Chapter 6

THE EFFECT OF TANNINS ON DIGESTION IN THE BRUSHTAIL POSSUM

6.1 Introduction

The ability of some plant polyphenols to precipitate proteins is central to the development of many of the recent theories of plant defense against herbivores. Most of the early theories (Feeney, 1976, Rhoades and Cates, 1976) classified tannins as generalized digestibility reducing compounds which precipitate salivary and dietary proteins and inactivate microbial enzymes. However, these authors did not recognize the specificity of tannin-protein interactions nor the variety of factors affecting the occurrence or stability of protein-tannin complexes (Hagerman and Butler, 1981).

Tannins have been suggested to be the basis of food choice in a range of eutherians (e.g. *Colobus guereza* : Oates *et al.* 1977) and marsupials (e.g. *Trichosurus vulpecula* : Freeland and Winter, 1975). Generally the approach in these studies has been to look for correlations between food choice and tannin content, although in some cases tannins are implicated in food choice without their presence having been demonstrated (e.g. Glander, 1978).

Eucalyptus foliage contains a wide variety of tannins and other polyphenolic compounds in high concentrations (Hillis, 1966, MacCauley and Fox, 1980). However, Fox and MacCauley (1977) were unable to show any effect on the growth, survival, or efficiency of nitrogen use in *Paropsis atomaria* larvae fed eucalypt foliage of various species and tannin concentrations. This may be because insects have several adaptations for coping with dietary tannins not found in mammalian herbivores. These include the maintenance of high pH in the hindgut (Berenbaum 1980) and the surfactant nature of some insect gut fluids (Martin and Martin, 1984). Also, Bernays (unpub. in Bernays, 1981) has shown that some

eucalypt-feeding ascarids can sequester tannins in special cells in the midgut.

Initially the approach taken in the studies described in this chapter was to correlate various measures of tannin content with parameters of nitrogen and fibre digestibility in both possum species. This approach yielded no useful relationships, possibly because the range of tannin intakes was so limited. Consequently, an experimental approach was adopted by using the tannin decomplexing agent PEG 4000 added to the drinking water.

6.2 **Materials and methods**

6.2.1 **Leaf phenolics**

The total phenol and leucoanthocyanidin content and the astringency of extracts of both *E. radiata* and *E. melliodora* were determined as described in Section 2.4.8. Total daily intakes of these substances were regressed against data on the intake and digestibility of dry matter, cell walls, and nitrogen from Chapters 4 and 5.

6.2.2 **PEG Supplementation.**

A preliminary experiment was conducted to assess the possibility of supplementing the drinking water of both Greater Gliders and Brushtail Possums with PEG 4000. Greater Gliders refused all water with PEG added. Brushtail Possums would not accept solutions of greater than 15% PEG (w/v). Mannitol solutions of comparable osmolarity were tested as control treatments but this resulted in diahorrea in one Brushtail while two other animals refused to drink after the first two days. Subsequently, control animals were offered only tap water.

In Experiment 1, three Brushtail Possums were supplemented with PEG at a level of 100% of estimated condensed tannin intake. The

concentration of PEG was varied so that variation among animals in their water intakes resulted in similar intakes of PEG. Animals were offered *E. melliodora* foliage *ad libitum* together with the PEG solution for 21 days. The first week served as an adaptation period and collections of faeces and urine were made during the second and third weeks

In Experiment 2, four Brushtail Possums were allocated between two treatments. The test animals were supplemented with PEG at a level of 100% of estimated condensed tannin intake. The other animals received tap water only. After a seven-day adaptation period, faeces and urine were collected for a further seven days. At the end of this collection period, the treatments were reversed and collections made for the last seven days of a further two-week period.

In both experiments, the intake and excretion of dry matter, cell contents, cell wall constituents, nitrogen and energy were determined as in Chapters 4, 5 and 8.

6.2.3 **Statistical.**

When intakes of dry matter, energy, fibre or nitrogen were significantly different between treatments, analysis of covariance (AOCV: Snedecor and Cochran 1967) was used to adjust all dependent means. The low numbers of observations of each treatment in Experiment 2 lowered the sensitivity of tests for differences between treatments. Consequently, comparisons of AOCV adjusted means were made between all PEG treatments and all other experiments (See Chapters 4,5 and 8) in which no PEG was offered (n = 22, fibre; n = 20, energy and nitrogen). This increased the power of the tests by increasing the degrees of freedom but it also increased between-experiment error. Also, in some cases, the slopes of the relationships between adjusted dependent and independent variables were not equal between the two treatments.

TABLE 6.1: *Content of total phenols, leucoanthocyanidins and the relative astringency of methanolic extracts of E. radiata and E. melliodora foliage fed to Greater Gliders and Brushtail Possums respectively (values exptessed as % DM)*

Experiment	Total phenols[1]	Leucoantho-cyanidins	Relative astringency
E. radiata			
P1	21.8	8.8 ± 0.6	9.9 ± 1.1
P2	14.3	3.2 ± 0.9	4.8 ± 0.9
P3	22.7	9.6 ± 0.8	8.7 ± 1.1
P4[2]	23.5	9.9	5.9
P5[2]	18.4	16.7	10.2
P6[2]	18.6	5.6	6.4
P7	9.2	5.7 ± 1.0	5.1 ± 1.9
P8	11.1	2.1 ± 0.6	3.2 ± 0.7
E. melliodora			
T1	27.9	22.2 ± 0.7	4.0 ± 0.7
T2	29.1	23.6 ± 1.5	3.5 ± 0.2
T3	27.9	20.0 ± 1.8	2.7 ± 0.8
T4[2]	27.0	19.0	2.1
T5[2]	27.1	20.0	3.0
PEG1	24.8	17.6 ± 0.3	3.1 ± 0.9
PEG2	19.9	16.8 ± 0.4	3.0 ± 0.9

[1] From Tables 4.1a and 4.1b

[2] Single bulked samples

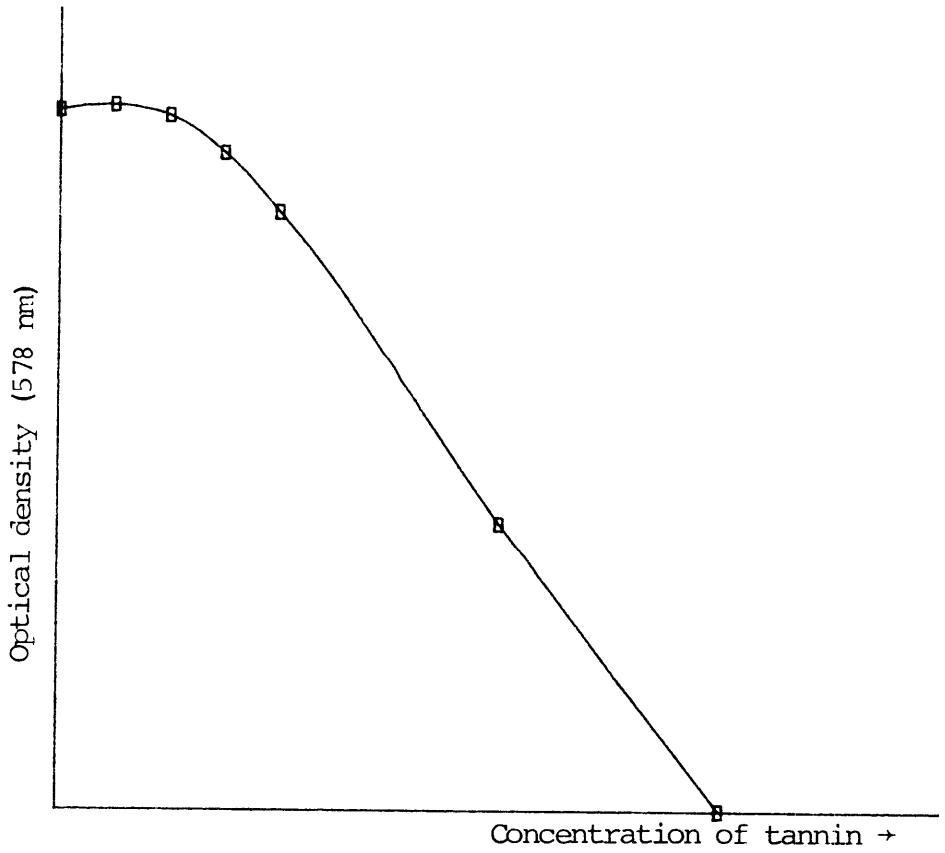

FIGURE 6.1 Effect of adding graded levels of eucalypt tannin on the optical density (578nm) of a solution of ovine blood in Tris-HCl buffer.

TABLE 6.2: *Intake, excretion and apparent digestibility of dry matter by Brushtail Possums supplemented with PEG (values expressed as* $g.kg^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment/ treatment		Intake	Faecal excretion	Digestibility (%)	Digestible intake
1	1(+PEG)	44.9 ± 1.9	21.6 ± 1.4	51.9 ± 2.0	23.3 ± 1.3
	2(+PEG)	44.5 ± 3.3	20.3 ± 1.0	54.1 ± 1.5	24.2 ± 2.4
2	3(+PEG)	52.3 ± 2.6	29.0 ± 1.7	44.5 ± 1.0	23.2 ± 1.1
	4(-PEG)	43.0 ± 2.7	23.1 ± 1.5	46.0 ± 2.3	19.8 ± 1.7
Mean (treatments 1-3)		47.8 ± 1.9	24.2 ± 1.5	49.6 ± 1.6	23.5 ± 0.8
Range (treatments 1-3)		38.4-56.9	18.8-32.5	42.7-56.0	19.6-27.4

TABLE 6.3: *Intake, excretion and apparent digestibility of cell contents by Brushtail Possums supplemented with PEG (values expressed as* $g.kg^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment/ treatment		Intake	Faecal excretion	Digestibility (%)	Digestible intake
1	1 (+PEG)	31.3 ± 1.3	14.4 ± 0.8	54.0 ± 2.3	17.0 ± 1.1
	2 (+PEG)	31.0 ± 2.3	14.0 ± 0.5	54.5 ± 2.0	17.0 ± 1.9
2	3 (+PEG)	36.9 ± 2.1	20.5 ± 0.6	44.1 ± 2.6	16.4 ± 1.8
	4 (-PEG)	30.4 ± 2.0	14.3 ± 1.1	52.8 ± 1.8	16.1 ± 1.2
Mean (treatments 1-3)		33.4 ± 1.4	16.7 ± 1.1	50.2 ± 2.1	16.7 ± 0.9
Range (treatments 1-3)		26.7 - 40.1	13.2 - 21.7	37.3 - 58.5	11.5 - 19.8

TABLE 6.4: *Intake, faecal excretion and apparent digestibility of E. melliodora cell wall constituents in Brushtail Possums supplemented with PEG (values expressed as* $g.kg^{-0.75}.d^{-1}$*) (Mean ± SE)*

Constituent	Experiment/ treatment		Intake	Faecal excretion	Digesti- bility (%)	Digestible intake
NEUTRAL DETERGENT FIBRE	1	1(+PEG)	13.6 ± 0.6	7.2 ± 0.6	46.9 ± 2.4	6.4 ± 0.2
		2(+PEG)	13.5 ± 0.9	6.3 ± 0.5	53.3 ± 1.4	7.2 ± 0.5
	2	3(+PEG)	15.4 ± 0.6	8.7 ± 1.1	43.9 ± 5.3	6.7 ± 0.6
		4(-PEG)	12.6 ± 0.7	8.8 ± 0.5	29.7 ± 3.7	3.8 ± 0.6
	Mean (treatments 1-3)		14.3 ± 0.5	7.5 ± 0.6	48.1 ± 2.7	6.8 ± 0.3
	Range (treatments 1-3)		11.7-16.7	5.1-10.9	35.2-63.7	5.9-8.9
HEMI-CELLULOSE	1	1(+PEG)	1.8 ± 0.3	-0.5 ± 0.0	126.2 ± 4.2	2.3 ± 0.3
		2(+PEG)	2.2 ± 0.1	-0.7 ± 0.1	130.3 ± 3.8	2.8 ± 0.1
	2	3(+PEG)	3.2 ± 0.2	-1.3 ± 0.7	137.7 ± 17.6	4.5 ± 0.8
		4(-PEG)	2.6 ± 0.3	1.2 ± 0.1	55.4 ± 4.0	1.5 ± 0.2
	Mean (treatments 1-3)		2.5 ± 0.2	-0.8 ± 0.3	131.1 ± 6.0	3.3 ± 0.4
	Range (treatments 1-3)		1.3-3.6	-2.8--0.2	108.4-177.4	1.8-6.4

ACID DETERGENT FIBRE	1	1(+PEG)	11.8 ± 0.6	7.7 ± 0.6	34.6 ± 3.3	4.1 ± 0.4
		2(+PEG)	11.4 ± 0.9	7.0 ± 0.4	38.5 ± 2.1	4.4 ± 0.5
	2	3(+PEG)	12.2 ± 0.7	10.0 ± 0.6	17.6 ± 2.2	2.2 ± 0.3
		4(-PEG)	10.0 ± 0.5	7.7 ± 0.4	22.9 ± 4.7	2.3 ± 0.5
	Mean (treatments 1-3)		11.8 ± 0.4	8.3 ± 0.5	29.9 ± 3.1	3.5 ± 0.4
	Range (treatments 1-3)		9.7-13.5	6.4-11.5	15.4-41.4	2.1-5.1
CELLULOSE	1	1(+PEG)	7.7 ± 0.4	3.4 ± 0.4	56.0 ± 6.2	4.3 ± 0.6
		2(+PEG)	6.8 ± 0.6	2.7 ± 0.3	58.7 ± 7.7	4.1 ± 0.8
	2	3(+PEG)	6.2 ± 0.3	3.8 ± 0.3	38.5 ± 4.1	2.4 ± 0.3
		4(-PEG)	5.0 ± 0.4	3.6 ± 0.3	28.0 ± 9.0	1.5 ± 0.5
	Mean (treatments 1-3)		6.7 ± 0.3	3.2 ± 0.2	51.4 ± 3.8	3.5 ± 0.4
	Range (treatments 1-3)		5.2-8.3	2.4-4.5	32.4-67.8	2.1-5.6
LIGNIN	1	1(+PEG)	4.1 ± 0.4	4.4 ± 0.3	-6.8 ± 5.2	-0.3 ± 0.2
		2(+PEG)	4.6 ± 0.3	4.2 ± 0.6	1.2 ± 4.3	0.1 ± 0.2
	2	3(+PEG)	6.0 ± 0.4	6.3 ± 0.5	-3.5 ± 1.6	-0.2 ± 0.1
		4(-PEG)	4.9 ± 0.1	5.1 ± 0.2	16.8 ± 2.5	0.8 ± 0.1
	Mean (treatments 1-3)		5.1 ± 0.4	5.1 ± 0.4	0.0 ± 3.1	0.0 ± 0.1
	Range (treatments 1-3)		3.5-7.0	3.1-7.5	-14.4-21.2	-0.6-0.8

TABLE 6.5: *Intake, excretion and digestibility of nitrogen in Brushtail Possums supplemented with PEG (values expressed as $g.kg^{-0.75}.d^{-1}$ unless stated otherwise)*

Experiment/ treatment	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Nitrogen balance	Apparent digestibility (%)	Non-dietary faecal nitrogen			True digestibility (%)	Truly digestible nitrogen intake
						$g.kg^{-0.75}.d^{-1}$	g.kg DMI	g.kg DMO		
1 +PEG	0.78 ± 0.04	0.43 ± 0.02	0.23 ± 0.03	0.12 ± 0.04	44.6 ± 0.6	0.32 ± 0.03	7.2 ± 0.3	14.9 ± 0.5	85.8 ± 1.3	0.67 ± 0.04
2 +PEG	0.81 ± 0.06	0.40 ± 0.02	0.22 ± 0.03	0.19 ± 0.17	50.9 ± 1.7	0.31 ± 0.02	7.0 ± 0.2	15.2 ± 0.03	89.5 ± 0.8	0.72 ± 0.06
3 +PEG	0.75 ± 0.04	0.49 ± 0.01	0.17 ± 0.02	0.09 ± 0.03	41.7 ± 4.6	0.30 ± 0.03	5.8 ± 0.2	10.4 ± 0.4	75.1 ± 3.1	0.57 ± 0.05
4 -PEG	0.61 ± 0.03	0.45 ± 0.03	0.21 ± 0.04	-0.05 ± 0.05	26.6 ± 2.1	0.25 ± 0.04	5.7 ± 0.5	10.5 ± 0.9	66.4 ± 2.5	0.41 ± 0.04
MEAN (treatments 1-3)	0.78 ± 0.02	0.44 ± 0.02	0.20 ± 0.01	0.13 ± 0.03	42.6 ± 2.4	0.31 ± 0.01	6.6 ± 0.3	13.2 ± 0.8	82.6 ± 2.4	0.64 ± 0.03
RANGE (treatments 1-3)	0.65-0.91	0.37-0.51	0.13-0.27	-0.01-0.32	30.1-53.3	0.23-0.37	5.2-7.7	9.6-15.5	66.2-90.9	0.43-0.81

TABLE 6.6: *Intake and excretion of energy by Brushtail Possums supplemented with PEG (values expressed as $MJ.kgW^{-0.75}.d^{-1}$) (Mean ± SE)*

Experiment/ treatment		Gross energy intake	Digestible energy intake	Metabolizable energy intake	Faecal energy excretion	Urinary energy excretion
1	1 (+PEG)	0.94 ± 0.04	0.52 ± 0.03	0.43 ± 0.03	0.42 ± 0.03	0.09 ± 0.00
	2 (+PEG)	0.94 ± 0.07	0.56 ± 0.05	0.48 ± 0.05	0.38 ± 0.02	0.07 ± 0.01
2	3 (+PEG)	1.10 ± 0.06	0.54 ± 0.02	0.44 ± 0.03	0.56 ± 0.04	0.10 ± 0.02
	4 (-PEG)	0.91 ± 0.06	0.38 ± 0.04	0.28 ± 0.06	0.54 ± 0.04	0.10 ± 0.03
Mean (treatments 1-3)		1.00 ± 0.04	0.54 ± 0.02	0.45 ± 0.02	0.47 ± 0.03	0.09 ± 0.01
Range (treatments 1-3)		0.81-1.23	0.46-0.62	0.34-0.55	0.35-0.65	0.04-0.14

TABLE 6.7: *Effect of PEG4000 on the determination of cell wall constitutents of Brushtail Possum faeces*

Fibre component[1] (% faecal DM)	Control	+10% PEG	+20% PEG
NDF	42.88	42.60	42.62
"Hemicellulose"	6.03	3.70	3.28
ADF	36.85	38.90	39.45
"Cellulose"	14.26	16.01	18.07
Lignin	22.59	22.89	21.27

[1] Means of duplicate samples (difference between duplicates <1.5%)

6.3 Results

6.3.1 Relationships between leaf phenolics and leaf intake

Table 6.1 gives details of the content of total phenolics and leucoanthocyanidins and the astringency of extracts of the foliages used in all feeding experiments. Leucoanthocyanidins comprised a greater proportion of the total phenolic fraction of *E. melliodora* than E. *radiata* but in contrast the astringency of *E. radiata* phenolics was a greater proportion of both the total phenolics and leucoanthocyanidins than *E. melliodora*. There was no significant relationship between any of the measures of tannin content (total phenolics, leucoanthocyanidins, or astringency) and the intake and digestibility of dry matter, cell wall constituents and nitrogen. The effect of graded levels of eucalypt tannin on the optical density of a solution of ovine blood in Tris-HCl buffer is shown in Figure 6.1. Tannin concentration had to reach a threshold level before precipitation of the haemoglobin occurred.

6.3.2 Brushtail Possums: PEG Supplementation.

Tables 6.2 to 6.6 give details of the intake and excretion of dry matter, fibre components, total cell contents, nitrogen and energy in Brushtail Possums supplemented with PEG in Experiment 1 and Experiment 2.

