INTRODUCTION

Few insects and even fewer mammals feed to a large extent on *Eucalyptus* foliage. This is in spite of the leaves being an easily apparent, abundant, year-round resource. Several reasons have been suggested to explain this situation. Firstly, eucalypt leaves are generally high in lignified fibre and low in nitrogen (Ullrey *et al.*, 1981a, Cork, 1984). Secondly, the leaves are perceived to be well defended against herbivores by polyphenolic compounds and essential oils (Freeland and Winter, 1975, Fox and MacCauley, 1977, Morrow and Fox, 1980). Both these groups of secondary plant compounds have the potential to reduce the palatability of the diet (Tamir and Alumot, 1970, Farentinos *et al.*, 1981), exert toxic effects against the herbivores (Dollahite *et al.*, 1962, Cleland, 1946) and inhibit microbial activity in the gut (Lyford *et al.*, 1967, Nagy and Tengerdy, 1968).

Several of the species that do feed on eucalypt foliage are strict folivores and in some cases can severely defoliate their food trees. For example, Journet (1981) showed that grazing by insects consumed 40-70% of *E. blakelyi* foliage annually. Similarly, Koalas have been responsible for the defoliation and death of *E. ovata* trees in Victoria (Martin and Lee, 1984). Clearly, some animals are able to cope with the low nutritive value and potential "defences" of the leaves. This thesis examines the effect of both the low nutritive value and the presence of secondary plant compounds on the utilization of *Eucalyptus* foliage by two marsupial arboreal folivores, the Greater Glider (*Petauroides volans*) and the Brushtail Possum (*Trichosurus vulpecula*).

The Greater Glider is one of the smallest mammals to feed exclusively on tree foliage (Eisenberg, 1978). The larger Brushtail Possum on the other hand, is more a generalist feeder, including species from the herb layer and a variety of non-eucalypt shrubs and trees together with varying proportions of eucalypt leaf (Freeland and Winter, 1975, Lintermans, 1979). Feeding on the ground would result in increased risks of predation for Brushtails as well as increased foraging times. Freeland

and Winter (1975) have suggested that ground feeding is necessary for Brushtail Possums since they are unable to maintain themselves on foliage diets alone. This idea has not yet been tested.

In recent years, increasing attention has been paid to the relationships between body mass, food intake, digestibility and rate of passage of digesta in herbivores. The high mass-specific energy requirement of small herbivores is considered to preclude them from eating high-fibre diets that require long digestion times (Janis, 1976, Parra, 1978, Van Soest, 1982). Animals such as the Greater Glider seem to be an exception to this generalization and the study of the interactions between these factors forms a central part of the present work.

The high fibre content of eucalypt leaf may also "dilute" the content of available energy (McNab, 1978). Additional energy may also be required for the excretion of ingested plant secondary compounds such as essential oils (Hinks and Bolliger, 1957a,b). These factors have led some authors (e.g. McNab, 1978) to suggest that arboreal folivores live close to the limits of their energy budgets. A low basal metabolic rate (BMR) has been suggested to be important in the maintenance of energy balance in arboreal folivores. Data on BMR are available for the Brushtail Possum but not for the Greater Glider. A study of the energy metabolism of both species including the contribution of fermentative energy to energy intake was a second central theme of the work.

Although some aspects of nitrogen metabolism have been studied in Brushtail Possums fed semi-purified diets (e.g. Wellard and Hume, 1981a), leaves may contain secondary plant compounds that have the potential to affect the nitrogen requirement of the animal. In particular, tannins have been shown to complex with dietary proteins hence rendering them unavailable to the animal (McLeod, 1974, Price and Butler, 1980). Similarly, nitrogen compounds (e.g. glycine) may be required for detoxification reactions. These factors, combined with the generally low nitrogen content of eucalypt foliage, made necessary the study of the nitrogen requirements of both species.

In view of the paucity of data on the digestion and metabolism of natural diets by small herbivores, a broadly based study encompassing both primary and "secondary" plant nutrients was considered most appropriate. It was hoped that this would provide a basis for further studies of the reasons for diet selection by arboreal mammals and help elucidate some of the factors limiting the utilization of high fibre diets by herbivores.

