precarious. Potoroines are no exception. Land-clearing and the introduction of feral predators and competitors has caused the extinction of *C. campestris* and *P. platyops*, severely endangered *B. leseur* and *B. penicillata*, and significantly compressed the distributions of *B. gaimardi*, *A. rufescens* and *P. tridactylus*. Too little is known of *B. tropica* and *P. longipes* to pass judgement on changes in their status.

Although several studies have been made of different aspects of potoroine marsupials, this is the first pertaining to an integrated study of their nutrition, metabolism and digestive physiology. It was, therefore, a general study. Its primary aim was to provide a platform for future research on more specific subjects — for example, carbohydrate metabolism, protein turnover and foregut buffering mechanisms. Unfortunately, Australia is a country governed by economics, where knowledge of natural history holds a low priority; a country that has one of the highest mammalian extinction-rates in recent times; and a country in which students are made to pay various taxes to conduct studies such as this. In view of these facts, one must remain pessimistic about the future of Australian wildlife and wildlife research.

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Suggestions for future research

The research reported in this thesis was hampered, somewhat, by our lack of knowledge of the natural diets of potoroine marsupials, especially that of *A. rufescens*. Since the start of the study a number of papers have been published on the diet of free-living *Potorous spp.* These indicate unanimously that *P. tridactylus* and *P. longipes* rely heavily on hypogeous fungi. It is pertinent that a similar detailed study of *Aepyprymnus* be conducted because this species still occupies much of its former range. When this has been achieved, I would suggest that some of the studies reported in this thesis — for example, Chapters 5, 7 and 8 be repeated with diets that conform more with those eaten by free-living potoroine marsupials. I suspect that direct comparisons between species might be inappropriate because *A. rufescens* is probably a rhizophagous species.

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