Experiment 1

There were no significant differences between collection periods in the intake, excretion and digestibility of any measured component. PEG appeared to interfere with the determination of some components of dietary fibre. Adding PEG to the neutral-detergent extractions of control faecal samples had no effect on NDF content (Table 6.7). However the ADF content of faecal samples fraction was higher when PEG was added to extractions and so the hemicellulose was underestimated and the cellulose overestimated. There was no apparent effect on the determination of lignin. PEG was soluble in both acid- and neutral-detergents but PEG-eucalypt tannin complexes were insoluble in acid detergent solutions. Pre-extraction of

TABLE 6.8: *Means of all feeding experiments with Brushtail Possums supplemented with or lacking PEG*

Parameter	+PEG[1]	-PEG[2]	Significance of differences between means at similar levels of intake
Dry matter intake (g)	47.8 ± 1.9	36.8 ± 1.2	--
Faecal dry matter	24.2 ± 1.5	18.4 ± 0.7	n.s.
DM digestibility (%)	49.6 ± 1.6	50.0 ± 0.6	n.s.
Digestible DM intake	23.5 ± 0.8	18.4 ± 0.6	n.s.
NDF intake (g)	14.3 ± 0.5	10.6 ± 0.4	--
Faecal NDF	7.5 ± 0.6	7.6 ± 0.2	***
NDF digestibility (%)	48.1 ± 2.7	27.1 ± 2.1	**
Digestible NDF intake	6.8 ± 0.3	3.0 ± 0.3	***
Cell contents intake (g)	33.4 ± 1.4	26.3 ± 0.9	--
Faecal cell contents	16.7 ± 1.1	10.8 ± 0.6	*
Cell content digestibility (%)	50.2 ± 2.1	59.1 ± 1.1	*
Digestible cell content intake	16.7 ± 0.9	15.5 ± 0.5	*
Nitrogen intake (g)	0.78 ± 0.02	0.59 ± 0.02	--
Faecal excretion	0.44 ± 0.02	0.40 ± 0.02	n.s.
Urinary excretion	0.20 ± 0.01	0.17 ± 0.01	*
Nitrogen balance	0.13 ± 0.03	0.02 ± 0.02	n.s.
Apparent digestibility (%) of nitrogen	42.6 ± 2.4	31.9 ± 1.7	n.s.
Non-dietary faecal nitrogen	0.31 ± 0.01	0.25 ± 0.01	n.s.
True digestibility (%) of nitrogen	82.6 ± 2.4	74.9 ± 1.5	*
Truly digestible intake	0.64 ± 0.03	0.44 ± 0.01	*
Gross energy intake (MJ)	1.00 ± 0.04	0.77 ± 0.02	--
Digestible energy intake	0.54 ± 0.02	0.35 ± 0.01	***
Metabolizable energy intake	0.45 ± 0.02	0.27 ± 0.01	***
Faecal energy excretion	0.47 ± 0.03	0.43 ± 0.02	***
Urinary energy excretion	0.09 ± 0.01	0.08 ± 0.01	n.s.

[1] Tables 6.2-6.6

[2] Chapters 4, 5 and 8, including treatment 4 (Experiment 2) this chapter.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$; n.s. Not significant

the faecal samples with water had no effect on the apparent interference.

Experiment 2

The intakes of dry matter, nitrogen, and NDF were higher ($P<0.05$) in those animals offered the PEG solutions instead of the normal drinking water. At similar levels of intake, there were no significant differences in faecal dry matter excretion, dry matter digestibility or the intake of digestible dry matter between treatments. Similarly, there were no significant differences between controls and PEG treatments in faecal nitrogen excretion, urinary nitrogen excretion, nitrogen balance, apparent and true digestibility of nitrogen, truly digestible nitrogen intake or non-dietary faecal nitrogen excretion.

Although not significant, there were trends towards lower faecal excretion ($P<0.08$) and higher digestibility ($P<0.11$) and digestible intake ($P<0.08$) of NDF in the PEG supplemented animals. However, the intake of apparently digestible energy was higher ($P<0.01$) with PEG supplementation but there were no significant differences in faecal or urinary energy losses or in metabolizable energy intake. On the other hand the faecal excretion of cell solubles was higher ($P<0.01$) and the digestibility of this fraction lower ($P<0.05$) in the PEG supplemented group.

Comparison of all Observations

Table 6.8 summarizes the means of all experiments with and without PEG supplementation. The intake of all measured constituents (dry matter, nitrogen, NDF, cell contents, and gross energy) was higher ($P<0.001$) in the PEG supplemented animals than in the controls and so comparisons of dependent means were made at similar levels of intake.

There were no significant differences in faecal dry matter excretion, dry matter digestibility or intake of digestible dry matter between the two groups. However, there was a greater truly digestible nitrogen intake ($P<0.05$) and true digestibility of nitrogen ($P<0.05$) with

PEG supplementation compared with control treatments. In addition, there were trends ($P<0.15$) towards lower faecal nitrogen excretion and higher apparent digestibility of nitrogen with PEG supplementation.

On the other hand, faecal excretion of NDF was lower ($P<0.001$) and NDF digestibility ($P<0.01$) and intake of digestible NDF ($P<0.001$) higher in the animals that received PEG. DE and ME intakes were higher ($P<0.001$) on the PEG treatments as were faecal energy losses but there were no significant differences in urinary energy losses between the two treatments. The faecal excretion of cell contents was higher ($P<0.05$) and their digestibility lower ($P<0.05$) on the PEG treatments but there was still a higher ($P<0.05$) intake of digestible cell contents in the PEG supplemented group.

6.4 **Discussion**

The lack of a relationship between any parameter of dry matter, fibre or nitrogen digestibility and phenolic content among the feeding experiments, together with the later finding of significant effects on fibre digestibility in the Brushtail Possum, suggests that the three measures of "tannin" employed here do not describe completely the biological actions of eucalypt tannins. Alternatively, it may reflect the limited range of tannin intakes exhibited by the animals in these experiments.

There are as yet no completely satisfactory methods for determining the tannin content of a particular plant tissue. Much of the problem lies in defining, both chemically and biologically, exactly what constitutes a tannin and in the variability of extraction of tannins from different plants. The extraction and analytical procedures used here were similar to those recommended for eucalypt foliage by Fox and MacCauley (1977). However, these techniques have several limitations.

The measurement of total phenols by the Folin reaction has often been equated with "total tannin" (Burns, 1963). However, the technique

detects all -OH groups (including those of the amino acids tyrosine and tryptophan) and hence provides neither a direct nor an indirect measure of tannins. Nonetheless, phenolics need not necessarily precipitate protein to have nutritional effects (Jung and Fahey, 1983). The leucoanthocyanidin assay measures the content of condensed tannin precursors. This assay also measures non-tannin monomeric flavonoids and the extent of colour development depends on the molecular structure as well as the total amount of the compound present (Martin and Martin, 1982).

The modified haemanalysis assay of Bate-Smith (1973) was chosen as a measure of the astringency or protein precipitating ability of the plant extract. This technique, together with others based on β-glucosidase (Goldstein and Swain, 1965) and bovine serum albumin (BSA) (Hagerman and Butler, 1982) aim to measure the biological activity of tannins. It was significant then, that eucalypt tannins had to reach a threshold concentration (Figure 6.1) before haemoglobin precipitation commenced. However, it is questionable whether proteins such as BSA and haemoglobin are representative of the types of proteins found either in plant tissues or as microbial enzymes. Perhaps an assay based on ribulose bisphosphate carboxylase might be suitable as a measure of the biological activity of a tannin, since this is specifically a plant enzyme (Martin and Martin, 1983).

Polyethylene glycol 4000 is a water soluble compound which is widely used as an inert digesta marker (Hyden, 1955, Teeter and Owens, 1983). However, it has been shown to be unsuitable for use with feeds containing tannins (Kay, 1969) since it complexes with and is precipitated by those tannins. This property has been exploited and used to improve plant enzyme extraction techniques (Loomis and Bataille, 1966) and to counter the effects of tannins in nutritional studies (Jones and Mangan, 1977, Ford and Hewitt, 1979).

While several of the measured parameters of nitrogen metabolism in Brushtails were apparently affected by PEG supplementation, this influence cannot be unequivocally attributed to a reduced influence of tannins. Although truly digestible nitrogen intake and the true digestibility of

nitrogen were significantly higher than the controls when all experiments were compared, this can be attributed to the higher NDF digestibility found on the PEG treatments. Non dietary faecal nitrogen (NDFN), and hence the true digestibility of nitrogen, was estimated using the indirect method of Mason (1969) which assumes that the only undigested dietary nitrogen present in faeces is that associated with plant cell walls (see Section 5.4 for further discussion). Hence the higher the NDF digestibility, the higher the true digestibility of nitrogen and the truly digestible nitrogen intake as determined by Mason's (1969) method. The true digestibility of nitrogen and the truly digestible nitrogen intake could be overestimated if dietary proteins formed complexes with leaf tannins and were excreted in the faeces. This may also explain the trend towards a higher apparent digestibility of nitrogen with PEG supplementation. Alternatively, Tamir and Alumot (1970) showed that the production of proteolytic enzymes in the hindgut of guinea pigs was stimulated by the inclusion of carob (*Ceratonia siliqua*) tannins in the diet. However, the similarity in NDFN excretion between PEG treatments and controls suggests that higher apparent nitrogen digestibilities may have been due to prevention of binding of tannins with dietary proteins rather than to a reduction in endogenous secretions.

Much of the nitrogen found in *Eucalyptus* leaf occurs as non-protein nitrogen, particularly free amino acids (Journet and Cochrane, 1976, Chapter 4), a form of nitrogen not expected to be bound by tannins (McLeod, 1974, Zucker, 1983). For example, Hagerman and Butler (1981) have shown that the amino acids glycine, proline and alanine have affinities for sorghum proanthocyanidins which are several thousand times lower than those of proteins such as bovine serum albumin or rat parotid saliva. Much of the remaining leaf nitrogen appears to consist of the enzyme ribulose bisphospate carboxylase (Mooney *et al.*, 1978) and this protein would be expected to form complexes with a variety of tannins (Martin and Martin 1983). In fact Lomdahl (1983) has shown that cytoplasmic constituents of *E. ovata* leaf are complexed by vacuolar tannins in the stomach of the Ringtail Possum. The formation of complexes depended on the release of tannins from cell vacuoles and this was effected by mastication or by the action of HCl on intra-cellular membranes.

Using histochemical techniques, Lomdahl (1983) was able to show that cytoplasm-tannin complexes were stable in the small intestine but that a mixture of bacteria appeared to degrade the complexes in the caecum. A similar situation may occur with *E. melliodora* in the Brushtail Possum. While bacteria capable of utilizing condensed tannins as a sole carbon source are known from soil (Grant, 1976), nothing is known of their occurrence in the gastrointestinal tract flora. The value of this process to the animal's nutrition would depend on the nature of the substrates that the bacteria were actually degrading in Lomdahl's (1983) study. Presumably, if nitrogenous compounds were released from the tannin complexes, they would be rapidly fermented and incorporated into microbial protein or absorbed from hindgut as ammonia.

The effect of tannin complexing may be to shift the site of digestion of cytoplasmic proteins from the small intestine to the hindgut. Material digested in the small intestine would be expected to be of a higher biological value to the animal and so nitrogen balance should be improved when the effects of the tannins are removed. The data from the PEG supplementation experiments do not support this conclusion since at similar levels of nitrogen intake there was no difference in nitrogen balance between the two groups. Alternatively, if nitrogen was absorbed in the same form from both the small intestine and the hindgut, there would be no difference in the observed nitrogen balance. While there has been much speculation as to whether amino acids can be actively absorbed from the caecum (e.g. Slade *et al.*, 1971, Parra, 1978, Section 1.3.3) much of the data is equivocal and the balance of evidence suggests that any absorption from the hindgut is of minor quantitative significance.

The effects of the PEG treatment on digestion of cell walls were much more pronounced. When all observations were compared, the significant increase in NDF digestibility together with the higher intake of NDF led to a significantly higher intake of digestible cell walls. This suggests that fibre was cleared from the hindgut more rapidly and the animals were able to maintain higher dry matter intakes.

Little can be said about the digestion of ADF, hemicellulose and cellulose since there appeared to be some interference in the measurements of these fractions in the faeces due to the excreted PEG. Van Soest (1982) has suggested that while tannin-protein complexes are soluble in neutral detergent solutions, they are mostly recovered in acid-detergent residues. Similarly, some polyphenols may be condensed by acid boiling and be precipitated during the acid detergent fibre procedure.

The higher NDF digestibilities, together with the higher intake of dry matter, led to higher intakes of digestible and metabolizable energy. About half the differences in the mean DE intake could be accounted for by the increased intake of digestible cell walls. Cell walls contributed 178 kJ to the digestible energy intake when PEG was included in the diet compared with only 79 kJ on the control treatments. Since there was no difference in urinary energy losses between the two treatments, the absorbed energy must have been highly metabolizable and mostly retained in the body. Some may have been lost as heat and gas during the more extensive fermentation that must have taken place during the digestion of the extra fibre but these sources of energy loss were not measured.

Although the digestibility of NDF was increased by the PEG treatments, digestibility of lignin and cell contents decreased, resulting in no increase in the digestibility of total dietary dry matter. There are several possible reasons for this. Much of the soluble faecal dry matter will consist of microbial cells (Mason 1969, Ørskov *et al.*, 1973, Van Soest 1982). If the higher NDF digestibilities reflect higher hindgut microbial activity, then microbial cell output in the faeces should also be higher and the apparent digestibility of dry matter lower. Secondly, Gaillard and Richards (1975) have shown that soluble lignin-carbohydrate complexes occur in rumen fluid, and it has been suggested (Fahey and Jung, 1983) that this is part of the reason for the occurrence of positive lignin digestibilities. If a similar situation exists with *E. melliodora* lignins, PEG could have precipitated these soluble complexes and they would be recovered in the faeces. Finally, since the majority of the cell contents consisted of total phenolics, this too is likely to have been precipitated by PEG and recovered in the faeces (Mould and Robbins, 1982).

The higher digestible intake of cell walls with PEG supplementation may be due to the removal of the inhibitory effects of tannins on microbial cellulases. A similar explanation has been advanced by Barry and Duncan (1984) to explain the low digestibility of the cellulose and hemicellulose of high tannin varieties of *Lotus pedunculatus* by sheep. As with the present study, supplementation with PEG resulted in increased intakes of organic matter and increased digestibilities of the fibre fraction.

The inhibition of cellulases by tannins *in vitro* is well documented (Smart *et al.*, 1961, Mandels and Reese, 1965, Lyford *et al* 1967, Griffiths and Jones, 1977). However, it could be argued that, unlike the situation in foregut fermenters, by the time tannins reach the hindgut they would already have been complexed with dietary proteins or with proteins from the gut wall or with enzymes such as proteases and so would not be free to complex with microbial cellulases.

There are at least three possible explanations for tannin binding to cellulases in the hindgut of the Brushtail Possum. First, the tannins responsible for the inhibition could be very specific for cellulases. Hagerman and Butler (1981) have shown that the degree of affinity of tannins for proteins depends on the structure of the tannin as well as the isoelectric point of the protein and the extent of exposed peptide bonds of the protein. Second, condensed tannins could occur bound to the cellulose and pectins of the cell wall (Zucker, 1983). This would help to ensure a high specificity for microbial enzymes. Third, tannins could be released from protein-tannin complexes either by bacterial degradation (Lomdahl, 1983), or else by the effect of a change in pH (Jones and Mangan, 1977) in the hindgut.

No evidence is available to assess the merits of any of these scenarios. Nonetheless, it is worth noting that enzymes that are complexed with tannins *in vitro* still retain significant catalytic function (Goldstein and Swain, 1965), suggesting that some active sites must be shielded from the actions of the complexing tannins. Treatment with decomplexing agents such as PEG 4000 or polyvinylpyrrolidine restore the

full catalytic effect of the enzyme (Goldstein and Swain, 1965). It was not possible to tell from Lomdahl's (1983) study whether tannins formed complexes with cellulases secreted by bacteria attached to or near the cell wall. However, an electron dense matrix, similar in appearance to tanned cytoplasm constituents, was often seen surrounding bacteria which were attached to the cell wall.

PEG supplementation resulted in an increase in dry matter intake by the Brushtail Possum to a level similar to that found in Greater Gliders, Koalas, and Ringtail Possums (Chapter 4). It was suggested earlier that one reason why the Brushtail Possum fed to only a limited extent on the highly fibrous tissues of *Eucalyptus* foliage was that it lacked a separation mechanism in the hindgut to rapidly clear dietary fibre. It appears that once the interfering action of tannins was eliminated by the action of PEG, this barrier to intake was removed and dry matter intake increased. In Lomdahl's (1983) study, it was only after the cell wall was degraded that the caecal bacteria were able to attack the tanned cytoplasm elements. Hence, material would have to be retained in the caecum long enough for cell wall digestion to occur and then for a further period for these complexes to be broken down.

Before the importance of dietary tannins can be assessed more completely in animals such as the Brushtail Possum, much more information is needed on the structure of eucalypt tannins. The information needed includes the molecular weights and structures of tannins from various eucalypt species, the factors that affect the binding of various proteins, the stability of tannin complexes at various pH's and the fate of tannins degraded in the hindgut.

6.5 **Summary**

Supplementation of eucalypt diets with PEG 4000 resulted in higher intakes of dry matter and metabolizable energy compared with control treatments in Brushtail Possums. Similarly, animals on PEG treatments had higher NDF digestibilities but lower digestibilities of cell contents

compared with controls. There was little effect of PEG on the digestibility and retention of nitrogen. It was suggested that *E. melliodora* tannins had complexed with microbial cellulases and hence reduced the digestibility of plant cell walls. The need to investigate the structure and binding characteristics of eucalypt tannins was stressed.

Chapter 7

ESSENTIAL OIL METABOLISM

7.1 Introduction

There has long been speculation about the effects that essential oils of *Eucalyptus* spp. might have on phytophagous animals (Pratt, 1937, Fleay, 1937, Betts, 1978). Most of these studies have used a correlative approach to look for relationships between the level and/or composition of leaf oils and feeding preferences of some mammals (e.g. Koala: Southwell, 1978) and insects (*Paropsis* : Morrow and Fox, 1980). The lack of associations has led some authors (e.g. Degabriele, 1981) to dismiss eucalypt oils as having no influence on the regulation of animal populations.

However, even if oils do not affect gross food preference, their ingestion still results in a metabolic cost for detoxification (Cleland, 1946, Hinks and Bolliger, 1957a,b) and their biological actions still have the potential to affect animal gut microbial populations (Freeland and Janzen, 1974). Several studies have demonstrated a deleterious effect of mono and sesquiterpenes on ruminal fermentation (Nagy *et al.*, 1964, Nagy and Tengerdy, 1968, Oh *et al.*, 1967, 1968). However, all these studies have been performed *in vitro* without allowing for absorption of the oils. In other cases, the concentrations of oils used have clearly been too high. In any case, hindgut fermenters like the Greater Glider and Brushtail Possum may be able to absorb and detoxify essential oils before they reach the site of microbial activity and thus avoid oil-microbe interactions (Section 1.3.5).

In this chapter, the pattern of absorption of essential oils from the gut of both the Greater Glider and Brushtail Possum was assessed by extraction of the volatile material and analysis by gas-liquid chromatography. This suggested that there was indeed little oil reaching the hindgut. Consequently, the hypothesis that efficient mastication might

result in a loss of oil during ingestion was tested in Greater Gliders by collecting and analysing samples of expired air.

7.2 Materials and Methods

7.2.1 Experiment 1

Three Greater Gliders (one male, two females) were maintained on *E. radiata* foliage as outlined in Chapter 4. The animals were offered foliage from only a single tree for 10 days and the drinking water was supplemented with Cr-EDTA (0.28 mg Cr/ml) prepared according to Binnerts *et al.* (1968). Faeces were collected for the last five days and then the animals were killed by an overdose of sodium pentobarbitone. The digestive tract was quickly excised, and the contents of the stomach, small intestine, caecum, proximal colon, distal colon and rectum removed and stored in plastic bags at -15° C. The samples were steam distilled and analysed as described in Section 2.4.7.

7.2.2 Experiment 2

Six Greater Gliders (one male, five female) and three male Brushtail Possums were fed foliage from an individual *E. radiata* or *E. melliodora* tree respectively for 14 days. Leaf and faecal samples were collected for the last five days and stored and analysed as described above. Mass spectrometric analyses were performed as described in Section 2.4.7.

7.2.3 Experiment 3

Three female Greater Gliders and three male Brushtail Possums were used in this experiment which was a repeat of that described in Experiment 1, except that no Cr-EDTA was added to the drinking water. Animals were killed one day apart at 1200 hours, five hours after feed was last available. Samples of leaf and digesta from each section of the gastro-intestinal tract were bulked within each species.