Chapter 1

LITERATURE REVIEW

1.1 *Eucalyptus* - Evolution and Life History

1.1.1 Distribution and leafing patterns

Eucalyptus is a genus of evergreen woody plants restricted originally to Australia with minor occurrences in Papua New Guinea and adjacent Indonesian islands. The majority of the 500 or so species are trees and it has been estimated that 90% of the forests and woodlands of Australia are dominated by eucalypts (Pryor and Johnson, 1971).

Eucalypts have been classified into five sub-genera between which there is no interbreeding. Extensive hybridization can occur within the sub-genera. Most eucalypts are insect pollinated, others are pollinated by birds while at least two species are known to be wind pollinated (Pryor, 1976).

Leaf production can occur by four means. Leaf axils contain two types of buds; naked buds from which the normal lateral branches arise and accessory buds which are dormant until the destruction of the naked buds by events such as drought stress (Jacobs, 1955). Some of these accessory buds will remain dormant staying just below the surface of the bark. These buds are then known as epicormic buds and can rapidly produce leafy shoots if the tree crown is severely damaged by fire or by insect defoliation. The fourth source of leafy shoots develops from what is known as a lignotuber. This is an underground mass of buds and associated vascular tissue, mostly found in seedlings and small saplings, that can develop shoots if the aerial part of the plant is destroyed by fire or grazing (Penfold and Willis, 1961).

Eucalypt leaves can have different physiological forms; the juvenile and adult leaf phases. Although there is great variation between species, the juvenile leaf phase may persist to the 20th or 30th node of a sapling before the transition to the adult phase (Pryor, 1976). Juvenile phase leaves are most often opposite and sessile whereas the adult phase leaves are alternate and petiolate. However, the terms juvenile and adult do not refer to the relative age of a leaf. The juvenile leaf phase may contain necrotic tissue along with newly expanded leaves. Eucalypt leaves can be retained on a tree for between 6 months and 3 years, depending on the species concerned, the weather and timing of growth flushes (Jacobs, 1955). Growth flushes also vary within species and sites.

1.1.2 **Evolution of *Eucalyptus***

Although the current day Australian flora is dominated by *Eucalyptus,* it is only a recent colonizer. It is generally accepted that *Eucalyptus* developed in Australia largely during the Tertiary. During the Eocene Period climates were warm with predominantly high rainfalls and most fossil records suggest that closed canopy rainforests were extremely widespread with *Nothofagus* spp. types occupying the cooler sites and broad leafed rainforest communities occupying the warmer areas (Kemp, 1978).

However, the middle Eocene saw a marked increase in *Nothofagus* and gymnosperm pollen types suggestive of a cooling of temperatures. This was accompanied by a decrease in floral diversity. During the early Oligocene temperatures were still low and *Nothofagus* - type vegetation dominated. However, the later Tertiary was marked by vast changes in soil parent materials and climate. The formation of laterite was one of the major pedological events of this period, implying a warm, wet climate over most of the continent (Barlow, 1981).

The land mass was also probably drifting northward at this time initiating the semi-arid and arid zones (Beard, 1977). The increase in aridity led to the reduction of the *Nothofagus* - gymosperm (e.g. *Podocarpus)* dominated assemblages. It was during the Oligocene that *Eucalyptus* first appeared (Gill and Ingle, 1975) although the genus is not

thought to have become a dominant part of the flora until the late Miocene (Kemp, 1978). The early eucalypt communities were probably interspersed with the contracting rainforests but the reduction in rainfall associated with increasing aridity, later led to the fragmentation of the eucalypt communities (Beadle, 1981). In the driest areas they were replaced by *Acacia* spp. The current day Australian flora is a mixture of many groups with diverse histories including many early groups, e.g. *Nothofagus* and *Atherosperma* and very recent colonizers such as the Indo-Malay genera *Ficus, Eugenia* and *Terminalia* (Specht, 1948). The most striking aspect of the flora is however, the development of a special kind of xeromorphy, i.e. scleromorphy. Sclerophyllous plants are generally characterized by small leaves with a thick cuticle and often with pungent points. Beadle (1954, 1966) has shown experimentally, that sclerophylly is a response to low levels of soil phosphorus. Sclerophylly is thought to have evolved during the mid-Tertiary possibly as an adaptation for minimizing leaf nutrient losses (Westman, 1978) or even as a means of reducing the phosphorus requirement for protein synthesis (Loveless, 1962). However, in *E. gummifera* communities, insoluble soil phosphorus can be released by tannic acid or by water extracts of leaves (Mullette, 1976). This may be an important adaptation to low phosphorus soils. In any case, the present day nutrient poor soils can probably be traced back to the intense leaching of the lateritic soils formed under warm, wet Miocene climates and the lack of significant subsequent tectonic activity (Kemp, 1978). The adaptation to low phosphorus soils results in a reduction in the number of cells formed and a reduction in the length of internodes. This results in smaller leaves and smaller plants (Beadle, 1966). Nonetheless, the thick cuticle and high proportion of sclerenchymous tissue found in sclerophylls have the potential to exert significant impact on herbivores.

1.1.3 **Herbivory and *Eucalyptus***

Few insects and even fewer mammals consume eucalypt leaves. The damage that phytophagous insects inflict on *Eucalyptus* is well documented (Morrow, 1977; Springett, 1978). Forest eucalypts in Australia can lose from 20-50% of their foliage annually (Kile, 1974; Carne *et al.*, 1974; Burdon and Chilvers, 1974). The loss is similar in woodland situations.

Journet (1981) has recently shown the annual loss of *E. blakelyi* leaves to be 40-70%. Morrow (1977) has pointed out that these losses are about ten times greater than typical estimates of insect derived foliage losses in various Northern Hemisphere forest types. Furthermore, since many eucalypts can produce new leaves continuously throughout the growing season, defoliation may continue for a prolonged period of time (Mazenec, 1967) although part of the loss is probably due to the premature shedding of damaged leaves.

While these high levels of damage may cause significant mortality to particularly sensitive species (e.g. *E. delegatensis*, Shepherd, 1957), other effects may include increased light penetration to the understorey (Pryor, 1976), reduction in competition and alteration of community structure, changes to the rates and intensities of nutrient flows (Springett, 1978) and growth depression. For example, partial defoliation of *E. regnans* by the phasmatid *Didymuria violescens* resulted in a 20% depression in diameter increment (Readshaw and Mazenec, 1969). Morrow (1977) has concluded that, as a group, eucalypts suffer from chronic and heavy levels of insect damage. Although this may often impair tree growth, it does not generally cause significant mortality.

There are few data on the degree and severity of mammalian folivory. Marples (1973) estimated that the annual consumption of leaf, bark and bud material by Greater Gliders (*Petauroides volans*) in southern N.S.W. was about 6 $kg \cdot ha^{-1}$. This is only a minor loss compared with the annual leaf production rates (1800-2000 kg per hectare) measured by Ohmart *et al.* (1983) in similar forest types. On the other hand, Koalas (*Phascolarctos cinereus*) have caused severe defoliation of eucalypt forest and woodlands in parts of Victoria (McNally, 1957; Martin and Lee, 1984). While this has mainly been in island situations, it does suggest that mammalian folivores have the potential for major impacts on their food trees.

1.1.4 **Evolution of gliding mammals and eucalypt allelochemicals**

The extensive climatic and vegetational changes in the Tertiary

also led to changes in the forest dwelling fauna. The vegetation changed structurally as well as botanically with the rainforests being replaced by more open-crowned sclerophyll forests and woodlands. Gill and Ingle (1975) have contended that it was this opening of the forest crowns that provided opportunities for the evolution of volant mammals. For example, *Gymnobelideus leadbeateri* (Leadbeaters Possum) is a non-volant petaurid remarkably similar to *Petaurus breviceps* - the sugar glider. Wakefield (in Gill and Ingle, 1975) has shown that during the last 20,000 years, there has been an elongation of the forearm bones of *Petaurus* compared with those of *Gymnobelideus*. A similar situation can be found with the closely related (Kirsch, 1977) *Petauroides* and *Pseudocheirus*. *Petauroides* (the largest of the marsupial gliders) has a significantly elongated tibia, fibia and femur compared with *Pseudocheirus*. The large number of gliding, arboreal, folivorous mammals (Eisenberg, 1978) has led some authors to speculate that this may be an energetically efficient form of locomotion (Emmons and Gentry, 1983). No data are available to test this hypothesis.