TABLE 7.1: *Yield (v/w) of essential oil from the foliage and from different parts of the gut of the Greater Glider and Brushtail Possum (% dry matter)*

Experiment/ species		Leaf	Stomach	Small intestine	Caecum/ proximal colon	Distal colon	Faeces	Urine (v/v)
1	Greater Glider	*E. radiata* 7.45 (3.20)[3]	--	--	--	--	0.1[1] (0.05)[3]	--
	Brushtail Possum	*E. melliodora* 0.82 (0.40)[3]	--	--	--	--	0.02[2] (0.01)[3]	--
2	Greater Glider[2]	*E. radiata* 11.05	6.59	Trace[4]	0.32	Trace	0.09	0.14
	Brushtail Possum[2]	*E. melliodora* 1.35	0.66	Trace	0.28	0.04	0.03	Trace

[1] Bulked samples of six animals

[2] Bulked samples of three animals

[3] Percentage wet weight

[4] Trace = < 0.01% or < 0.025 ml oil recovered

7.2.4 **Experiment 4**

The amount of terpene lost during mastication of leaf by Greater Gliders was measured after conversion of the respirometers described in Chapter 8 to an open flow system. Expired air was bubbled through two cyclohexane flasks which had been shown in preliminary studies to trap expired terpenes. At 0600 h on Day 1, leaves of an individual *E. radiata* tree were placed in the chamber and the pump started. At 1800 h a Greater Glider was placed in the chamber and allowed to feed normally. Fresh cyclohexane was placed in the traps. At 0600 h on Day 2, uneaten leaves, faeces and urine were removed from the chamber, fresh cyclohexane was placed in the traps and the animal left until 1800 hours when the experiment was terminated. This procedure was replicated three times. Samples for the two controls (leaf only, animal only) and the experimental treatment were bulked over the three replicates. Two runs in which a known volume of *E. radiata* essential oil was evaporated in the chamber were conducted to check recoveries. The cyclohexane was removed from each sample by fractional distillation on a series of Vigreaux and packed columns. The remaining material was analysed by gas-liquid chromatography (Section 2.4.7) using n-dodecane as an internal standard.

7.3 **Results**

7.3.1 **Experiment 1**

Only trace amounts of steam volatile material could be distilled from the faeces and gut contents of the three Greater Gliders that had received Cr-EDTA in the drinking water. All this material appeared oxidized and polymerized and would not pass through the GLC column even when taken up in di-ethyl ether or acetone. Therefore, in subsequent experiments no Cr-EDTA was offered but this lack of a reference marker meant that absorption could not be determined quantitatively.

TABLE 7.2a: *Major components (>1.0%) of the steam volatile oil from E. radiata and the concentration in digesta from different parts of the gut of the Greater Glider*

Peak number (Fig. 7.1a)	Identification	Experiment 2		Experiment 3			
		Leaf (percentage composition)	Faeces (percentage leaf)	Leaf (percentage composition	Percentage leaf		
					Stomach	Caecum	Faeces
1	α-pinene	4.9	23	6.6	81	34	5
5	α-phellandrene	9.3	40	16.5	103	8	40
6	α-terpinene	4.4	42	6.8	101	?[1]	74
8	1,8 cincole	4.3	43	5.1	100	36	192
9	γ-terpinene	7.5	30	14.6	105	?[1]	33
10	p-cymene	10.5	23	3.4	55	147	89
11	Terpinolene	2.2	41	4.3	105	37	49
15	Trans-p-menth-2en-1ol	4.5	107	2.6	124	157	190
16	Terpinen-4-ol	19.8	17	15.6	146	14	38
17	Cis-p-menth-2en-1ol	3.3	79	1.8	127	?[1]	200
18	Cis-piperitol	1.4	77	-	-	-	-
19	α-terpineol	2.1	42	1.5	117	10	19
20	Piperitone	-	-	1.3	172	121	58
21	Trans-piperitol	2.6	111	1.2	111	181	247
22	Unknown	1.0	32	1.8	69	?[1]	98
23	4-phenyl butanone	1.5	12	-	-	-	-
24	Unknown	1.1	63	-	-	-	-
26	γ-eudesmol	1.2	84	2.3	44	18	129
27	α-eudesmol	1.1	85	1.2	63	49	215
28	ß-eudesmol	1.6	84	1.2	79	60	333

[1] Solvents comprised >60% of area and peaks could not be accurately defined

TABLE 7.2b: *Major components (>1.0%) of the steam volatile oil from E. melliodora and the concentration in digesta from different parts of the gut of the Brushtail Possum*

Peak number (Fig.7.1b)	Identification	Experiment 2		Experiment 3			
		Leaf (percentage composition)	Faeces (percentage leaf)	Leaf (percentage composition)	Percentage leaf		
					Stomach	Caecum	Faeces
2	Iso-valeric aldehyde	2.0	124	-	-	-	-
3	α-pinene	7.5	101	3.8	96	83	19
5	Limonene	4.9	64	-	-	-	-
6	1,8 cineole	63.1	5	56.7	99	29	10
8	p-cymene	2.2	70	1.2	153	143	48
9	Terpinolene	-	-	4.2	69	144	95
13	α-terpineol	1.7	62	2.1	180	143	120
-	Unknown	-	-	1.1	130	281	220
-	Unknown	-	-	2.0	-	-	-
19	Unknown	1.8	87	5.1	-	-	-
-	Unknown	-	-	1.6	24	28	-

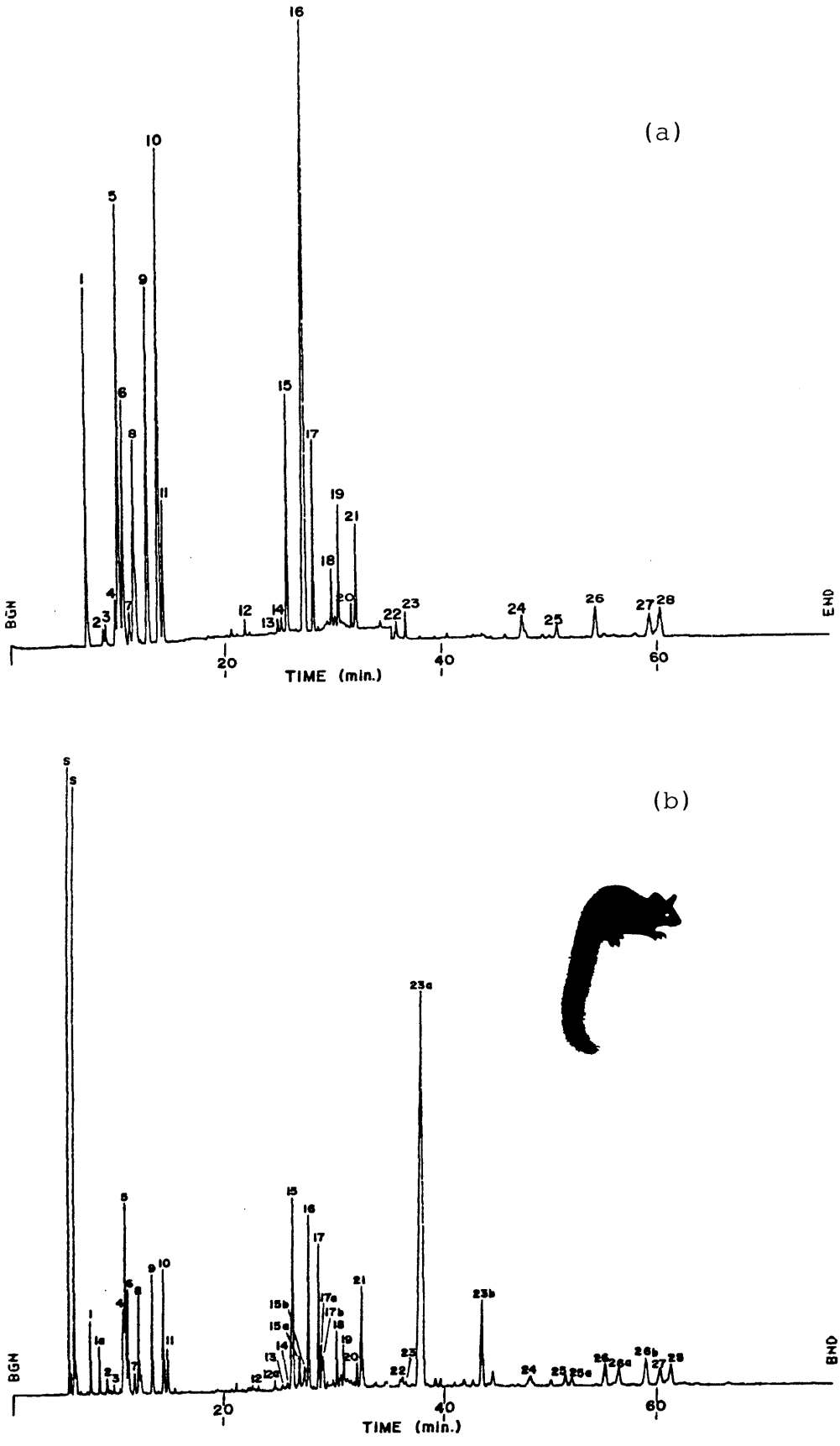

FIGURE 7.1a: *GLC traces of steam volatile essential oils from -*

(a) E. radiata *foliage; and*
(b) Greater Glider faeces. *(Exp 2).*

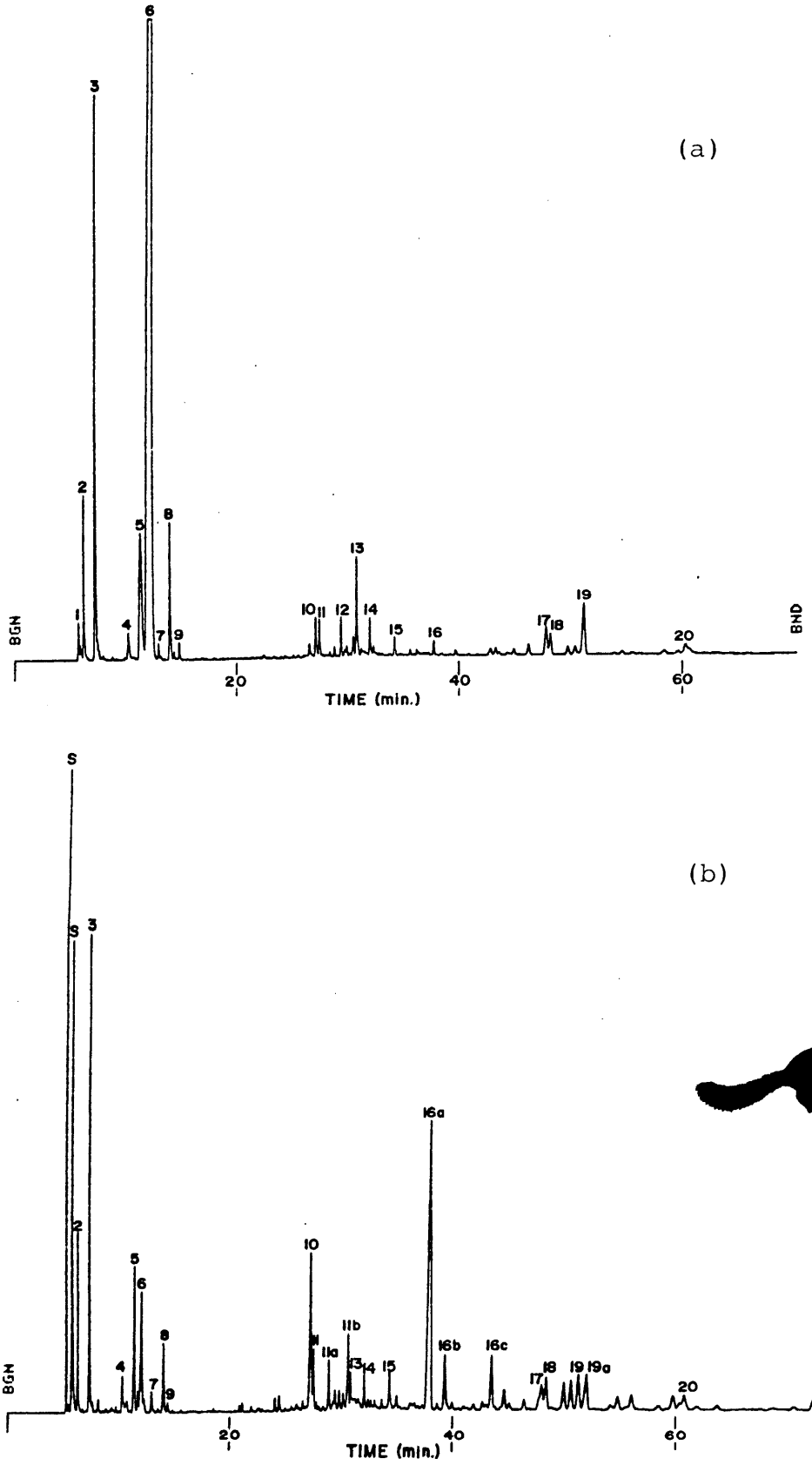

FIGURE 7.1b: *GLC traces of steam volatile essential oils from -*

(a) E. melliodora *foliage; and*
(b) Brushtail Possum faeces. *(Exp 2).*

7.3.2 **Experiment 2**

The yield and percentage composition of the major components of the steam volatile oils from *E. radiata* leaf and the corresponding Greater Glider faeces and from *E. melliodora* leaf and Brushtail Possum faeces are given in Tables 7.1 and 7.2. Full details of percentage composition of leaf and faeces oils are given in Appendix 1. GLC traces of the oil from leaf and faeces of each species are shown in Figures 7.1a and 7.1b. *E. radiata* oil was complex, consisting primarily of terpinen-4-ol (19.8%), p-cymene (10.5%), α-phellandrene (9.3%) and γ terpinene (7.5%). *E. melliodora* oil was much simpler, being dominated by 1,8-cineole (63%) with smaller amounts of α-pinene (7%) and limonene (4%). Both oils contained only small amounts of sesquiterpenes (3-5%).

Only minor amounts of oil were recovered from the faeces of both species. However, these oils were much more complex than the corresponding leaf oils. In both species, the percentage composition of most faeces oil components differed from that of the leaf oil. The faeces oil from the Brushtail Possum was notable for the almost complete absence of the 1,8 cineole peak (3% vs 63% in the leaf). In the faeces oil from the Greater Glider, the peaks representing terpinen-4-ol, p-cymene, α-pinene amd α-phellandrene were greatly reduced although no oil component passed through the gut without some apparent digestion.

Several peaks appeared in the faeces oil of both species that did not occur in the leaf oils. While many of these were quite minor (e.g. Figure 7.1a, peaks 12a, 15a, 17a), peak 23a was the largest component (21%) of the Greater Glider faeces oil. Interestingly, the same component appeared in the faeces oil of the Brushtail Possum (Figure 7.1b, peak 16a) where it comprised 14% of the total oil. Detailed chemical and mass spectral examination of this peak by Dr J. Brophy (School of Chemistry, University of New South Wales) (Appendix 2) confirmed that the component was non-terpenoid and most likely a dibutyroyl ester of an octane-diol. It has not yet been possible to assign a definite structure to this or any other peaks present in the oil of faeces but not of the leaf.

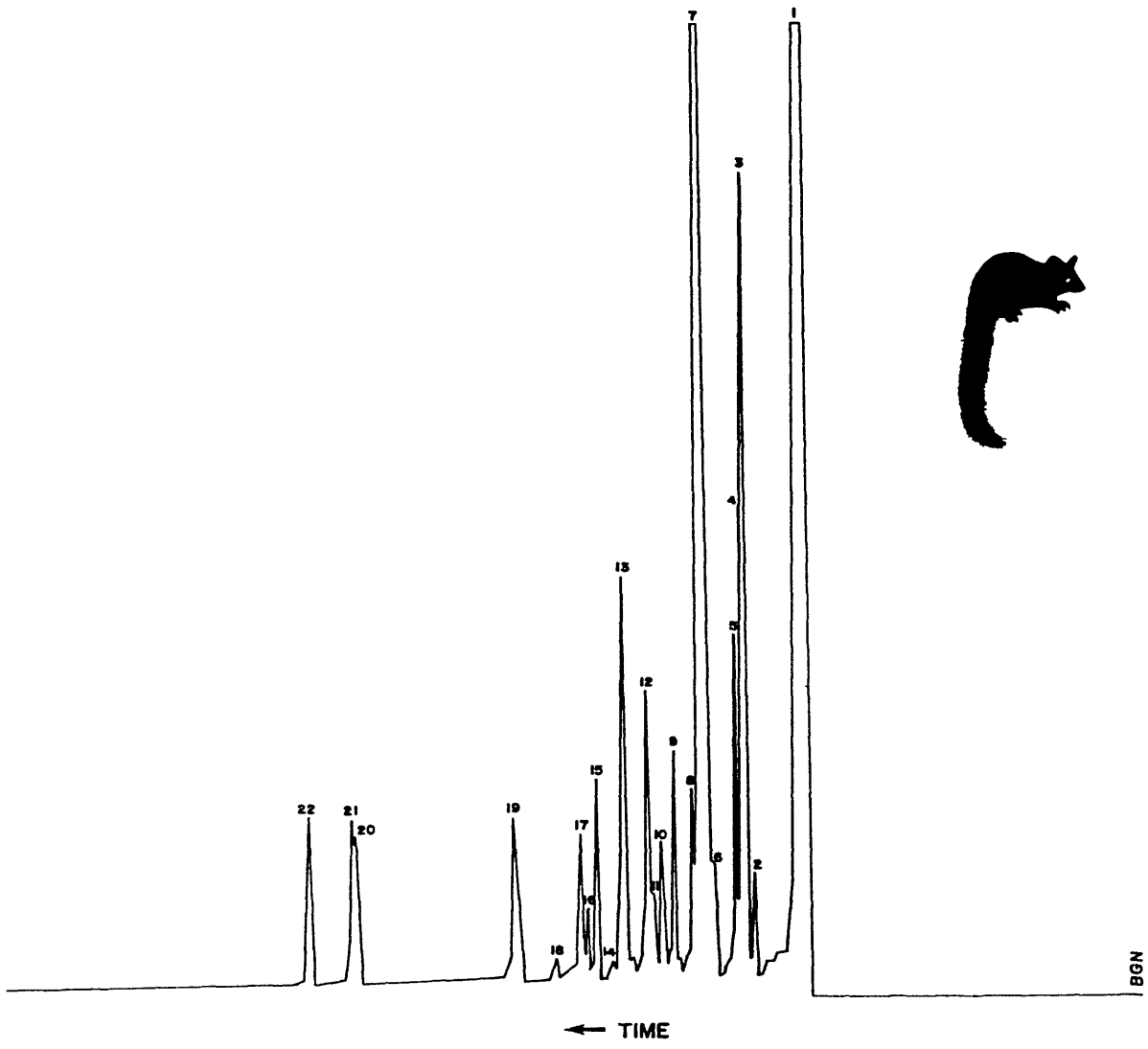

FIGURE 7.2: *Qualitative GLC trace of cyclohexane soluble fraction from respired air of Greater Gliders eating* E. radiata *foliage.*

Figure 7.2 Key to peak numbers of GLC trace of cyclohexane soluble material from expired air of Greater Gliders.

1	Solvent	12	$C_{12}H_{26}$
2	Unknown	13	ψ-terpinene
3	Unknown	14	ar. alkyl benzene
4	$C_{11}H_{24}$	15	p-cymene
5	Unknown	16	ar. alkyl-benzene
6	Myrcene	17	terpinolene
7	α-phellandrene	18	unknown
8	α-terpinene	19	$C_{13}H_{28}$
9	d-limonene	20	$C_{14}H_{30}$ (part)
10	1,8 cineole	21	Unknown
11	ar. alkyl-benzene	22	terpinen-4-ol

7.3.3 **Experiment 3**

Details of the yield and percentage composition of the steam volatile oils recovered from different parts of the digestive tracts of the two species are given in Tables 7.1 and 7.2. Although the yields of oils from the leaves were notably higher in this experiment than in Experiment 2, the yield of oil from the faeces was similar. On the other hand, the percentage composition of the leaf oils was similar to in Experiment 2, but the faeces oils isolated in the present experiment were quite different. For example, the major (unknown) faeces peak found in Experiment 2, although present in this sample, comprised only 9% of the Greater Glider faeces oil and 3% of the Brushtail Possum faeces oil.

7.3.4 **Experiment 4**

The GLC trace of cyclohexane-soluble material from expired air of Greater Gliders from the preliminary experiment is shown in Figure 7.2. The terpene peaks were identified by their mass spectra but this also indicated that some of the other peaks represented aliphatic straight chain hydrocarbons resulting from impurities in the cyclohexane solvent.

No peaks representing terpenes were apparent in the GLC traces of the samples from the leaf alone in the chamber, the leaf plus Greater Gliders, or the Greater Gliders alone in the chamber. Recoveries of the evaporated terpenes were 28 and 35% in the two runs with *E. radiata* essential oils.

7.4 **Discussion**

Some workers (e.g. Von Rudloff, 1975) have criticized steam distillation as a means of extracting essential oils because of the possibility of inducing artefactual rearrangements of oil components (Lassak, 1974). This was unlikely to have been a serious problem in the present study. Lassak (pers. comm.) has shown that the steam volatile oil of *E. dives* foliage, a close relative of *E. radiata* (Ladiges *et al.* 1983)

is chromatographically identical to that extracted from individual oil glands with a fine capillary needle.