Earlier it was argued that the sclerophyllous habit of *Eucalyptus* was a response to low nutrient soils (see Section 1.1.2). Recently it has been suggested that the allelochemical profile of the genus is also influenced by the generally low levels of soil phosphorus and nitrogen (Mattson, 1980) since the two major groups of allelochemicals found in *Eucalyptus* (the essential oils and polyphenols) generally do not contain these elements. The work of Gartlan *et al.* (1980) tends to support the idea that the allelochemical profile of a particular community can be influenced by the soil nutrient content. These workers showed that the mature leaves of rainforest species growing on low fertility sandy soils in West Africa produce more phenolic compounds and less alkaloids than the same species growing on nutrient-rich soils in East Africa. The effect of this on the folivorous mammals of the two areas will be discussed in Section 1.2.4.

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Eucalyptus foliage is a valuable food resource for several species of insects and mammals. Many aspects of the evolution and life history of the genus are likely to be important in determining the nature and extent of herbivory viz. the continuous leaf growth, the sclerophyllous nature of the foliage and the possibility that the content of allelochemicals is influenced by the soil nutrients. In the next section, some of the characteristics and problems facing arboreal, folivorous mammals will be considered with a view to examining the types of adaptations likely to be found in those mammals utilizing *Eucalyptus* foliage as a food resource.

1.2 **Problems Faced by Arboreal Folivores**

1.2.1 **Characteristics of arboreal folivores**

Recently, arboreal folivores have come to be recognized as a distinct ecological and physiological group (Eisenberg, 1978). The arboreal folivores are a diverse group including some primates (both simians and prosimians), rodents, edentates, marsupials and a dermopteran. There are several convergent patterns among these groups and it has been suggested (McNab, 1978, Eisenberg, 1978) that this group of mammals have difficulty satisfying their energy requirements.

Most arboreal folivores have body masses that fall in a range of 1-12kg. Among primates, there is a strong positive correlation between body mass and the proportion of foliage in the diet (Clutton-Brock and Harvey, 1977). There are however, some notable exceptions to this. *Lepilemur* is one of the most strictly folivorous of the primates yet weighs only 700g (Hladik, 1978). There are several exceptions to the general relationship between body mass and degree of folivory among the arboreal marsupials. While the Koala is the largest member of the group, and also an apparently obligate folivore, (Bergin, 1978) several medium-sized possums (2-5kg) (e.g. Cuscuses (*Phalanger* spp.) and Brushtail Possums (*Trichosurus* spp.)) are not always highly folivorous (Hume, 1982). On the other hand, while all arboreal marsupials smaller than about 600g (e.g.

Petaurus, Acrobates and Dactylopsila) are nectar or insect feeders, *Petauroides* at 1.2kg is a strict folivore.

In primates, the trends between body mass and diet have been explained in terms of, firstly, the protein content of tree foliage, and secondly, of a requirement for a fermentative digestion. Hladik (1978) suggested that it was unrealistic for large arboreal mammals to forage for insects or pollen and instead had to satisfy their protein requirements from tree foliage. On the other hand, Clutton-Brock and Harvey (1977) suggested that a large body mass was advantageous to an animal feeding on leaves high in cellulose since this would reduce the animal's mass-specific energy requirements and allow time for fermentation of the cellulose. Among the primates, home range size and time spent feeding are negatively correlated with the proportion of foliage in the diet. Insufficient data exist to assess the strength of this relationship in marsupials. Similarly, Mace *et al.* (1981) have shown that primate and rodent arboreal folivores have a smaller relative brain size than terrestial mammals of comparable taxa. No marsupials were included in these analyses but Haight's (1984) data on Koala brain size and structure support this idea. Grand (1978) has hypothesized that arboreal folivores exhibit a reduced muscle mass compared to terrestial mammals though only two species of each group were included in his analyses. Eisenberg (1978) has highlighted the number of gliding genera found among arboreal folivores and pointed out that most members of this group produce very small (usually 1 or 2) litters.