The yield and composition of eucalypt essential oils has been shown to vary with season (Berry *et al.* 1937), physical form of the tree (Penfold and Willis, 1961) and leaf age (McKern *et al.* 1951). In the present study, no examination of leaf oil variation was necessary since leaves were collected fom only one or two branches of individual *E. radiata* or *E. melliodora* trees. Although several chemical variants of *E. radiata* have been described (cineole form: Penfold and Morrison, 1935; phellandrene form; Penfold and Morrison, 1936), specimens with terpinen-4-ol as the principal component, as in this study, have not previously been described.

Using the mean intake and dry matter digestibility figures from Chapter 4, together with the data in Tables 4.6a and 4.6b, it can be calculated that Greater Gliders apparently digested 97% of the essential oils of *E. radiata* while Brushtail Possums apparently digested 96% of *E. melliodora* essential oils. Using similar techniques, Eberhard *et al.* (1975) found that Koalas apparently digested 70-97% of the essential oil of *E. punctata*. Similarly, Southwell *et al.* (1980) found only traces of essential oil in the faeces of Brushtail Possums dosed with 5 ml of purified oil components (p-cymene and 1,8 cineole) daily for five days. Igimi *et al.* (1974) detected only 10% of the [^{14}C] label in the faeces of rabbits fed [^{14}C] d-limonene. That components of these essential oils are readily absorbed is not surprising in view of their small molecular weight and high lipid solubility. The important question is where they are absorbed.

The apparent interaction between Cr-EDTA and essential oils in the gut meant that the site of absorption could not be accurately ascertained. Whether the Cr-EDTA complex was disrupted or whether the chelated chromium catalyzed the autoxidation of oil components (Lassak, 1974) could not be determined. However, analysis of oils from different parts of the gut showed that the quantity of oil in the stomach contents was only 49% of what would be expected (on the basis of digesta mass) in Brushtail

Possums and 59% in the Greater Gliders. Similar discrepancies have recently been observed in the rumen contents of Mule Deer (*Odoecoileus hemionus*) (Cluff *et al.*, 1982) and stomach ingesta of Pygmy Rabbits (*Brachylagus idahoensis*) (White *et al.*, 1982). There are two posible explanations for this. First, lipid soluble material such as terpenes could be rapidly absorbed across the mucosa of the stomach of both ruminants and hindgut fermenters (Cook *et al.*, 1952, Alexander and Chowdhury, 1958). Igimi *et al.* (1974) has shown that there is rapid disappearance of [^{14}C] d-limonene from the rat stomach after dosing by stomach tube. Similarly, Narjese (1981, in Welch *et al.*, 1982) was unable to detect monoterpenes in goat rumen contents three hours after direct infusions.

An alternative explanation might be that volatile oils are lost during mastication of the leaf. If this is the case, it is surprising that the percentage loss from stomach contents was greater in the Brushtail Possum than in the Greater Glider since mastication in the Greater Glider produces finer particles (Tables 3.2a,b). However,the coarse grinding action of Brushtail Possum teeth may be more effective in disrupting oil glands than is the fine cutting action employed by Greater Gliders (Gipps, 1980).

The experiments designed to measure terpene losses during mastication by Greater Gliders suggested that this route of loss was of only minor importance. Although preliminary qualitative experiments had detected terpenes arising from expired breath (see Figure 7.2), only traces of oils were detected in the quantitative experiment. This was unexpected since several steps were taken to maximise the recovery of oil components in this second experiment. This involved decreasing the rate of air flow through the chamber, bulking of samples from three animals, and distilling the cyclohexane through longer packed columns. Although recoveries of standards evaporated in the chamber averaged only 32%, the losses during mastication cannot explain the low concentration of oil in the stomach contents of the Greater Gliders relative to that ingested.

Using a similar collection system (but with di-ethyl ether) White *et al.* (1982) found that twice as much monoterpene was trapped when *Artemesia tridentata* foliage was in a chamber with Pygmy Rabbits compared with *Artemesia* alone. They concluded that losses during mastication may be important in explaining the 77% reduction in concentration of monoterpene between the leaves and stomach contents. The lack of agreement between the present experiments and those of White *et al.* (1982) may reflect differences between the masticatory efficiencies of Pygmy Rabbits and Greater Gliders, differences in the efficiency of the collection systems, differences in the volatility of the oils or differences in the way the oils are held in the glands of *Eucalyptus* and *Artemesia* foliage. However, close examination of White *et al*'s (1982) results shows that the amount of monoterpene collected is still only a minor proportion (0.5%) of the total missing fraction. Even assuming that the efficiency of collection was only 5%, makes little difference to the conclusion. It would seem that in both studies, although losses through mastication can occur, these are of minor quantitative importance and absorption from the stomach must be the principal means of loss.

Further absorption must take place in the small intestine, since the amount of terpene reaching the hindgut is of the order of 1% of that ingested in both Greater Gliders and Brushtail Possums. There would thus seem to be little chance of major disruption to the microbial ecosystem in the hindgut. On the other hand, it is interesting that the major unknown faeces peak was found in both the Greater Glider and Brushtail Possum. Eberhard *et al.* (1975) found a major new peak in a similar position and at similar concentrations in the steam volatile material from the faeces of Koalas feeding on *E. punctata* foliage. While no material was available for comparison, it is likely that this is the same component as that found in the present study. No examination was made of the faeces of control animals in the present experiments, but Southwell *et al.* (1980) did not detect any similar metabolite in the faeces of Brushtail Possums fed a fruit diet alone or fruit supplemented with 1,8 cineole, p-cymene or α-pinene.

The fact that the major unknown faeces peak was detected only in caecal contents or distal to the hindgut suggests that microbial metabolism is responsible. Bacteria capable of using essential oils as a sole carbon source are known (Baum and Marr, 1972, Bertram, 1977) and terpene degradation pathways are known from a wide range of micro-organisms (Lassak, 1974). Although the unknown compound was non-terpenoid and most likely a dibutyroyl ester of an octane-diol (Appendix 2), it may have arisen by microbial fermentation of terpenes. For example, Joglekar and Dhavalikar (1969) isolated the 10-carbon compound 3,7-dimethyl-1,7-octane-diol from the fermentation of citral by a soil pseudomonad. Similarly, Bhattacharyya and Dhavalikar (1965) found a complex nine-carbon dicarboxylic acid from the *Aspergillus niger* fermentation of a number of terpenes including camphene and β-santalene.

New faeces peaks could also arise by absorption and subsequent biliary excretion. Eberhard *et al.* (1975) suggested that biliary excretion would be important, together with urinary excretion, in dealing with those compounds greater than MW 150 (i.e. monoterpenoids and sesquiterpenoids). The amount of digesta in the small intestine of both species was too small to recover any oil and the gall bladders of both species contained only a minor amount of bile. Igimi *et al.* (1974) found that 25% of [^{14}C] from ingested [^{14}C] d-limonene in rats was excreted in the bile within 14 hours. However, since only 5% of dose was eventually excreted in the faeces, much of the biliary excretion must have been fermented or reabsorbed lower in the gut and excreted in the urine.

The absorption of various components of the ingested oils appeared to be selective in both species. Most striking was the almost complete disappearance of 1,8 cineole during passage through the gut of the Brushtail Possum. Cleland (1946) has calculated that the toxic dose of 1,8 cineole in Brushtail Possums is 3-4 ml/d. In the present study, Brushtail Possums ingested about 0.7 ml of cineole each day of which about 90% was absorbed, resulting in a tissue load of only 0.6 ml. This does not approach Cleland's (1946) lethal level and Brushtail Possums feeding on *E.*

melliodora leaf would not appear to be in danger of cineole poisoning.

While only traces of oil were detected in Brushtail Possum urine, a large quantity of steam volatile material was recovered from Greater Glider urine. Eberhard *et al.* (1975) have reported similar yields from Koala urine. In both instances, some oil could have resulted from contamination by faeces or leaves dropped by the animals or by hydrolysis during steam distillation. The steam volatile material isolated from the Greater Glider urine was a complex mixture, containing dietary terpenes as well as a variety of unknown compounds (W.J. Foley and J. Brophy, unpub.) Nevertheless, oil recovered from the faeces, urine and expired air account for only minor amounts of the ingested oil in both species. Some oil may have been sequestered in external glands (e.g. sternal glands: K. Greenfield, pers. comm., 1981), and it is possible that another portion may have been used as an energy source by micro-organisms (Igimi *et al.*, 1974). However, these losses are likely to be of minor quantitative importance.

It is likely that the majority of the oil ingested in both species has been absorbed, detoxified and excreted in the urine. While the identification of the detoxification pathways is beyond the scope of this work, it is clear that pathways other than conjugation with glucuronic acid are important in the excretion of absorbed terpenes (Williams, 1959, Flynn and Southwell, 1979, Southwell *et al.*, 1980). Southwell *et al.* (1980) concluded that as the consumption of terpenes in the natural diet increases, so does the oxidase activity which is part of the detoxification process. Future studies using labelled terpenes would be necessary to quantify the routes of loss of ingested oils and the pathways of detoxification.

The possibility that dietary essential oils could have deleterious effects on gut micro-organisms has been raised by many authors (e.g. Freeland and Janzen, 1974, Bryant and Kuropat, 1980). Most of these speculations have been based on the work of Nagy *et al.* (1964, Nagy and Tengerdy, 1968) and Oh *et al.* (1967, 1968) (see Section 1.1.1). However, this work has been challenged (Welch *et al.*, 1981, 1982, Welch and McArthur, 1979) on the grounds that the volumes of oil used to demonstrate

microbial inhibition were too high in relation to the amounts normally expected to be ingested. Also, the *in vitro* systems did not allow for absorption of the oil. For example, Oh *et al.* (1967) found that microbial inhibition occurred at an essential oil concentration of 1.2% of rumen fluid. This is about 20 times greater than the concentration of oil found in the hindgut of the Greater Glider and the Brushtail Possum in this study.

On the other hand, Sadler (1983) found that pure compounds and ether dilutions down to 10^{-4} of 1,8 cineole, d-limonene, terpinen-4-ol and α-terpineol inhibited the growth of cellulolytic bacteria which had been previously cultured on *Eucalyptus viminalis* leaf *in vitro*. Similarly, while ether extracts of *E. viminalis* and *E. blakelyi* inhibited both "adapted" and "non-adapted" cellulolytic bacteria, extracts of *E. radiata* did not differ from controls even though this leaf (from the same batch as that used in Experiment 3) contained substantial proportions of terpinen-4-ol and α-terpineol. Thus antimicrobial effects of essential oils may well be due to synergistic effects of particular components (see also Akimov *et al.*, 1977). Andrews *et al.* (1980) have suggested that the antimicrobial action of terpenes results from disruption of the cytoplasmic membranes and that gram-negative organisms are more resistant than gram-positive microbes. Nothing is known of the occurrence of each of these groups in the hindgut of Greater Gliders and Brushtail Possums, although London (1981) found the caecal flora of the Koala to be predominantly gram-positive.

7.5 Summary

Even allowing for the fact that some species of eucalypt may not affect micro-organisms, it is unlikely that the volumes of eucalypt essential oil reaching the hindgut would have had a major effect on the microbial population. In this study the apparent digestibility of essential oils was 96-97% in both the Greater Glider and the Brushtail Possum. However, the occurrence of butylated metabolites in the faeces of both species indicated that some oil-microbe interaction may have occurred.

Absorption of the essential oils oral to the fermentation site would reduce interactions with hindgut micro-organisms to a minimum, but would involve a metabolic cost to the animal in detoxification and excretion processes.

Chapter 8

ENERGY METABOLISM AND BALANCE IN THE GREATER GLIDER AND BRUSHTAIL POSSUM

8.1 Introduction

Many arboreal folivores have in common, characteristics that have been interpreted as energy saving strategies. These include a reduced litter size (Eisenberg, 1978), the use of gliding as a form of locomotion (Emmons and Gentry, 1983) and reduced activity patterns. Consequently, there is a widespread belief that arboreal folivores have difficulty in meeting their energy requirements. This is because of the "low available energy of foliage diets" (McNab, 1978), the extra energy demands for the detoxification of dietary allelochemicals (Freeland and Winter, 1975) and limited access to sources of free water (Degabriele *et al.*, 1978). On the other hand, it has been argued that less energy is expended in searching for leaves compared with fruits or seeds (Mace *et al.*, 1981). McNab (1978) has suggested that a lowered basal metabolic rate is the best way for arboreal folivores to conserve energy.

This chapter describes some aspects of the energy balance of Brushtail Possums and Greater Gliders fed *Eucalyptus* foliage. Although there have been several measurements made of basal metabolic rates in Brushtail Possums, similar information is not available for Greater Gliders. Rübsamen *et al.* (1984) have examined the response of Greater Gliders to heat loads, but all measurements were made on fed animals. Initially, then, the heat production of fasting Greater Gliders was measured using indirect respirometry. Faecal and urinary energy losses were then measured for both species and the intake of digestible and metabolizable energy determined. This showed that there was a very large loss of energy in the urine of the Greater Gliders. Consequently, a more detailed study was made of the energy balance of Greater Gliders and of the efficiency with which they utilized the metabolizable energy of *E. radiata*.

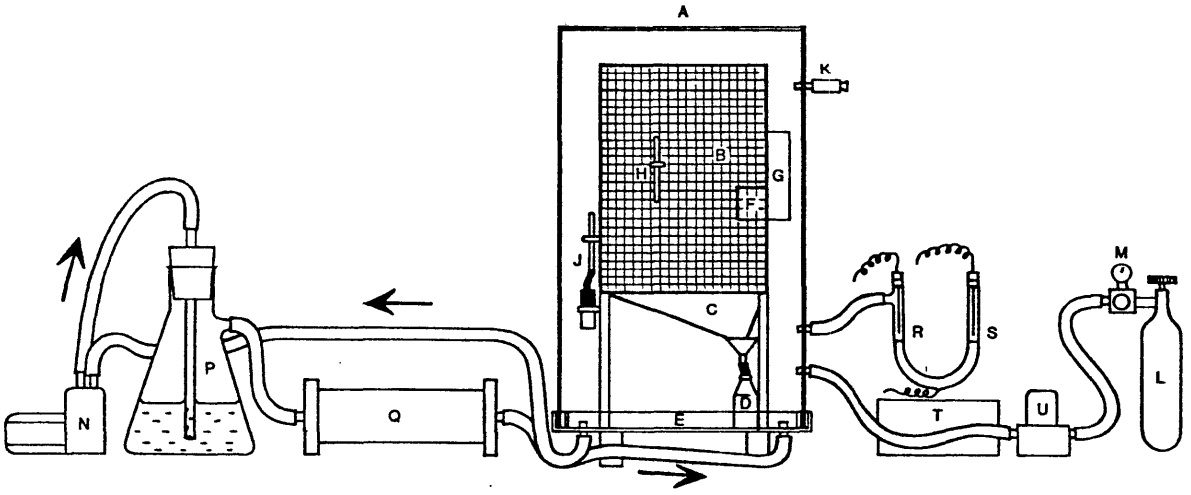

FIGURE 8.1: *Diagrammatic representation of a closed circuit respirometer used for measurement of heat production of Greater Gliders.*

A Glass chamber
B Wire mesh cage 0.3 x 0.6 x 0.8 m
C Faeces and urine collection tray
D Urine collection bottle (containing glacial acetic acid)
E Trough containing water
F Water container
G Cylinder for holding foliage
H One of three dry bulb mercury thermometers
J Wet bulb mercury thermometer
K Gas sample outlet
L Oxygen cylinder
M Oxygen regulator and reducing valve
N Pump
P Flask containing KOH solution
Q Silica gel train
R Glass manometer containing $NaHCO_3$
S Steel contact in manometer open arm
T Relay system
U Solenoid valve

Subsequent chapters consider the importance of microbial digestion in the energy balance of both species as well as the energy expenditure of free-living Greater Gliders.

8.2 **Materials and Methods**

8.2.1 **Energy intake and excretion**

The intake and excretion of energy in both Greater Gliders and Brushtail Possums was determined in conjunction with the feeding experiments already described. The animals and diets used and the procedures followed during these measurements have been described (Section 4.2). The analytical techniques are described in Section 2.4.

8.2.2 **Heat production**

Measurements of heat production and energy balance were made only on Greater Gliders. All animals used in these measurements had been maintained for at least 5 weeks at 20° C on a 12 h/12 h light cycle and fed *E. radiata* foliage *ad libitum*.

8.2.2.1 **Respirometers**

Three closed circuit respiration chambers based on indirect calorimetric principles were used (Figure 8.1). The design and operation of these chambers was described by Farrell (1972) with modifications of Farrell (1974). The system consisted of a pump which continuously circulated chamber air through a flask containing a KOH solution (to remove CO_2) and through a cartridge containing silica gel (to remove H_2O). Oxygen was bled into the system in response to a drop in pressure due to absorbance of the CO_2.

8.2.2.2 **Analysis**

Oxygen consumption was determined by weighing the oxygen cylinder

before and after each run. CO_2 was recovered as $BaCO_3$ after neutralizing residual KOH with excess NH_4Cl_2. A sample of chamber air was taken into a gas-tight brass syringe at the start and end of each run. The O_2 and CO_2 content of this sample was determined by gas chromatography (Beckman GC-2A) using helium as the carrier gas and used to correct for differences in the initial and final volumes of gas in the chamber.

All temperature and pressure values were converted to STP. Heat production was calculated according to Brouwer (1965) without correction for CH_4 or urinary nitrogen.

8.2.3 **Fasting heat production**

Fasting heat production was measured in 10 Greater Gliders (five females, five males). Two observations were made on each of four animals of each sex, with only one observation on the remaining animals. All measurements were made in the winter and spring of 1982 and all animals were given a period of adaptation in the chambers of at least two days prior to experiments. Food was withdrawn 24 h before the start of each run; water was available continuously. The animals were weighed and placed in the unsealed chamber at 1800 h and left overnight. At 0600 h the next day, the chambers were sealed and heat production measured for 12 hours. The animals were not reweighed prior to commencement of the run since they became very active after being handled, but observations were made of the animal's behaviour during the measurement periods. A preliminary experiment assessed the effect of fasting for 48 h. A minimum period of two weeks during which the animals were fed *E. radiata* foliage *ad libitum* was allowed before an individual was remeasured.

8.2.4 **Energy retention**

Energy retention was determined in four Greater Gliders at different levels of energy intake in a series of experiments in spring and summer, 1982/83. Three animals were used in Runs 1 and 2, but one of these lost considerable condition between Runs 2 and 3 and was replaced by an animal of similar body mass. The experiment was initially designed as a

3 x 3 Latin square, but after replacement of the animal, the treatments were re-randomized and recommenced. Hence, there was a total of five periods in which two of the animals received two treatments twice. The three treatments were intended to be *ad libitum* intake of energy, ≃ 85% of *ad libitum* intake and ≃ 70% of *ad libitum* intake. However, it proved impossible to accurately estimate the mass of leaves on a particular branch and this, combined with the animals' propensity to consume the bark and the wood when fed resticted levels of foliage, led to difficulties in standardizing the energy intake on the restriction treatments.

The animals were fed at their allocated level for three days prior to measurement of heat production. At 1200 h on the fourth day, they were weighed, and placed in the chamber. At 1600 h they were fed, the chamber was sealed and heat production measured for 22-23 hours. The diet and diet sampling was as previously described (Section 2.2 and 2.3). At 1400 h the next day, the pumps were stopped, a fresh drying train and CO_2 trap connected to each chamber, and faeces and urine collected, weighed and stored at -10° C (For full description see Section 2.3.2). This procedure was repeated for another two days. The animals were reweighed only at the end of three days in the respiration chambers since urine was easily lost if they were handled outside the chamber cage. A period of two to three weeks during which all animals were fed *ad libitum* was allowed between runs.

8.2.5 **Methane production**

Methane production was determined in six fully-fed and six fasted Greater Gliders by measuring the methane content of the chamber air at the end of a run. Methane concentration was determined on a thermal conductivity gas chromatograph (Series 150, Gow Mac Instument Co. New Jersey U.S.A.) using argon as the carrier gas.

8.2.6 **Plant Respiration**

The contribution of plant respiration to measured gas volumes was assessed by running the system with *E. radiata* foliage only in the chamber.