The types of relationships described above are most often interpreted as a response to feeding on a ubiquitous and widespread food resource in contrast to a patchy or unpredictable resource such as seeds or fruits. For example, Mace *et al.* (1981) suggest that detecting and "capturing" tree leaves requires much less brain organizational complexity than that required for the search, detection and capture of fruits or insects.

Several problems exist with this type of explanation. Firstly, in spite of the variety of studies on feeding behaviour in primates (see Section 1.2.4) few data exist on the distribution of various potential food

items for these animals and indeed, young leaves of some tree species may be just as patchy and unpredictable as fruits and seeds. The second problem is the lack of data on the feeding ecology of arboreal marsupials and so some of the relationships described above may not reflect the diversity found within the group as a whole.

1.2.2 **The problem of body size**

Although body mass and surface area are ecological and physiological constraints on all animals, arboreal folivores may face two opposing influences on body mass. Arboreal folivores must exist in essentially a three-dimensional habitat as opposed to the two-dimensional habitat of many terrestial herbivores. While this problem is not unique to arboreal folivores, (rock wallabies, (*Petrogale* spp.) for example, have preferred habitats on the steepest areas of rocky slopes and outcrops (Short, 1982)), it is likely to be at its most extreme for these mammals. This is because the energy required to move a mass against gravity is directly proportional to the mass of that object. Therefore, the smaller the body mass of an arboreal marsupial or primate, the smaller the energy expenditure (per unit body mass) for climbing vertically up a tree (Degabriele and Dawson, 1979). Additionally, shoots on the outer extremities of branches may be inaccessible to large animals.

The opposing influence on body mass for an arboreal mammal is related to the animal's need for fermentative digestion (Section 1.3.3) if the structural components of the tree leaves are to be utilized. Basal metabolic rate generally decreases non-linearly with body mass (Kleiber 1961), and the basal metabolic requirements of mammalian herbivores increases with body mass to the exponent 0.75. Therefore, large animals require more total energy but small animals require more energy relative to their body mass. Since fermentation rates seem to have upper limits, this effectively means that small herbivores must increase their food intake, increasing at the same time the rate of passage of food through the gut and thus decreasing digestibility of plant fibre (Parra, 1978). If the arboreal folivore must feed on fibrous material, a higher body mass would

TABLE 1.1: *Composition of plant parts available to arboreal mammals (% DM)*

Component	Bark	Leaves		Flowers	Fruit[b]	Seeds	Grasses
		Young	Mature				
Dry matter (% wet matter)	--	12-46[1,3]	28-54[2,3,15]	14-22[1]	13-57[1,7]	--	10-70[5,13]
Ash	--	2- 9[1]	2-26[4,15]	3- 7[1]	2-13[1,7]	1- 5[1,4]	6-18[5,14]
Crude protein	3[6]	11-55[1,4,16]	5-30[2,3,15]	10-27[1]	4-11[1,7]	3-20[4,5]	2-20[9,11]
TNC[a]	--	0-16[1,2,4]	4-15[2,3,4]	4-10[1]	8-56[1,7]	2-17[4]	3-19[13]
Lipid	--	2- 5[1]	1-19[15,16]	1- 4[1]	1-52[1,7]	4-57[17]	3-10[13]
NDF	76[6]	24-41[1]	33-69[2,3]	--	--	53[5]	13-85[10]
"Cellulose"	44[6]	15-25[1,3,4]	10-24[2,3]	15-28[1]	6-24[1]	19[5]	15-40[9]
Lignin	20[6]	9-28[1,3]	10-33[3,15]	19-32[1]	3-18[1]	20[5]	3-14[8]

[a] Total non-structural carbohydrates; [b] Pulp only

[1] Subramaniam (1981); [2] Milton (1979); [3] Oates *et al.* (1980); [4] Waterman *et al.* (1980); [5] Short and Epps; [6] Van Soest and Robertson (1976); [7] Williams (1982); [8] Harkin (1973); [9] Lyttleton (1973); [10] Van Soest and Robertson (1977); [11] Brown and Main (1967); [12] Smith (1973); [13] Bailey (1973); [14] Ensminger (1978); [15] Cork (1984); [16] Hladik (1978); [17] McKey *et al.* (1981)

be more advantageous whereas the effects of living in a three- dimensional habitat would tend to select for smaller body mass. Therefore, it is interesting to note that arboreal folivores fall into a fairly narrow range of body mass from about 12kg (*Colobus satanus*) to about 700g (*Lepilemur mustelinus*). It could thus be expected that arboreal folivores would exhibit physiological or behavioural adaptations to overcome these size related problems.