TABLE 8.1: *Body weight, heat production and respiratory quotients of fasted, resting Greater Gliders at 20°C (Mean ± SE)*

	Body weight (kg)	Heat production		RQ[1]
		W	$W.kg^{-0.75}$	
Males[2]	0.956 ± 0.031	2.32 ± 0.09	2.40 ± 0.05	0.748 ± 0.012
Females[3]	1.059 ± 0.029	2.47 ± 0.05	2.37 ± 0.04	0.762 ± 0.008
Mean	1.010 ± 0.024	2.40 ± 0.05	2.39 ± 0.03	0.755 ± 0.07
Range	0.830-1.148	2.07-2.75	2.20-2.69	0.712-0.792

[1] Respiratory quotient

[2] n = 8 (one observation omitted, see text)

[3] n = 9

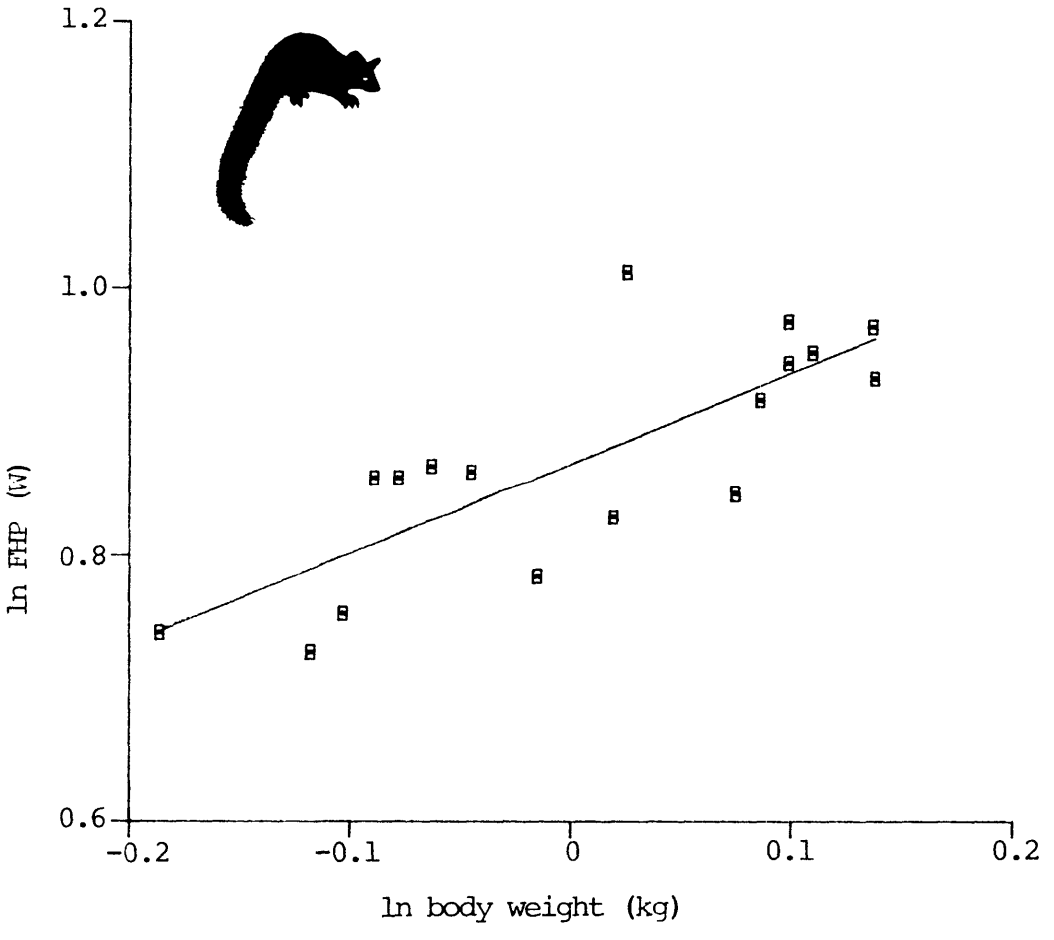

FIGURE 8.2: *Relationship between $\log_e$ body weight and $\log_e$ fasting heat production in Greater Gliders.*

Regression equation:
$y = 0.869 + 0.676x$, $r = 0.774$ ($P<0.001$), RSD = 0.055

TABLE 8.2a: *Intake and excretion of energy by Greater Gliders (values expressed as* $MJ.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Gross energy intake	Digestible energy intake	Metabolizable energy intake	Faecal energy excretion	Urinary energy excretion
P1	1.13 ± 0.03^{b}	0.66 ± 0.02^{ab}	0.41 ± 0.03^{a}	0.47 ± 0.02^{a}	0.25 ± 0.01^{ac}
P2	1.00 ± 0.01^{d}	0.51 ± 0.01^{e}	0.26 ± 0.01^{c}	0.49 ± 0.02^{a}	0.25 ± 0.00^{a}
P3	1.08 ± 0.03^{ab}	0.62 ± 0.02^{abc}	0.36 ± 0.02^{ab}	0.45 ± 0.02^{a}	0.27 ± 0.02^{a}
P4	1.06 ± 0.04^{ac}	0.61 ± 0.03^{bc}	0.36 ± 0.03^{ab}	0.45 ± 0.01^{a}	0.25 ± 0.02^{a}
P5	1.02 ± 0.04^{acd}	0.59 ± 0.02^{c}	0.32 ± 0.02^{b}	0.43 ± 0.02^{bc}	0.28 ± 0.01^{ab}
P6	1.12 ± 0.03^{ab}	0.67 ± 0.01^{ad}	0.37 ± 0.01^{ab}	0.45 ± 0.01^{ab}	0.30 ± 0.01^{b}
P7	1.13 ± 0.03^{ab}	0.72 ± 0.03^{d}	0.48 ± 0.03^{d}	0.41 ± 0.02^{c}	0.24 ± 0.02^{c}
P8	0.85 ± 0.03^{e}	0.48 ± 0.02^{e}	0.24 ± 0.03^{c}	0.36 ± 0.01^{d}	0.25 ± 0.01^{a}
Mean	1.05 ± 0.02	0.61 ± 0.01	0.35 ± 0.01	0.44 ± 0.01	0.26 ± 0.01
Range	0.77-1.24	0.42-0.82	0.16-0.54	0.33-0.55	0.19-0.36

a,b,c,d,e = statistical analysis code within columsn ($P < 0.05$)

TABLE 8.2b: *Intake and excretion of energy by Brushtail Possums (values expressed as* $MJ.kgW^{-0.75}.d^{-1}$*) (Mean ± SE)*

Experiment	Gross energy intake	Digestible energy intake	Metabolizable energy intake	Faecal energy excretion	Urinary energy excretion
T1	0.82 ± 0.02^{b}	0.40 ± 0.02^{b}	0.30 ± 0.02^{b}	0.42 ± 0.03^{a}	0.10 ± 0.02^{a}
T2	0.77 ± 0.02^{ab}	0.31 ± 0.01^{a}	0.24 ± 0.01^{a}	0.45 ± 0.02^{a}	0.08 ± 0.01^{a}
T3	0.70 ± 0.05^{a}	0.31 ± 0.01^{a}	0.25 ± 0.01^{ab}	0.39 ± 0.05^{a}	0.06 ± 0.01^{a}
T4	0.65 ± 0.06^{a}	0.29 ± 0.03^{a}	0.22 ± 0.03^{a}	0.36 ± 0.04^{a}	0.07 ± 0.00^{a}
T5	0.68 ± 0.03^{a}	0.31 ± 0.01^{a}	0.24 ± 0.01^{a}	0.37 ± 0.02^{a}	0.07 ± 0.01^{a}
Mean	0.74 ± 0.02	0.34 ± 0.02	0.26 ± 0.01	0.40 ± 0.02	0.08 ± 0.01
Range	0.53-0.93	0.23-0.44	0.16-0.35	0.30-0.53	0.05-0.16

a,b = statistical analysis code within columns ($P < 0.05$)

Fifteen runs were made over a range of leaf masses (50-750 g) on the same 12 h dark/12 h light cycle used in the experiments.

8.3 Results

8.3.1 Fasting heat production of Greater Gliders

In the preliminary experiment, the fasting heat production (FHP) of one adult female Greater Glider was 2.43 $W \cdot kg^{-0.75}$ (RQ = 0.79) after 24 h fasting. After fasting for 48 h FHP was 2.26 $W \cdot kg^{-0.75}$ (RQ = 0.74). However, on removal from the chamber, the animal became comatose, and was revived by intraperitoneal injections of glucose in saline. Hence all future experiments were conducted after 24 h fasting.

Details of the FHP measurements are given in Table 8.1. One observation was excluded since the animal appeared to fall into a deep sleep during the measurements and heat production dropped to 2.01 $W \cdot kgW^{-0.75}$. There were no significant differences in FHP or RQ between male and female Greater Gliders and the mean value was 2.39 $W \cdot kg^{-0.75}$ (207 $kJ \cdot kgW^{-0.75} \cdot d^{-1}$) at an RQ of 0.755. A relationship of the form $Y = aW^b$ was derived between body mass and FHP (Figure 8.2) and this allowed the mean FHP to be rewritten as ($FHP = 2.39W(kg)^{0.68}$)

8.3.2 Energy intake and excretion

Tables 8.2a and 8.2b give details of the intake and excretion of energy by Greater Gliders and Brushtail Possums respectively. The major route of energy loss in both species was via the faeces, with apparent digestibilities of gross energy of 57% in Greater Gliders and 46% in Brushtail Possums. Although ME intakes were similar proportions of GE intakes in both species (33-35%), Brushtail Possums retained more (76%) of their DE as ME than the Greater Gliders (57%). There was no relationship between urinary energy losses and gross energy intakes in either species. Faecal energy losses were higher ($P<0.001$) at higher levels of GE intake in both species (Figures 8.3a and 8.3b).

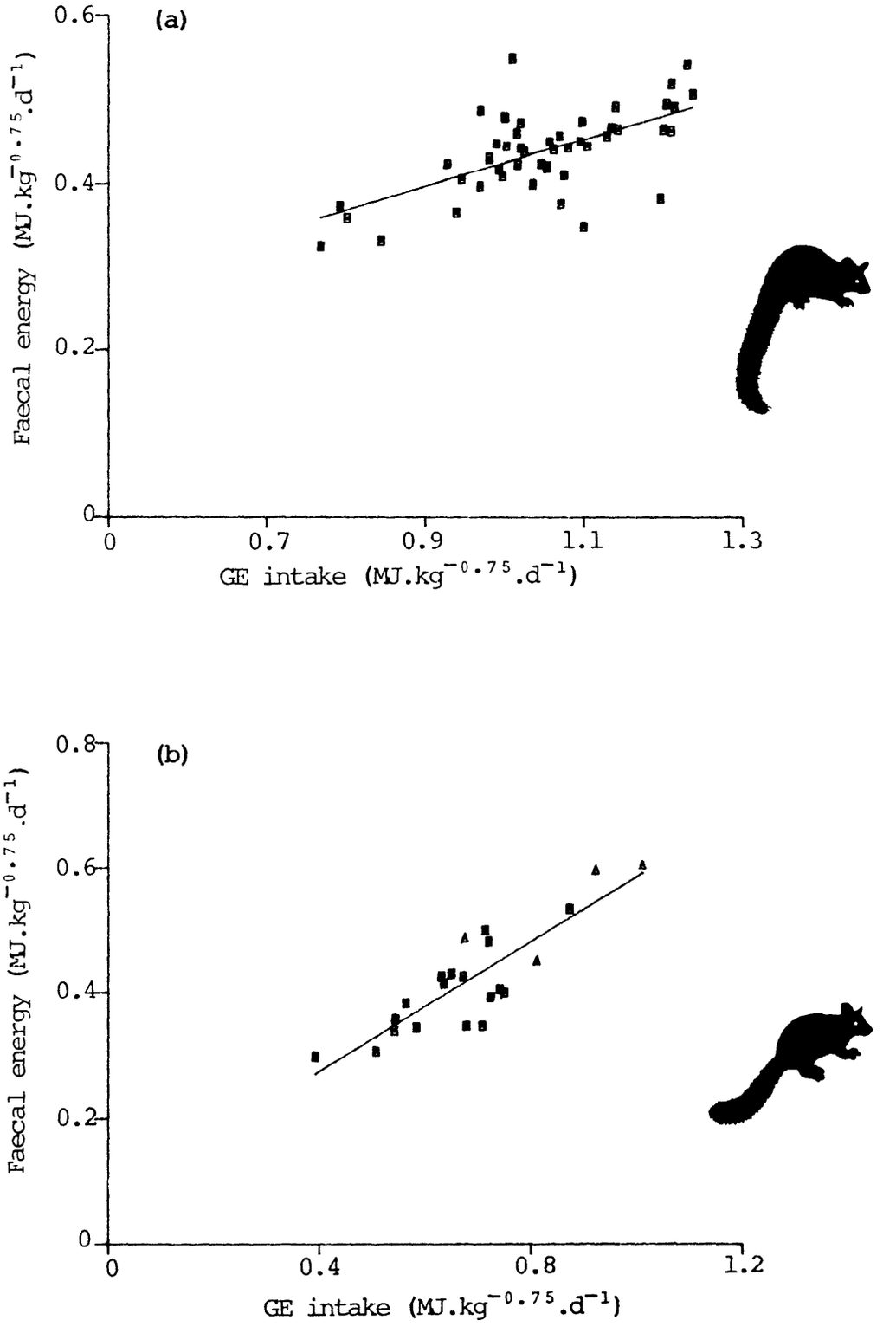

FIGURE 8.3: *Relationship between gross energy intake and faecal energy excretion in -*
(a) Greater Gliders; and
(b) Brushtail Possums.

Regression equations:
(a) y = 0.139 + 0.287x, r = 0.620 (P<0.001), RSD = 0.040
(b) y = -0.058 + 0.623x, r = 0.850 (P<0.001), RSD = 0.045

TABLE 8.3: *Energy intake, heat production and energy retention in Greater Gliders fed various levels of E. radiata foliage*

Experiment/ animal		Body weight Mean (kg)	Body weight Daily change (%)	Gross energy intake ($kJ.kg^{-0.75}.d^{-1}$)	Digestible energy intake ($kJ.kg^{-0.75}.d^{-1}$)	Metabolizable energy intake ($kJ.kg^{-0.75}.d^{-1}$)	Heat production ($kJ.kg^{-0.75}.d^{-1}$)	Energy retained ($kJ.kg^{-0.75}.d^{-1}$)	RQ[1]
	1	1.000	(-1.8)	955.9 ± 78.9	539.9 ± 72.4	332.7 ± 90.5	327.1 ± 15.2	5.6 ± 81.2	1.020 ± 0.021
1	2	0.982	(-1.6)	873.4 ± 80.5	489.9 ± 68.5	291.2 ± 55.1	303.4 ± 6.0	-12.3 ± 57.0	0.998 ± 0.008
	3	1.084	(-1.1)	921.7 ± 30.4	405.8 ± 10.9	170.3 ± 18.2	400.5 ± 6.3	-230.2 ± 14.8	1.030 ± 0.019
	2	1.033	(-0.8)	996.9 ± 21.8	591.3 ± 18.4	397.9 ± 53.2	344.5 ± 6.1	53.4 ± 47.3	0.920 ± 0.021
2	3	1.150	(-1.1)	1023.6 ± 5.7	587.4 ± 25.9	323.8 ± 14.6	354.1 ± 3.1	-30.3 ± 13.7	0.977 ± 0.065
	1	1.090	(-1.7)	893.6 ± 15.5	479.5 ± 9.1	212.8 ± 61.7	320.6 ± 7.2	-107.8 ± 30.1	0.989 ± 0.011

	1	1.058	(-2.3)	871.9 ± 16.5	422.8 ± 58.3	242.0 ± 61.8	357.7 ± 5.7	-115.8 ± 58.3	1.010 ± 0.0198
3	3	1.213	(-0.9)	1058.8 ± 41.9	583.2 ± 56.1	347.1 ± 62.0	400.8 ± 4.8	-53.7 ± 66.8	1.002 ± 0.003
	4	1.130	(-2.3)	725.7 ± 37.5	347.8 ± 36.1	175.0 ± 37.6	345.4 ± 11.7	-170.4 ± 39.4	0.985 ± 0.014
	1	1.058	(-1.4)	885.3 ± 87.1	389.8 ± 79.3	157.4 ± 83.2	352.0 ± 3.7	-194.7 ± 85.6	0.997 ± 0.012
4	3	1.088	(-2.5)	784.0 ± 92.6	378.9 ± 48.7	183.6 ± 71.5	312.0 ± 9.2	-128.5 ± 80.0	0.935 ± 0.020
	4	1.100	(-0.6)	773.4 ± 38.5	386.8 ± 27.3	201.0 ± 30.4	337.7 ± 9.2	-130.7 ± 35.1	1.000 ± 0.001
	1	0.925	(-1.6)	597.2 ± 22.3	300.9 ± 26.5	143.8± 2.8	321.3 ± 1.7	-177.6 ± 4.5	0.897 ± 0.002
5[2]	3	1.190	(-0.4)	1096.6 ± 86.3	590.3 ± 62.7	318.3 ± 89.4	389.3 ± 2.9	-71.1 ± 92.3	0.990 ± 0.009
	4	1.023	(-2.2)	477.0 ± 35.6	231.2 ± 81.6	-120.0 ± 4.5	310.2 ± 3.7	-430.2 ± 1.0	0.930 ± 0.049

[1] Respiratory quotient

[2] Two days only - all other experiments three days

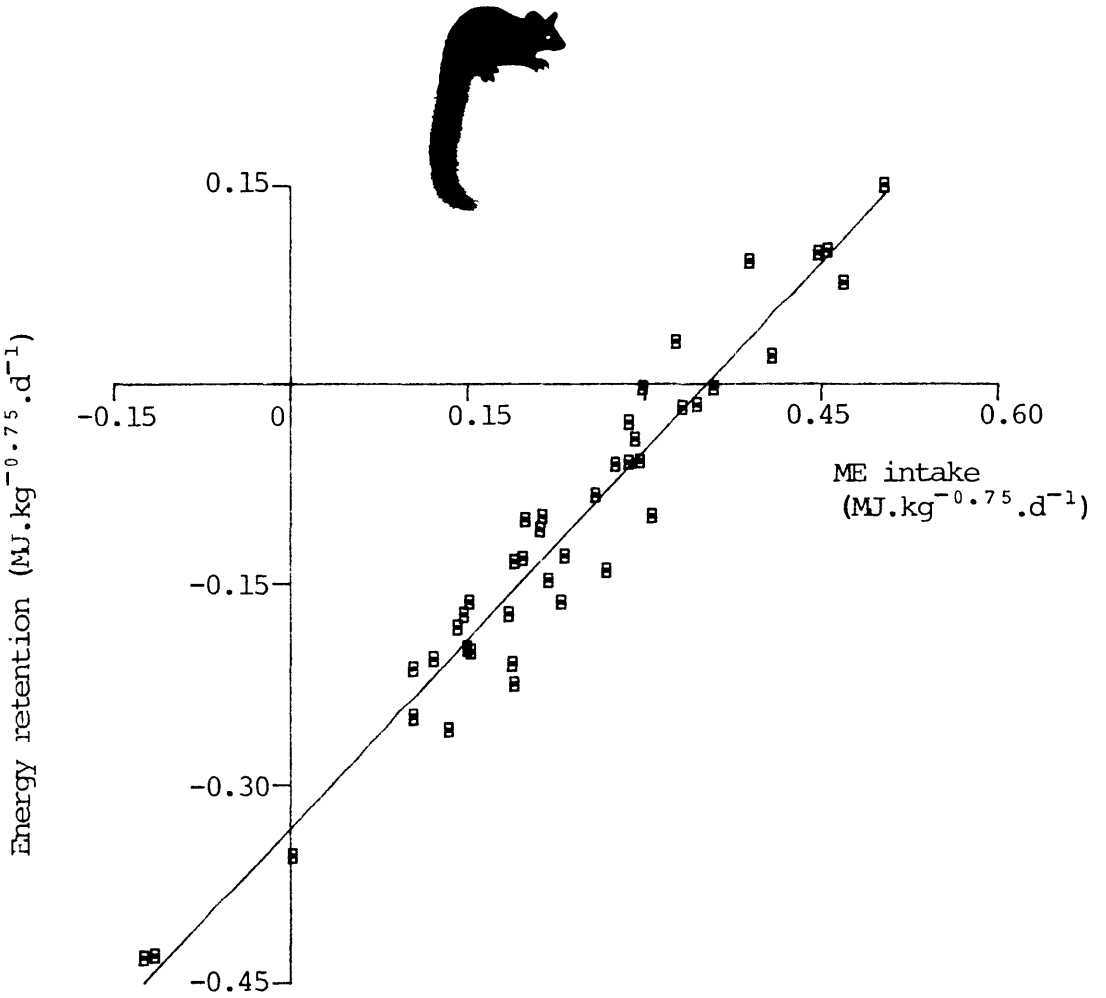

FIGURE 8.4: *Relationship between metabolizable energy intake and energy retention in Greater Gliders.*

Regression equation:
y = -0.332 + 0.944x, r = 0.971 (P<0.001), RSD = 0.032

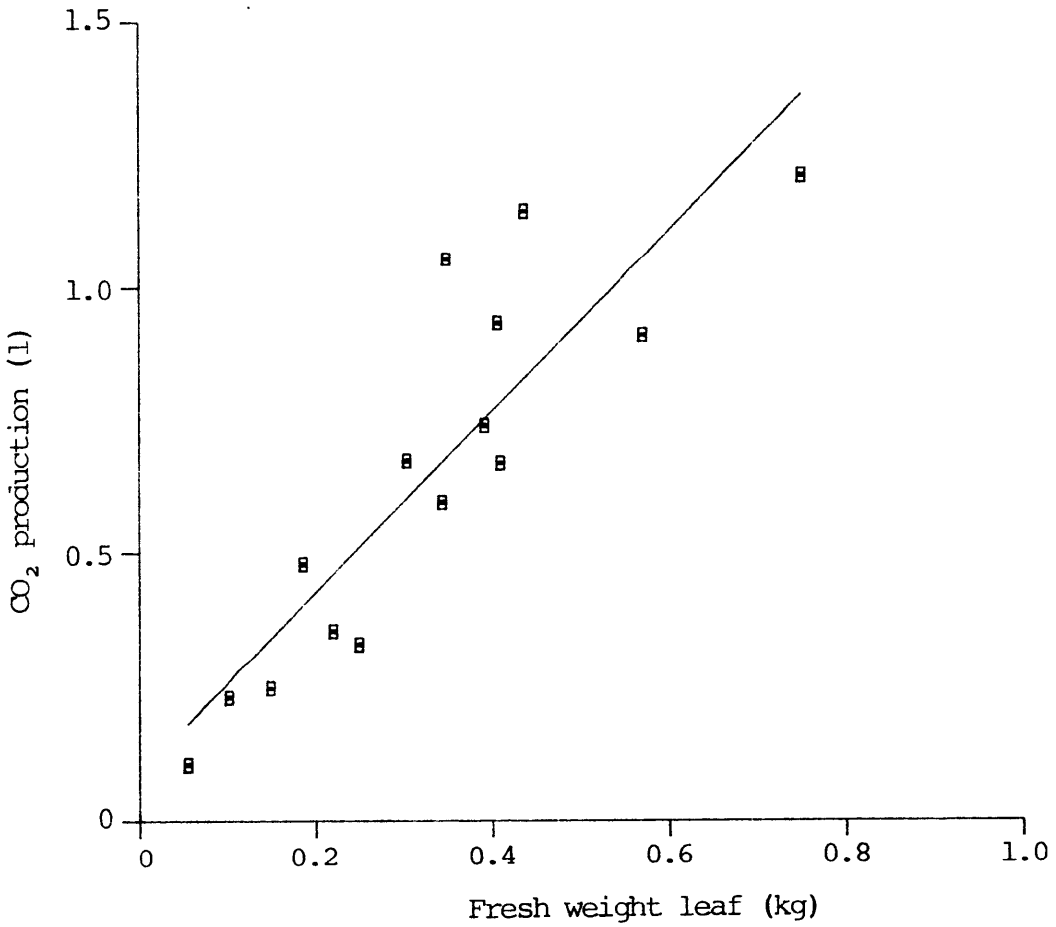

FIGURE 8.5: *Relationship between fresh weight* E. radiata *leaf and volume of CO_2 produced (12 h light:12 h dark) in respirometers.*

Regression equation:
$y = 0.088 + 1.698x$, $r = 0.868$ ($P<0.001$), RSD = 0.174

8.3.3 **Energy retention in Greater Gliders**

Details of energy intake, excretion and retention of Greater Gliders, together with concurrent measurements of heat production and RQ, are given in Table 8.3. Again, there was no relationship between urinary energy losses and GE intake. However, there was a significant relationship between faecal energy losses and GE intake ($y = 0.136 + 0.324x$, $r = 0.706$, RSD = 0.054), although in these experiments the digestibility of GE was lower ($P<0.01$) at lower GE intakes. This probably reflected the relatively greater contribution of endogenous energy losses at low GE intakes.

There was a significant relationship ($P<0.001$) (Figure 8.4) between ME intake and energy retention. ME intake at zero energy retention was 0.35 $MJ \cdot kg^{-0.75} \cdot d^{-1}$. The slope of this relationship was 0.944 hence the efficiency of utilization of ME for maintenance (Km) was 94.4%.

8.3.4 **Methane production**

No methane could be detected in the residual chamber air at the end of six observations of FHP of Greater Gliders. However, some methane production (106±21 ml) was recorded in conjunction with measurements of heat production of six Greater Gliders offered *E. radiata ad libitum*. This was equivalent to 0.4% of GE intake and 0.7% of DE intake.

8.3.5 **Plant respiration**

There was a significant ($P<0.001$) relationship between CO_2 production and fresh mass of *E. radiata* leaf (Figure 8.5), and between oxygen consumption and leaf mass ($Y = -0.037 + 0.002X$; $r = 0.865$, RSD = 0.327). The variation around the line is attributable to differences in the proportion of leaf and stem in each bunch of leaves tested; the dry matter content of all bunches was relatively constant (43±1%).

8.4 Discussion

8.4.1 Fasting heat production

Measurements of standard metabolic rate must satisfy four criteria.

1) The animal must be in a post-absorptive state:

Because of the very long digesta retention times of Greater Gliders (Section 3.3.2) small amounts of faeces were still voided after a 24 h fast. Fasting for 48 h was not feasible. The average RQ during the fasting measurements was 0.78 which suggested that the animals were catabolizing a mixture of fat and protein (Brody, 1945). Interestingly, the RQ of partially fed animals in later experiments (Table 8.3) (≈ 85% *ad libitum*) remained close to unity. These data, together with the lack of detectable methane production, suggest that even though post-absorptive conditions were not attained, they were closely approached.

2) The animal must be at rest:

The fasted animals were active during the dark phase but all rested during the 12 h light phase. One male Greater Glider fell into an apparent deep sleep during the measurement period and heat production droppped to only 2.01 $W \cdot kgW^{-0.75}$ with an RQ of 0.734. Degabriele and Dawson (1979) and May (1982) have noted similarly low levels of metabolism in sleeping Koalas and Sugar Gliders. All other animals were observed "awake" for the majority of the 12 h period in the chamber.

3) The animal must be in its thermoneutral zone:

Rübsamen *et al.* (1984) showed that the Greater Glider has only a point of thermoneutrality at 20° C rather than a range of thermoneutral temperatures. In these experiments, the chamber temperature was controlled only by the room temperature. The minimum temperature at the start of a run was 19.9 ± 0.2° C, and the maximum temperature at the end of a run was

TABLE 8.4: *Standard metabolic rate in some arboreal mammals*

Species	Body weight (kg)	Standard metabolic rate		Reference
		W	$W.kg^{-0.75}$	
MARSUPIALS				
Koala	3.5-4.8	5.12	1.75	Degabriele and Dawson (1979)
Spotted Cuscus	3.1-4.8	5.42	1.93	Dawson and Degabriele (1973)
Brushtail Possum	2.0	3.49	2.09	Dawson and Hulbert (1970)
Ringtail Possum	0.7-1.0	2.85	2.55	Kinnear and Shield (1975)
Greater Glider	0.8-1.2	2.40	2.39	Present study
Sugar Glider[1]	0.13	0.50	2.31	Dawson and Hulbert (1970)
Leadbeaters Possum[1]	0.17	0.46	2.15	Smith *et al.* (1982)
EUTHERIANS				
Two-toed Sloth	4.3	4.71	1.59	McNab (1978)
Three-toed Sloth	3.8	3.83	1.41	McNab (1978)
Colobus Monkey	9.4-11.5	16.65	2.86[2]	Müller *et al.* (1983)
Howler Monkey	3.2-6.1	11.18	3.56	Milton *et al.* (1979)
Tree Hyrax	2.2	4.20	2.32	Hildwein (1972)
Blue Monkey[1]	8.0-9.0	18.96	3.81[2]	Müller *et al.* (1983)

[1] Omnivores - all others folivores/herbivores

[2] Measurements made on sleeping animals

20.7 ± 0.3° C. The errors introduced by these variations from the thermoneutral point could be expected to be minimal (Rübsamen *et al.* 1984).

4) Measurements should be made only on non-growing animals:

All animals used were sexually mature (Bancroft, 1970, Griffith, 1973) and maintained a constant body mass (±2%) for four weeks prior to the experiment.

* * *

Thus these four conditions for the measurement of standard metabolic rate were closely approached and the fasting heat productions measured should be close estimates of the standard metabolic rate of adult Greater Gliders. Table 8.4 shows standard metabolic rates for a number of marsupial and eutherian folivores. Estimates of the fasting heat production of the Brushtail Possum vary widely. Dawson and Hulbert (1970) found values of only 2.09 $W \cdot kgW^{-0.75}$, while H.E. Bayley and D.J. Farrell (pers. comm., 1982) recorded values of 2.48 $W \cdot kgW^{-0.75}$ after a 48 h period of fasting. In growing Brushtail Possums from New Zealand, Harris and Dellow (1983) found the SMR to be 2.35 $W \cdot kgW^{-0.75}$. Some of this variation is probably due to methodological differences. For example, Dawson and Hulbert (1970) measured only oxygen consumption and assumed an RQ value to convert this to heat production.

While most marsupials have standard metabolic rates about 30% lower than most eutherians, there is considerable overlap between the two groups - particularly among desert animals (Hume, 1982). Previously it had been suggested that the marsupial-eutherian differences were a result of a lower marsupial body temperature and various Q_{10} factors were derived to correct for the temperature difference (Hulbert, 1980). However, Q_{10} effects appear to be merely coincidental, since wider comparisons over all vertebrate orders yield unrealistic values (Poczopko, 1980) and it must be concluded that there are other more important determinants of BMR.

Although McNab (1978) has suggested that arboreal folivores should display lower metabolic rates, the available evidence does not support this contention. Much of the variation can be attributed to differences in the measurement conditions in various studies. For example, Müller *et al.*'s (1983) measurements of "SMR" in *Colobus* were made on sleeping animals. On the other hand, there seems no *a priori* reason why low basal (as opposed to field) metabolic rates should be a feature of arboreal folivores generally. It is widely accepted that the two major contributions to fasting heat production are the energy required for maintenance of membrane transport functions and for tissue protein synthesis (Buttery and Boorman, 1976). The lower relative brain size (Mace *et al.*, 1981) and lowered body muscle mass (Grand, 1978) of at least some arboreal folivores might be reasons for supposing that the body protein turnover component of BMR could be lower. Data to test this idea are few, but Dellow and Harris (1984) have shown that whole body protein synthesis rates are identical in Brushtail Possums and domestic rabbits. While examination of some of the more highly folivorous species could be valuable, in the meantime it is probably most appropriate to regard lowered BMRs in some arboreal folivores (e.g., Koala) as a useful preadaptation to folivory rather than as a consequence.

There has always been some debate about the most appropriate exponent to use in modelling energy metabolism (Kleiber, 1961, Thonney *et al.*, 1976) and $BW^{0.75}$ has been commonly applied to both inter- and intra-specific comparisons. Heusner (1982) has shown that the exponent 0.67 provides a better description of intra-specific variation and he dismissed $BW^{0.75}$ as a "statistical artefact". The relationship between fasting heat production and body mass derived in this study ($HP = 2.39W^{0.68}$) would tend to support Heusner's contention. However, while $BW^{0.67}$ may be appropriate for intra-species comparisons, the balance of both experimental and theoretical evidence (Thonney *et al.*, 1976, Feldman and McMahon, 1983) suggests that $BW^{0.75}$ is the most appropriate factor to use when making inter-species comparisons.

8.4.2 **Energy intake and excretion**

It is likely that the gross energy intake of both the Brushtail

TABLE 8.5: *Digestible, metabolizable and net energy coefficients in several eutherian and marsupial herbivores*

Species	Diet	Metabolic state	DE/GE (%)	ME/DE (%)	NE (%)	Reference
EUTHERIAN - Foregut Fermenters						
Sheep	Browse (sage)	M	41	54[1]	-	Cook *et al.* (1952)
Eland, Wildebeest	Hay and concentrates	M/G	62/69	82/83[1]	81/56	Rogerson (1968)
Moose	Browse	M	-	78[1]	68	Regelin *et al.* (1981)
White-tailed Deer	Concentrates	M + G[2]	76	87[1]	53	Thompson *et al.* (1973)
	Browse	M	41	86[1]	78	Mautz *et al.* (1975)
EUTHERIAN - Hindgut Fermenters						
Rodents	Grains	G	-	97	89	Jagosz and Drozdz (1968)
Snowshoe Hare	Browse and concentrates	M + G	50	86	79	Holter *et al.* (1974)
Mountain Hare	Browse	M	22/39	87	-	Pehrson (1983b)
Howler Monkey	Fig fruit and leaves	M	37/43	88/90	-	Nagy and Milton (1979a)
MARSUPIAL - Foregut Fermenters						
Red-necked Pademelon		M	56	-	-	
Eastern Grey Kangaroo		M	59	-	-	Foley *et al.* (1980)
MARSUPIAL - Hindgut Fermenters						
Koala	Eucalypt leaf	M	51	86	-	Cork *et al.* (1983)
Ringtail Possum	Eucalypt leaf	M	51	79	-	Chilcott and Hume (1984a)
Greater Glider	Eucalypt leaf	M	57	55	94	Present study
Brushtail Possum	Eucalypt leaf	M	46	77	—	Present study

[1] ME corrected for methane losses; all other values uncorrected

[2] Fawns (including growing and wintering seasons)

M = maintenance; G = gain

Possums and Greater Gliders have been underestimated in the present study due to losses of essential oils in the drying process. Freeze-dried *E. radiata* foliage contained 38% less oil than fresh foliage, while oven drying lost up to 60%. (W.J. Foley unpub.) On the other hand, Woodland (1972) found no difference in the caloric content of freeze- and oven dried *E. viminalis* leaf.

The digestible energy content of *E. radiata* was higher than that found in other eucalypt species ingested by Koalas and Ringtail Possums (Table 8.5). However, the DE content of some other browse species fed to ruminants is much lower (Table 8.5) and this has been attributed to their high ash content (Cook *et al.*, 1952) and their large and poorly digested fibre fractions (Cook *et al.*, 1952, Ullrey *et al.*, 1972). The low ash content of eucalypt foliage is therefore one factor which might enhance its DE content compared to other foliages. The lower DE content of *E. melliodora* leaf may have been due to a relatively higher endogenous energy loss per unit faecal dry matter in the Brushtail Possums. This would have resulted from their lower intakes of dry matter. Similarly, the lack of an effective digesta separation mechanism in the hindgut of the Brushtail Possum might be part of the reason for their higher faecal energy losses compared with the Greater Gliders. The difference in essential oil content of the two eucalypt species would have had minimal effects on their digestible energy contents since little of the ingested oil was excreted in the faeces (Chapter 7).

However, differences in leaf oil content are likely to have been one of the principal reasons for the striking differences in the metabolizability of the two foliages. The urinary energy losses of the Greater Gliders were much greater than in any other eucalypt-feeding mammal. Cook *et al.* (1952) found similarly high urinary energy loses in sheep feeding on two browses noted for their high essential oil content.

The ME intakes in Tables 8.2a and 8.2b are overestimates because they do not allow for losses of combustible gases (mainly methane) and of heat formed during the fermentation of cell walls in the hindgut. However, methane losses in the Greater Glider were less than 1% of DE intake, and

TABLE 8.6: *Methane production (% DE) in several non-domesticated herbivores*

Species	Diet	Methane production (%)	Reference
FOREGUT FERMENTERS			
Eland, Wildebeest	Hay and concentrates	12	Rogerson *et al.* (1968)
Red Deer	Hay and concentrates	7	Simpson *et al.* (1978)
White-tailed Deer	Browse	5	Mautz *et al.* (1975)
White-tailed Deer	Concentrates	7	Thompson *et al.* (1973)
Grey Kangaroo	Lucerne hay	0.4	Kempton *et al.* (1976)
Tammar Wallaby	Lucerne hay	< 1	Englehardt *et al.* (1978)
HINDGUT FERMENTERS			
Snowshoe Hare	Browse and concentrates	0.6	Holter *et al.* (1974)
Rock Ptarmigan	Pelleted diet	0.3[1]	Gasaway (1976)
Greater Glider	*Eucalypt* foliage	0.7	Present study

[1] Proportion of metabolizable energy intake

there seems little reason to suspect that losses via this route would be any greater in the Brushtail Possums since the rates of fermentation in the hindgut were so similar to those in the Greater Glider (Section 9.3). While methane loss may be up to 15% of digestible energy intake in some wild ruminants (Robbins, 1983), it does not seem to be an important avenue of energy loss in hindgut fermenters (Table 8.6). Low concentrations (9×10^{-4} M) of d-limonene (a component of the essential oils of both *E. radiata* and *E. melliodora*: Chapter 7, Appendix 2) have been shown to be toxic to some rumen methanogens (Crane *et al.*, 1957). However, there seems to be little interaction between terpenes and hindgut micro-organisms (Chapter 7).

The excretion of steam volatile material in the urine of the Greater Glider (Chapter 7) accounted for only 6 kJ or about 2% of the total urinary energy loss (assuming a caloric density of 6.5 MJ/mole monoterpene (Weast, 1979)). The amount of glucuronic acid, which is one of the main compounds used in the conjugation and excretion of terpenes (Section 1.3.4) was estimated in urine samples from six Greater Gliders and six Brushtail Possums collected in conjunction with these experiments (J.A. Dash pers. comm.). Greater Gliders excreted 600 ±30mg per day which would have accounted for about 5% of the heat of combustion of the urine. In the Brushtail Possum, glucuronic acid excretion accounted for a similar amount of urinary energy but a greater proportion (15%) of the total urinary energy output. However, marsupials do not excrete free glucuronic acid (Marsh, 1969), but glucuronides; the heat of combustion of these may be much higher than the free acid depending on the structure of the molecule.

There are a large number of other terpene detoxification products found in the urine of Brushtail Possums and Koalas (Southwell, 1975, Flynn and Southwell, 1979), and similar complex aromatic compounds could be expected to be formed by Greater Gliders. These compounds too will be partly responsible for the high heat of combustion of the urine. However, without detailed knowledge of the composition of the compounds excreted, little more can be said of the contribution of terpene detoxification products to the total urinary energy loss.

Urinary nitrogenous compounds would also be responsible for a proportion of the urinary energy, and differences in the composition of the urinary nitrogen between the two species may be responsible for differences in the ME concentration of the two diets (Table 5.7). The excretion of 1 g of nitrogen as ammonia involves the loss of 38% more energy than the excretion of the same amount of nitrogen as urea (Blaxter *et al*, 1966). Creatinine excretion costs even more energy per gram nitrogen, and loss of N as hippuric acid costs about 14 times as much as urea-N excretion (Blaxter *et al.*, 1966). Although no hippuric acid was detected in the urine of Greater Gliders, it was a common (but very variable) component of the urine of the Brushtail Possums. On the other hand, the higher ratio of ammonia to urea in the urine of Greater Gliders would tend to increase the urinary energy losses of this species compared with the Brushtail Possums. Comparison of the ratios of urinary nitrogen excretion to urinary energy excretion between the two species (Greater Gliders = 0.65 MJ/g N; Brushtail Possums 0.45 MJ/g N) suggests that with the differences in urinary N composition, nitrogenous compounds contributed a greater proportion of the total heat of combustion of the Brushtail Possum urine than the Greater Glider urine.

8.4.3 **Energy retention in Greater Gliders**

Respiration of *E. radiata* leaf in the calorimeters produced significant amounts of carbon dioxide (and hence consumed oxygen) and corrections were necessary to the animal gas volumes and heat productions. This is in contrast to results reported by Cork (1981) who could not detect any change in oxygen consumption due to *E. punctata* respiration in an open flow system. Either the flow rates used in Cork's study were too high, or the equipment used not sensitive enough to detect the small changes in gas composition due to leaf respiration. The respiration rates of the *E. radiata* foliage were within the range of values in live eucalypt seedlings from a range of growth habits (Mooney *et al.*, 1978).