1.2.3 **Tree foliage as a food resource**

Much of the recent work on the nutritive value of tree foliage (e.g. Waterman *et al.*, 1980, 1983) has been focussed on the relative concentrations of plant allelochemicals (e.g., tannins and alkaloids) and primary nutrients (protein and energy). However, some authors (e.g., McNab, 1978; Glander, 1978) have implied that animals feeding on tree foliage face greater problems in "detoxifying" allelochemicals than other animals. Whether this idea has sprung from the increased attention paid by phytochemists to woody plants rather than herbs is unknown (Swain, 1978). However, it is probably true that tannins occur in greater concentrations in woody plants than herbs (Bate-Smith, 1957).

Table 1.1 summarizes several studies that allow comparisons to be made of the composition of different plant parts available to arboreal animals. Tree leaves are generally higher in structural carbohydrates such as cellulose, hemicellulose, and lignin than other plant parts. On the other hand, non-structural carbohydrates (sugars and starches) are usually higher in fruits than leaves but the content in seeds is intermediate. While the lipid content of seeds is generally higher than that in leaves, some leaf species (e.g., *Eucalyptus,* Cork, 1984) are very high in crude lipid. However, it is likely that not all this crude lipid is available to animals since it occurs as essential oils or waxes.

Cork (1981) has highlighted a number of studies that show that the fibre of tree foliage is less digestible than the fibre of other herbaceous plants. For example, Wilson (1977) showed that the acid detergent fibre (and dry matter) of a mixture of tree foliage fed to either goats or sheep

was of much lower digestibility than shrubs or herbaceous legumes of similar ADF content. Much of this difference could be attributed to the higher lignin content of the tree leaves. Similarly Short *et al.* (1974) concluded that browse was of lower nutritive value to ruminants than herbage at similar fibre levels. This effect is also apparent in comparisons of grasses and legumes. Bosman (1970) showed that over a wide range of forages, organic matter digestibility was, on average, 15 percentage units higher in grasses than in legumes at the same level of neutral detergent fibre (NDF). It would be of interest to know whether this trend carried through to woody legumes and how these in turn compared to non-legume woody trees.

The final aspect of tree leaves that is noteworthy is the generally low nitrogen content of mature foliage. Several authors (e.g., Hladik, 1978; Milton, 1979) have remarked on the generally low levels of tree foliage nitrogen. Leaf nitrogen content is usually highly correlated with the photosynthetic capacity of the plant (e.g., *Eucalyptus,* Mooney *et al.*, 1978). This relationship has several important consequences. If reduced nitrogen content is seen as an antiherbivore "defence" (McNeill and Southwood, 1978; Fox and MacCauley, 1977) then it also imposes the cost of a reduced photosynthetic rate. If a plant invests heavily in "defensive" allelochemicals containing nitrogen, then this could divert resources from the carboxylating enzyme and thus reduce potential photosynthetic gain. On the other hand, if the plant was to invest heavily in lipid based (e.g., resins and essential oils) or phenolic (e.g., tannins and lignins) based compounds, then it requires more carbon; construction costs for lipids (3g glucose/g lipid) and phenolics (1.9g glucose /g phenolics) being greater than for nitrogenous compounds (Mooney and Gulmon, 1982). Attention needs to be paid to the role of nitrogen in the nutrition of the plant before its role in plant-herbivore interactions can be better assessed.