The relationship between ME intake and energy balance has been represented by a single linear equation (Figure 8.4) both above and below maintenance. Theoretically (Blaxter, 1962, Reid *et al.*, 1980, Webster,

1980, Blaxter and Wainman, 1961) the relationship should be curvilinear; above maintenance each increment in metabolizable energy intake should result in progressively smaller increments in energy retention. Below maintenance, curvilinearity in the relationship is mostly due to increases in endogenous energy losses relative to undigested dietary energy. There was no advantage in fitting other than a straight line to points below maintenance and there were too few points above maintenance to fit a second component. Blaxter and Wainman (1961) found that there was no serious error introduced if the relationships between ME intake and energy balance were represented as straight lines. In this model, the slope of the line below maintenance is the efficiency of utilization of metabolizable energy for maintenance or the net energy coefficient. This is the efficiency with which metabolizable energy substitutes for tissue energy in meeting maintenance requirements (Robbins, 1983). The ME intake at zero energy balance is the maintenance energy requirement. Although this method of representing energy transactions is widely recognized (Blaxter, 1962) it ignores the fact that anabolic and catabolic processes proceed simultaneously (Blaxter, 1971).

The efficiency of utilization of ME of *E. radiata* for maintenance by Greater Gliders is high compared with the utilization of hay and concentrate diets and browses by large wild ruminants at maintenance (Table 8.5). It is also high compared with the efficiency of use of ME of a browse and concentrate diet for maintenance and gain by the snowshoe hare (Holter *et al.*, 1977), a hindgut fermenter of similar size to the Greater Glider.

Net energy coefficients (as a proportion of DE) are generally regarded as being lower in foregut fermenters than in other herbivores because of the inefficiencies of foregut fermentation; (e.g. the fermentation of dietary glucose to short-chain fatty acids (SCFA)). This suggests that in the Greater Glider SCFA are not a major metabolizable energy source and data to be reported in the next chapter suggest that SCFA make only a minor contribution to the energy economy of this species. However, in domestic ruminant species, acetate alone is utilized less efficiently than either propionate alone or butyrate (Blaxter, 1971), but the overall efficiency depends on the molar proportions of these SCFA.

There are several factors which affect the efficiency of use of ME:

1) Net energy coefficients for maintenance and gain decrease as the dietary ME concentration decreases (Reid *et al.*, 1980).

2) In ruminants, the higher the amount of nutrients that escape fermentation, the higher the efficiency of use of ME (Armstrong and Blaxter, 1961, Reid, 1974).

3. The form and nature of absorbed nutrients. Lipid is used more efficiently than carbohydrates which, in turn, are used more efficiently than protein for both maintenance and gain in both foregut and hindgut fermenters (Blaxter, 1971, Nehring *et al.*, 1964).

4) The relative proportions of fat and protein being either deposited (above maintenance) or mobilized (below maintenance). Lipid deposition normally occurs with an efficiency of about 75% (Webster, 1980), while the efficiency of protein deposition is typically around 45% (Kielanowski, 1976).

5) Reserves available to the animal. Van Soest (1982) has suggested that economies of underfeeding is one of the reasons why net energy coefficients below maintenance are higher than those above maintenance. For example, fasting in fat sheep results in a higher heat production than a lean animal of similar body mass (Marston, 1948).

Therefore, in view of the well established inverse relationship in (1) above, the high efficiency of use of ME is surprising since the ME concentration of *E. radiata* was so low. Similarly, the high efficiencies do not seem to be associated with the metabolism of lipid, since the RQ during all periods, including those of food restriction, remained close to 1.00 which is indicative of carbohydrate catabolism. Cork (1981) suggested that Koalas might obtain up to 27% of their digestible energy intake from the crude lipid fraction of *E. punctata*. However, this is likely to be an overestimate since the crude lipid fraction would also have included

components of the essential oils. Similarly some of the faecal lipid would be expected to be bound as calcium soaps (Grace and Body, 1983) and would not be extracted by the non-polar solvents used.

8.4.4 **Maintenance Energy Requirement**

The maintenance energy requirement (MER) of the Greater Glider was estimated to be 0.35 MJ ME·kgW$^{-0.75}$·d^{-1}. This is the same as the mean ME intake recorded during the feeding experiments (Table 8.2a). Similarly, the DE requirement for maintenance (0.58 MJ DE·kgW$^{-0.75}$·d^{-1}) is close to that ingested during the earlier experiments. Again, no account has been taken of methane losses but the measurements reported in Section 8.2.5 suggest that this omission will have only a minor effect on estimates of energy requirements. Therefore, during most of the feeding experiments the animals were in positive energy balance. The significant relationship between DE intake and nitrogen balance (Figue 5.7) suggested that the maintenance nitrogen requirement may have been overestimated due to catabolism of body proteins to compensate for an inadequate ME intake. While this is likely to have occurred in experiments P2 and P8, the extra urinary nitrogen may have been associated with requirements for the maintenance of acid-base equilibrium rather than with the degradation of body tissues to meet energy requirements (Chapter 5).

This estimate of the maintenance energy requirement of the Greater Glider is about 1.7 times their FHP. Cork (1981) found the MER of captive Koalas under similar conditions to be 1.8 times FHP. However, heat production in the Koalas was determined only at one level of feed intake. The animals in Cork's (1981) study were ingesting energy at a rate of 2.8 times FHP. Although body mass was constant, the accuracy of the weighing procedures was such that it was not possible to rule out the deposition of fat as the fate of this extra energy.

Extrapolation of the relationship between ME intake and energy balance to zero ME intake indicates a negative tissue energy balance in the Greater Gliders of -0.33 MJ·kgW$^{-0.75}$·d^{-1}. This is markedly different from the fasting heat production of 0.207 MJ·kgW$^{-0.75}$·d^{-1}. The line would

intersect the Y axis at the FHP if there was an input of ME of about 123 kJ. Although the animals were probably still absorbing some ME from fermentation even after 24 h fasting it is likely to have provided only a small fraction of the 123 kJ.

The remainder could be partly due to endogenous energy losses during the fasting measurements. Animals did not defecate or urinate during the measurement period, but even so, the long digesta retention times meant that it would not have been possible to estimate the endogenous fraction. Also animals on the restriction treatments were expending energy in prehension, mastication and gut motility. Hence, even though both the fasting measurements and some of the restriction experiments represented zero ME intake, energy balance for those fed restricted amounts of foliage included an "activity" expenditure as well.

8.5 Summary

The standard metabolic rate of the Greater Glider was similar to that found for the majority of marsupials but slightly higher than estimates of SMR in the Brushtail Possum. There were marked differences in the partitioning of ingested energy between the two species. The Greater Glider digested 57% of the gross energy intake compared with only 46% by the Brushtails. However, the Brushtails lost only 24% of this in the urine whereas 43% of the energy digested by Greater Gliders was excreted in the urine. The low metabolizability of *E. radiata* was mostly attributable to its high content of essential oils, though the excretion of most of the urinary nitrogen of the Greater Glider as NH_4-N would also have increased urinary energy losses. In spite of the low metabolizability of *E. radiata*, Greater Gliders could maintain themselves in energy balance since the net energy co-efficient for maintenance was high.

Chapter 9

MICROBIAL DIGESTION IN THE GREATER GLIDER AND BRUSHTAIL POSSUM

9.1 Introduction

Greater Gliders and Brushtail Possums digested 34% and 27% respectively of the cell walls of their eucalypt foliage diets (Chapter 4). Since no vertebrates are known to produce endogenous cellulases, degradation of plant fibre must be achieved by microbial fermentation. On anatomical grounds, microbial digestion in both species would be expected in the caecum (and proximal colon of the Brushtail Possum). Observations with a scanning electron microscope (Chapter 4) showed that these organs, in both species, harboured numerous micro-organisms that attached to and degraded plant cell walls.

The large proportion of lignified fibre, together with the presence of tannins and essential oils in eucalypt foliage, could be expected to limit the extent of microbial digestion. Both eucalypt essential oils and polyphenolics have been shown to possess antimicrobial activity (Chapter 1). It was concluded (Chapter 7) that there was little interaction between essential oils and the microbial population in either species. However, in the Brushtail Possum, the formation of complexes between tannins and microbial cellulases may have been responsible for the depressant effects of tannins on NDF digestion (Chapter 6).

Short chain fatty acids (SCFA) are the principal non-gaseous end products of microbial digestion. This chapter reports measurements of the concentration and rate of production of SCFA *in vitro* in the gut contents of both Greater Glider and Brushtail Possum. The production of methane (a gaseous by-product of fermentation) in the Greater Gliders was reported in Chapter 8.

9.2 **Materials and Methods**

The concentration and production of SCFA was measured in six Greater Gliders and four Brushtail Possums. Animals were maintained as described in Section 2.3 and fed *E. radiata* and *E. melliodora* foliage respectively. Two Greater Gliders were killed at each of three times during the day; during the feeding period (≃ 2300 h), after feeding (≃ 0900 h), and just before feeding (≃ 1800 h). Two Brushtail Possums were killed after feeding but only one measurement was made at each of the other two times.

Animals were killed with an overdose of sodium pentobarbitone. The gut was rapidly removed and cut into segments. These segments were the forestomach, hindstomach, duodenum, jejunum, ileum, caecum, proximal colon, distal colon and rectum. The pH of digesta in gut segments was determined with narrow range pH paper. SCFA production rates were measured in the caecum of the Greater Gliders and separately in the caecum and proximal colon of the Brushtail Possums. Each of these segments was weighed and the contents emptied into a warm (37° C) glass jar filled with CO_2. The digesta were mixed and a subsample taken for determination of the initial dry matter and SCFA concentration. The jar was then flushed with CO_2, capped, and incubated in a water bath at 37° C without any addition of substrate or buffer. Further subsamples were taken initially at 15 minute intervals and then half-hourly for 2-3 hours.

In both species, incubation of caecal contents commenced 4-5 minutes after death, with a further 3-4 minutes before incubation of the Brushtail Possum proximal colon contents. Sampling from other parts of the gastro-intestinal tract proceeded simultaneously.

Subsamples (≃ 7-10 g) from the incubation jars and from other gut segments were placed in glass vials with 0.5 ml saturated $HgCl_2$ to stop microbial activity, then stored at -20° C. These subsamples were extracted with distilled water and the supernatant separated by high speed centrifugation (12000 g) for 15 min. Total SCFA was determined by steam distillation (Annison, 1954) and titration against NaOH in CO_2-free

TABLE 9.1a: *Concentration and molar proportions of SCFA in digesta from different sites in the gut of Greater Gliders (Means ± SE)*

SCFA	Region of gut								
	Fore-stomach	Hind-stomach	Duodenum	Jejunum	Ileum	Caecum	Proximal colon	Distal colon	Rectum
Total SCFA ($mmol.l^{-1}$)	3.0 ± 1.2^{a}	2.5 ± 0.8^{a}	4.2 ± 1.6^{a}	6.0 ± 2.7^{a}	5.1 ± 1.1^{a}	36.1 ± 2.7^{b}	34.1 ± 2.8^{b}	32.9 ± 4.16^{b}	25.4 ± 6.2^{b}
Acetic	92.2 ± 2.4^{a}	91.9 ± 3.1^{a}	86.8 ± 2.5^{a}	88.2 ± 2.9^{a}	90.6 ± 3.8^{a}	73.8 ± 2.0^{b}	76.2 ± 1.9^{b}	77.6 ± 2.4^{b}	80.1 ± 2.7^{ab}
Propionic	5.6 ± 0.9^{a}	6.2 ± 0.9^{a}	8.8 ± 1.7^{a}	8.0 ± 0.6^{a}	7.8 ± 1.1^{a}	16.7 ± 1.8^{c}	14.4 ± 2.1^{cb}	11.5 ± 1.7^{b}	10.9 ± 1.9^{b}
Iso-butyric	<	<	0.1 ± 0.1^{a}	<	0.1 ± 0.1^{a}	0.1 ± 0.1^{a}	<	<	<
n-butyric	1.9 ± 0.5^{a}	1.8 ± 0.2^{a}	4.0 ± 1.2^{a}	3.7 ± 1.4^{a}	1.4 ± 0.7^{a}	7.6 ± 0.7^{b}	9.2 ± 0.9^{b}	10.7 ± 0.8^{b}	8.0 ± 1.4^{b}
Iso-valeric	<	0.1 ± 0.1^{a}	<	<	<	0.3 ± 0.2^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	<
n-valeric	0.1 ± 0.1^{a}	<	0.1 ± 0.1^{a}	<	<	0.3 ± 0.1^{a}	0.1 ± 0.1^{a}	<	<
pH	2.6 ± 0.1	2.0 ± 0.1	6.8 ± 0.2	7.0 ± 0.1	6.8 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.5 ± 0.2	6.2 ± 0.4

< Less than 0.1%

a,b,c = statistical analysis code within rows ($P < 0.05$)

TABLE 9.1b: *Concentration and molar proportions of SCFA in digesta from different sites in the gut of Brushtail Possums (Means ± SE)*

VFA	Region of gut								
	Fore-stomach	Hind-stomach	Duodenum	Jejunum	Ileum	Caecum	Proximal colon	Distal colon	Rectum
Total VFA ($mmol.l^{-1}$)	5.2 ± 1.2^{a}	7.7 ± 1.5^{a}	3.8 ± 1.0^{a}	4.7 ± 1.4^{a}	4.4 ± 0.6^{a}	60.8 ± 7.4^{b}	73.9 ± 10.2^{b}	64.6 ± 10.0^{b}	27.8 ± 6.8^{c}
Acetic	96.6 ± 3.4^{a}	98.2 ± 2.6^{a}	88.2 ± 6.4^{b}	95.8 ± 2.4^{a}	98.8 ± 1.1^{a}	74.7 ± 1.1^{b}	77.1 ± 1.4^{b}	75.6 ± 0.9^{b}	79.0 ± 4.2^{b}
Propionic	1.7 ± 0.7^{a}	1.0 ± 0.7^{a}	5.6 ± 3.2^{b}	2.3 ± 2.3^{a}	0.7 ± 0.7^{a}	6.8 ± 1.2^{b}	6.3 ± 1.1^{b}	6.8 ± 1.1^{b}	10.3 ± 1.5^{c}
Iso-butyric	<	<	<	<	<	0.1 ± 0.1	<	<	<
n-butyric	1.6 ± 1.0^{a}	0.8 ± 0.4^{a}	6.0 ± 2.5^{bc}	1.9 ± 0.9^{a}	0.5 ± 0.5^{a}	18.3 ± 1.1^{b}	16.3 ± 0.7^{b}	16.6 ± 0.3^{b}	10.5 ± 4.0^{bc}
Iso-valeric	<	<	0.2 ± 0.2^{a}	<	<	<	0.1 ± 0.1^{a}	0.7 ± 0.2^{a}	0.2 ± 0.1^{a}
n-valeric	<	<	<	<	<	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	<
pH	2.4 ± 0.3	1.8 ± 0.3	7.0 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	7.1 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.5 ± 0.3

< Less than 0.1%

a,b,c = statistical analysis code within rows ($P < 0.05$)

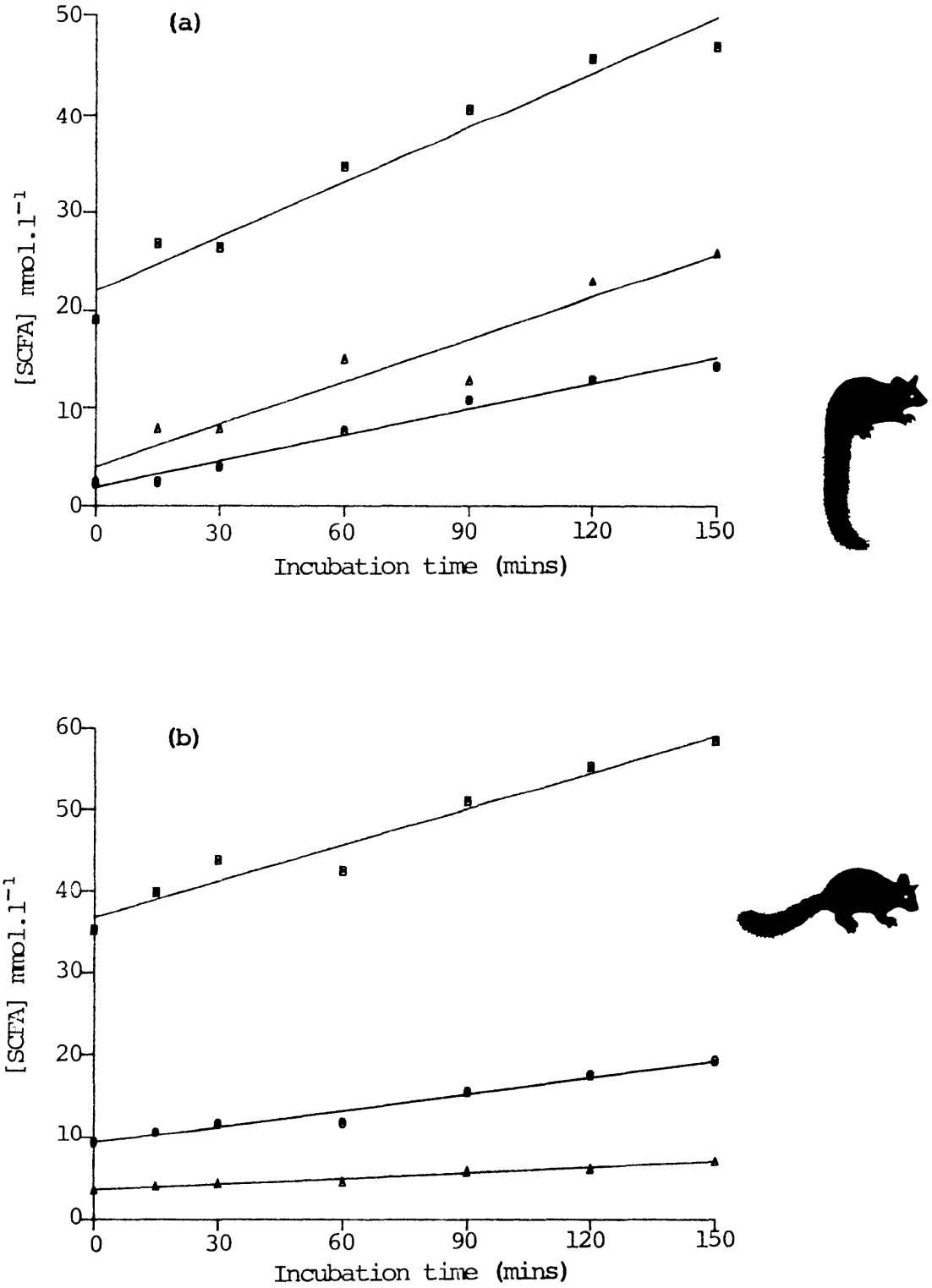

FIGURE 9.1: *The change in concentration with time of* □ *acetic,* △ *propionic and* ○ *butyric acid during* in vitro *incubation of caecal content of -*
(a) one Greater Glider; and
(b) one Brushtail Possum.

conditions. The molar proportions of individual SCFA in the distillate were determined by gas-liquid chromatography (Erwin *et al.*, 1961). The production rates of acetic, propionic and butyric acids and total SCFA were determined by the zero time method (Carroll and Hungate, 1954) from the slope of the linear regression of SCFA concentration versus time. The SCFA produced were converted to their energy equivalents using the calorific values of Blaxter (1962).

9.3 **Results**

9.3.1 **Concentration of SCFA at different sites in the gut**

Tables 9.1a and 9.1b show the concentration and molar proportions of SCFA and the pH at different sites in the gut of both species. The concentration of SCFA was higher ($P<0.001$) in the hindgut of both species than in the stomach or small intestine. There was no significant difference in the concentration of SCFA between the caecum and proximal colon in either species. The molar proportion of acetate was lower ($P<0.05$) in the hindgut than in the stomach and small intestine of both species. The proportions of butyrate and propionate showed the opposite trend. In the Greater Gliders there was a trend towards a higher proportion of acetate with increasing distance along the hindgut and a concomitant decrease in the proportion of propionate. While acetate showed a similar trend in the hindgut of the Brushtail Possums, it was accompanied by a decrease in the proportion of butyrate. In both species, the pH of the stomach digesta was acidic whereas that in all other gut segments pH was at or close to neutrality.

9.3.2 **SCFA production rates**

Figures 9.1a and 9.1b show an example of the rate of change of concentration of acetic acid, propionic acid and butyric acid during the incubation *in vitro* of caecal contents from one Greater Glider and one Brushtail Possum respectively. In all cases, the rates of change of SCFA concentration during incubations were linear with time. In both species

TABLE 9.2a: *In vitro production rates of SCFA in the caecum of two Greater Gliders at each of three times of day (Mean ± SE) (mmol.l^{-1}.h^{-1})*

Time	Acetic	Propionic	Butyric	Other[1]
Post-feeding	7.92_{a} 6.60^{a}	5.88_{a} 4.32^{a}	5.76_{a} 2.88^{a}	0.60_{a} 0.30^{a}
Pre-feeding	9.00_{a} 6.78^{a}	4.86_{a} 3.84^{a}	2.70_{a} 1.44^{a}	0.42_{a} 0.06^{a}
Feeding	10.62_{a} 12.90^{a}	8.34_{b} 7.80^{b}	5.52 3.42^{a}	0.66_{a} 0.60^{a}
Mean	9.0 ± 1.0	4.7 ± 0.4 (8.1 ± 0.3)[2]	3.6 ± 0.7	0.4 ± 0.1
Percentage total	51	27	20	2

[1] Valeric acid, iso-butyric acid and iso-valeric acid

[2] During feeding

a,b = statistical analysis code within columns (see Section 2.7) ($P < 0.05$)

TABLE 9.2b: *In vitro production rates of SCFA in the caecum and proximal colon*[1] *of the Brushtail Possum (values are Mean ± SE) (mmol.l^{-1}.h^{-1})*

Gut segment	Acetic	Propionic	Butyric	Other[2]
Caecum	11.9 ± 1.8	1.4 = 0.4	4.3 ± 0.7	0.2 ± 0.1
Proximal colon	13.1 ± 1.2	1.6 ± 0.3	5.0 ± 0.4	0.3 ± 0.1
Mean[3]	12.5 ± 1.0	1.5 ± 0.2	4.6 ± 0.4	0.2 ± 0.1
Percentage total	67	8	25	1

[1] There were no significant differences between the caecum and proximal colon

[2] Valeric acid; iso-butyric acid and iso-valeric acid

[3] No significant difference in production rates with time after feeding

TABLE 9.3a: *Weight of caecal digesta and the contribution of caecal SCFA to energy intake of Greater Gliders*

Animal/period		Body weight (kg)	Gut segment	Contents (g)[1]	SCFA production			% DEI[2]
					$mmol.d^{-1}$	$kJ.d^{-1}$	$kJ.kg^{-0.75}.d^{-1}$	
1	Post-feeding	0.968	C	97.3	36.6	55.0	56.4	9.3
2		1.099	C	113.0	30.8	42.9	40.0	6.6
3	Pre-feeding	1.171	C	137.7	46.4	61.4	54.5	8.9
4		1.188	C	119.5	28.8	36.5	32.1	5.3
5	Feeding	1.007	C	74.0	35.5	51.1	50.9	8.3
6		1.214	C	80.2	38.1	50.1	43.3	7.1

[1] Wet weight

[2] DE intake from Table 8.2a

TABLE 9.3b: *Weight of digesta in the caecum and proximal colon and the contribution of SCFA produced therein to energy intake of the Brushtail Possum*

Animal/time of death	Body weight (kg)	Gut segment	Contents (g)[1]	SCFA production			
				$mmol.d^{-1}$	$kJ.d^{-1}$	$kJ.kg^{-0.75}.d^{-1}$	% DEI[2]
1 0915	1.68	C	126.1	56.1	68.6	46.5	14.5
		PC	33.5	25.3	18.7	12.7	4.0
		Total	159.6	81.4	87.3	59.2	18.5
2 0920	2.82	C	123.2	36.2	48.2	22.2	6.9
		PC	61.2	27.7	36.1	16.6	5.2
		Total	184.4	63.9	84.3	38.8	12.1
3 1815	2.72	C	189.6	46.1	55.9	26.4	8.3
		PC	79.6	26.8	33.5	15.8	4.9
		Total	269.2	72.9	89.4	42.2	13.2
4 2310	2.06	C	112.4	45.5	59.4	34.6	10.8
		PC	55.0	37.7	49.7	28.9	9.0
		Total	167.4	83.2	109.1	63.5	19.8

[1] Wet weight

[2] DE intake from Table 8.2b

the low concentrations of valeric acid and the branched chain acids, iso-butyric and iso-valeric, meant that their production rates could not be determined with confidence.

Table 9.2a shows the estimated zero-time production rates of acetic, propionic and butyric acids at different times of day in the caecum of the Greater Glider. The production rate of propionic acid was higher ($P<0.05$) during the feeding period but there were no significant differences in total SCFA production rates during the day. Acetate was produced in the greatest quantities (51%) followed by propionate (38% during feeding; 27% other times) and butyrate (20%). However, the acetate production rate was lower than the proportion of acetate occurring in the caecal contents, while the proportions of propionate and butyrate produced were greater than their initial molar percentages. Acetate, propionate and butyrate productions were 0.7, 1.9 and 2.5 times the initial molar proportions respectively.

There were no significant differences in the production rates of any SCFA between the caecum and the proximal colon of the Brushtail Possum. Production rates of individual and total SCFA are shown in Table 9.2b. As with the Greater Gliders, acetate was the principal SCFA produced (67%), but in contrast to the pattern observed in the Gliders, butyrate comprised a greater proportion (25%) of the total SCFA produced than did propionate (8%). The ratios of the production rates of acetate, propionate and butyrate to their initial molar proportions in zero time digesta samples were 0.9, 1.2 and 1.5 respectively.

The mean daily production of SCFA (Table 9.3a and 9.3b) was calculated from the volume of hindgut digesta and the production rates of individual SCFA. The higher production rates of propionate during feeding in the Greater Gliders was assumed to apply for 6 hours. However, in this species, the total daily production of SCFA and its contribution to digestible energy (DE) intake was similar at all three times. SCFA production in the caecum contributed 7.6% of the mean intake of digestible energy (Section 8.3.2).

The total daily production of SCFA (Table 9.3b) was higher ($P<0.05$) in the caecum of the Brushtail Possum than in the proximal colon. This was a reflection of the relatively greater amount of digesta in the caecum. There were no significant differences in total SCFA production with time. Total daily SCFA production (caecum plus proximal colon) accounted for 15.9% of mean DE intake (Section 8.3.2).

9.4 **Discussion**

An *in vitro* incubation procedure was used in the present study for two reasons. Firstly, the rapid mixing of tracers with gut contents, which is required in *in vivo* procedures, could not necessarily be assumed in the hindguts of these two species. Secondly, caecal cannulation in other small, agile arboreal marsupials has not been successful (M.J. Chilcott, pers. comm.). Earlier, several attempts were made to measure caecal SCFA production rates *in situ* in both species in conjunction with the experiments of Rübsamen *et al.* (1983). Small dialysis bags filled with isotonic saline were inserted into caecal segments containing [^{14}C] acetate. However, in only one instance was equilibrium attained between the contents of the caecum and the contents of the dialysis bag.

There have been few comparisons of *in vitro* and *in vivo* techniques for measuring SCFA production. *In vitro* techniques invariably underestimate *in vivo* rates in digesta from the rumens of sheep and cattle (e.g. Whitelaw *et al.*, 1970). However, *in vitro* techniques should be more reliable estimates of hindgut fermentation rates since little rapidly fermentable substrate will be available in the hindgut. Faichney (1969) found similar production rates in the sheep caecum using isotope dilution simultaneously with incubation techniques. In contrast, Rübsamen *et al.* (1982) found that *in vivo* estimates of SCFA production in the hindgut of the Rock Hyrax were 8-9 times those found *in vitro*. Nevertheless, there is little reason to doubt the validity of comparisons made between species *in vitro*.

TABLE 9.4: *Short chain fatty acid production in several eutherian and marsupial herbivores*

Species	Diet	Site	Digesta Mass (kg)*	Digesta Volume (ℓ)	SCFA production mmol.ℓ$^{-1}$.h^{-1}	SCFA production μmol.g^{-1}.h^{-1}	DEI (%)	Reference
EUTHERIAN - Foregut fermenters								
Sheep	Chopped lucerne hay	R	5.2	--	23	--	29	Hume (1977a)
		C	1.8	--	16	--	7	Hume (1977a)
Black-tailed Deer	Browse	R	--	--	--	84	--	Allo *et al.* (1973)
		C	--	--	--	75	--	Allo *et al.* (1973)
Grey Duiker	Natural - browse	R	--	1.1	47	--	28[5]	Boomker (1983)
		C	--	0.1	70	--	4[5]	Boomker (1983)
EUTHERIAN - Hindgut Fermenters								
Rabbit	Alfalfa/concentrates (low-high fibre)	C	0.1	--	--	41-35	10-12[4]	Hoover and Heitman (1972)
	Rabbit pellets	C	--	--	--	--	30[2,4]	Parker (1976)
Porcupine	Natural	C	0.5	--	--	184	8[4]	Johnson and McBee (1967)
Howler Monkey	Fig leaves and fruit	C	--	--	--	1050-1490	≈30[4]	Milton and McBee (1983)
Elephant	Natural - browse and grasses	C+PC +DC	--	408	--	100	--	Van Hoven *et al.* (1981)
MARSUPIAL - Foregut Fermenters								
Red-necked Wallaby	Chopped lucerne hay	F	1.1	--	52	--	42	Hume (1977a)
		C	0.1	--	27	--	1	Hume (1977a)
Red-necked Pademelon	Chopped lucerne hay	F	0.5	--	39	--	21	Hume (1977a)
		C	0.1	--	29	--	2	Hume (1977a)
		F	--	--	99[2]	--	--	Dellow *et al.* (1983)
Tammar Wallaby	Chopped lucerne hay	F	--	--	113[2]	--	--	Dellow *et al.* (1983)
MARSUPIAL - Hindgut Fermenters								
Koala	Eucalypt leaf	C+PC	0.8	--	11	49	9	Cork and Hume (1983)
Brushtail Possum	Eucalypt leaf	C+PC	0.2	0.2	19	80	16	Present study
Greater Glider	Eucalypt leaf	C	0.1	0.1	20	77	8	Present study

* Wet

[1] R = rumen, C = caecum, PC = proximal colon, DC = distal colon, F = forestomach; [2] *In vivo* isotope dilution - all other values from *in vitro* fermentation of digesta; [3] Assuming 15% dy matter; [4] Production in proximal colon not measured; [5] Assuming DEI = 2 x basal metabolic rate

The rate of SCFA production in both Greater Gliders and Brushtail Possums was faster than that observed in the hindgut of the Koala (Cork and Hume, 1983) and sheep caecum (Hume, 1977a), but slower than that observed *in vitro* in the hindgut of a range of other herbivores (Table 9.4). Some of these differences may be due to differences in the interval from death to the commencement of the incubation of gut contents. For example, SCFA production rates in the Greater Gliders were greater than those found in wild animals shot while feeding by Cork and Hume (1978). Only 4-5 minutes elapsed between death and commencement of incubation in the present study but in Cork and Hume's (1978) work, transport of dead animals to the field laboratory, unavoidably took 25-30 minutes. This delay may have affected the rate of microbial activity. Similarly, removal of contents from the Koala hindgut in Cork and Hume's (1983) study took up to 30 minutes.

The very rapid fermentation rates in the hindgut of the Howler Monkey reported by Milton and McBee (1983) may be due to some of the sugars of the fig pulp diet reaching the hindgut. However, such rapid SCFA production rates are unexpected considering the high initial molar proportion of acetate (94%), the slow turnover of the Howler Monkey hindgut contents (Milton, 1981) and Milton and McBee's (1983) own conclusion that the substrate fermented was primarily plant cell walls.

The relatively slow rate of fermentation in the hindgut of the Koala, Greater Glider and Brushtail Possum may be a consequence of several factors. Little is known of the cellulolytic potential of the hindgut flora in either the Brushtail Possum or the Greater Glider. However, London (1981) found that purified cellulose was degraded only by mixed cultures of Koala hindgut micro-organisms and not by pure cultures. Secondly, the highly lignified nature of eucalypt fibre would tend to reduce its potential fermentability. SEM observations (Chapter 4) showed very few micro-organisms adhering to the lignified tissues such as xylem elements in the hindgut of either species.

Cork and Hume (1983) suggested that preferential retention of solutes and fine particles in the hindgut of the rabbit and Koala may have reduced the fermentable substrate per unit of digesta and hence the

fermentation rate. However, it could equally be argued that the larger surface area of the finer particles would increase the potential fermentability of the digesta. Selective retention of fine particles occurs in the hindgut of the Greater Glider but not in the Brushtail Possum (Chapter 3). While this difference had no apparent effect on the total SCFA production rates, it may be responsible for the different pattern of SCFA production in the two species.

Fermentation in the hindgut of the Brushtail Possums produced a greater proportion of acetate but only 25% as much propionate as in the Greater Gliders. The ratios between the proportional production rates of the three major acids was 51:27:20 in Greater Gliders, and 67:8:25 in Brushtail Possums, for acetate, propionate and butyrate respectively. The Brushtail pattern is typical of the fermentation of a higher fibre substrate (Hungate, 1966). Conversely, propionate production is high in ruminants when the diet contains a high proportion of soluble carbohydrates. There are conflicting reports on the effects of fibre on butyrate proportions. Champe and Maurice (1982) noted higher molar proportions of butyrate in rabbits fed higher levels of tropical grass fibre, but Hoover and Heitman (1972) found the opposite trend in rabbits fed diets of lucerne and concentrates. Nonetheless, the differences in proportional production rate of individual SCFA between the Greater Glider and Brushtail Possum are likely to be due to fermentation of a more fibrous substrate by the Brushtail Possums. This is consistent with the lack of a mechanism for clearing the hindgut of fibre in this species. Conversely, the higher rate of production of propionate found during feeding in the Greater Gliders may indicate that some easily fermentable material passes through the gut into the caecum very rapidly.

In this study, the production rate of butyrate was greater than the production rate of propionate in the Brushtail Possums, while the two acids were produced at similar rates (except during feeding) in the Greater Gliders. In most ruminant fermentations (except on molasses diets) and in macropods (Hume, 1977a) and equines (Glinsky *et al.*, 1976) production of propionate is much greater than butyrate. Butyrate production rates equal to or greater than propionate have been found in the hindgut of rabbits

(Hoover and Heitman, 1972), porcupines (Johnson and McBee, 1967), rats (Yang *et al.*, 1970) and Koalas (Cork and Hume, 1983). Until more is known of the biochemistry of fermentation in the hindgut, the significance of these differences is uncertain.

In both species, there was a difference between the initial molar percentage of acetate, propionate and butyrate and their proportional contributions to total SCFA production rate. Similar trends have been observed in the hindgut of a wide range of herbivores including the Koala (Cork and Hume, 1983), the rabbit (Hoover and Heitman, 1972) and both Rock and Willow Ptarmigan (Gasaway, 1976a,b). These trends suggest that selective absorption of SCFA occurs in the order butyrate > propionate > acetate. This conclusion is supported by the results of the *in vivo* perfusion experiments of Rübsamen *et al.* (1983). In both the Greater Glider and the Brushtail Possum, SCFA were absorbed in proportion to chain length. However, while the rate of SCFA absorption was similar in the caecum, proximal colon and distal colon of the Brushtail Possum, the proximal colon of the Greater Glider absorbed SCFA at rates nearly twice that found in the caecum (Rübsamen *et al.*, 1983).

The difference between the initial molar percentages of individual SCFA and their proportional production rates may also have been affected by end production inhibition of acetate production (Cork and Hume, 1983). However, this influence is likely to have been minor in the present study since the production rate of acetate was slow and linear in all cases. Similarly, the difference may have been accentuated by interconversions of SCFA. The synthesis of butyrate (but not propionate) from acetate has been demonstrated in the caecum of rabbits (Parker, 1976), and Faichney (1969) has shown the conversion of acetate to propionate in the sheep caecum. No data are available to assess the quantitative significance of these pathways in the Greater Glider and Brushtail Possum.

Hoover and Heitman (1972) and Cork and Hume (1983) have suggested that preferential absorption of butyrate rather than acetate or propionate, might benefit the rabbit and Koala by virtue of its high calorific value. However, there exists some uncertainty as to the extent of butyrate

metabolism in the gut wall. Henning and Hird (1972b) found that up to 36% of labelled butyrate was converted to ketone bodies in rabbit caecal tissue *in vitro*, but *in vivo* measurements indicated a conversion of only 4-6%. Similarly, Woodnut and Parker (1980) found only minor conversion of butyrate to ketone bodies in conscious rabbits. Extensive absorption of ketone bodies might benefit the animal in several ways. Firstly, the oxidation of butyrate to acetoacetyl CoA and subsequently to ketone bodies, could satisfy the energy requirements of the epithelial tissue (Roediger, 1980). This would "spare" coenzyme A for other metabolic purposes (Henning and Hird, 1972a). Secondly, ketone bodies could be used in place of glucose as a respiratory substrate for brain, kidney and skeletal tissues. This process may be important if absorbed glucose has to be diverted to conjugate detoxified allelochemicals.

The occurrence of high concentrations of SCFA in the gut of carnivores such as the dog (Banta *et al.*, 1979) suggests that plant polysaccharides are not the only source of SCFA. Meat, for example, contains complex carbohydrates (e.g. hyaluronic acid) which are fermented by intestinal bacteria (Salyers, 1979). From the data presented in Chapter 4, it can be calculated that the digestion of cellulose supplied up to 8% of the digestible energy intake (DEI) of both the Greater Gliders and Brushtail Possums (assuming a caloric density of 16.8 kJ/g cellulose (Brody, 1945)). This is similar to the present estimates of the contribution of SCFA to DEI in Greater Gliders but only half that estimated in Brushtail Possums. However, the conversion of carbohydrate to SCFA in the rumen occurs with an efficiency of only about 70-75% (Hungate, 1966, ARC, 1980), since energy is lost in the fermentative reactions. If a similar conversion efficiency is assumed for the hindgut, then up to 25% of Greater Glider SCFA production and up to 63% of Brushtail Possum SCFA must have arisen from the fermentation of substrates other than cellulose. While some hemicellulose may have been fermented, intestinal mucoproteins secreted by gut cells (Vercellotti *et al.*, 1978) and glucuronides excreted in the bile are two other likely sources of this extra fermentable carbohydrate.

From a knowledge of the concentration of faecal SCFA and the total faecal dry matter output (Chapter 4) it can be calculated that 99 and 98% of SCFA produced was absorbed by the Greater Gliders and Brushtail Possums respectively. Although the production rate of SCFA was similar in the two species, this absorbed SCFA represented only 8% of the mean DE intake of the Greater Gliders but 16% of DEI of the Brushtail Possums. This difference was principally due to the lower DEI of the Brushtail Possums. The higher production rate of butyrate in the Brushtails also increased the caloric value of the absorbed SCFA.

The contribution of absorbed SCFA to DE intake depends on several factors. These include the SCFA production rate, the rate of absorption of SCFA, the volume of digesta in the fermentation compartment, the rate of turnover of the compartment. Table 9.4 shows the SCFA production rates, digesta volumes and contribution of SCFA to DE intake in several herbivores. The slow rates of SCFA production in the hindgut of the Brushtail Possum and Greater Glider are offset by the large volume of digesta contained therein. Although production rates of SCFA in the caecum of macropods, sheep and the Grey Duiker were similar to or greater than the rates recorded here, the relatively small volume of digesta in the caecum of these three species meant that the contribution of SCFA to DEI was low. A rapid rate of turnover of digesta probably explains the low daily production of SCFA in the caecum of the pig (Farrell and Johnson, 1970) and rat (Yang *et al.*, 1970). Conversely, the slow rate of turnover of hindgut contents in the Greater Glider and Brushtail Possum, together with the large volume of digesta, compensates for the relatively slow production rate of SCFA.

Regardless of their contribution to the energy requirements of the Brushtail Possum and Greater Glider, SCFA may play a major role in the secretory and absorptive processes in the hindgut. Water absorption depends on the creation of an osmotic gradient generated by the active transport of sodium and SCFA (Stevens, 1978). Interrelationships between SCFA and sodium and water absorption have been shown in the hindgut of goats (Argenzio *et al.*, 1975), pigs (Argenzio and Whipp, 1979) and rats (Umesaki *et al.*, 1979). Rübsamen *et al.* (1983) found no relationship

between sodium and SCFA absorption in the hindgut of either the Greater Glider or Brushtail Possum. However, this may have been due to the differing buffering capacity of the solutions used in the absorption studies. Other important functions of the caecum may include the microbial disruption of tannin complexes (Lomdahl, 1983), and the possible absorption of amino acids and B-vitamins. Further research is needed to assess the significance of these functions and whether they have been important pressures in the evolution of the large hindgut in the Greater Glider and Brushtail Possum.

9.5 Summary

The caecum and the caecum/proximal colon was the principal site of microbial activity in the Greater Glider and Brushtail Possum respectively. SCFA production rates were similar *in vitro* in the two species and slower than in the hindgut of most other herbivores but this was offset by the large volume of digesta contained in the hindgut. The lower energy intakes of the Brushtail Possums meant that absorbed SCFA contributed twice as much to DEI (16%) in this species compared with the Greater Glider (8%). Fermentation of Brushtail hindgut contents produced a greater proportion of acetate and less propionate than in the Greater Glider but both species fermented substrates other than cellulose. The lignified nature of the eucalypt fibre, together with the possible inhibitory effects of plant tannins (at least in the Brushtail Possum), are likely to have been responsible for the low level of microbial activity.