1.2.4 **Food choice by arboreal folivores**

Much of the recent literature on food choice by arboreal folivores has focussed on the relative importance of primary nutrients (protein, available carbohydrate and energy) and plant allelochemicals (in particular

tannins) on leaf choice. Several recent studies of food choice in colobine monkeys have measured a variety of chemical features of the forest vegetation likely to be important to these mammals. Oates *et al.* (1980) found that the staple food items of *Presbytis johnii* were of low fibre and low condensed tannin content. Similarly, Baranga (1982, 1983) found that the preferred foods of *Colobus guereza* and *Colobus badius* in East Africa were higher in moisture, crude protein and phosphorus but lower in lignin than rejected leaves. McKey *et al.* (1981) suggested that *Colobus satanas* selected items partly on the basis of mineral and crude protein levels while Oates (1978) has suggested that *Colobus guereza* ingested soil and some aquatic plants partly to offset mineral deficiencies in the rest of the diet. Waterman *et al.* (1980) have stressed the importance of highly digestible items in colobine food choice. Similarly, Milton (1979) has implicated high digestibility in food choice by Howler monkeys and Montgomery and Sunquist (1978) showed a correlation between *in situ* digestion rate and leaf selection by Three-toed Sloths.

McKey *et al.* (1978) have implicated the different phenolic contents of rainforest vegetation in East and West African forests as factors controlling the different feeding patterns of colobines. The leaves of trees in a West African forest (Douala-Edea) are rich in tannins and of high fibre content whereas the same taxa in the Kibale Forest, Uganda are lower in tannins but higher in alkaloids. It has been suggested (Waterman, 1980; McKey *et al.*, 1978) that this is part of the reason why West African species of *Colobus* feed more on easily digested items such as fruits and seeds. However, this interpretation has been challenged by Gautier-Hion (1983) who suggested that the dietary differences merely reflected the broadness of the frugivorous niche in West Africa.

Other groups of arboreal folivores have not received the same attention. Degabriele (1981, 1983) has suggested that foliage nitrogen is the critical nutrient limiting the distribution and abundance of the Koala but little evidence was presented in support of this idea.

Much of the work on the colobine monkeys has been carried out under misconceptions of the nature of the forestomach fermentation of this

group. It is most often referred to as "ruminant-like" (McKey *et al.*, 1981; Waterman *et al.* 1980) or "semi-ruminant" (Curtin and Chivers, 1978) in much the same way as the early descriptions of macropod marsupial digestive physiology (Hume, 1982). For example, this has led to the idea that only food of a certain particle size will be able to leave the colobine forestomach, as is the case in ruminants (McKey *et al.*, 1981). It is likely that better interpretations could be made of the large amount of data available on the nutrient content of food items once some information is available on colobine digestive physiology.

In the next section, consideration will be given to the range of digestive strategies found in arboreal folivores and the effects of these on the metabolism of plant allelochemicals.

1.3 **Adaptations of Arboreal Folivores**

1.3.1 **Metabolic rate**

Many of the characteristics of arboreal folivores such as small litter sizes and reduced home range sizes have been interpreted as energy saving adaptations. Indeed, McNab (1978) has suggested that folivores should display reduced basal metabolic rates. McNab (1978) suggested three reasons why low basal metabolic rates should be found in folivorous mammals:

(i) Since tree leaves may have a high structural carbohydrate content, there is a limit on their rate of digestion and consequently a limit on the animal's energy expenditure.

(ii) Since tree leaves contain plant secondary compounds, energy must be expended in their detoxification.

(iii) The reduced muscle mass found in arboreal mammals (Grand, 1978) should lead to lower basal metabolic rates and a reduced metabolic scope.

While these arguments should not necessarily be restricted to arboreal folivores, McNab's (1978) hypothesis was supported by data from

only seven mammal species and some of these (e.g. *Capromys pilorides* and *Coendou prehensilis*) are doubtful candidates for the group "arboreal folivores". Others with reduced basal metabolic rates such as the sloths *Bradypus* and *Choloepus* are heterotherms. In addition, no details of the procedures used in the metabolism measurements were reported in McNab's (1978) study and so it is difficult to assess whether the conditions for "basal" measurements were satisfied.

Several reports of metabolism in arboreal folivores have been published since McNab's (1978) hypothesis was advanced. Degabriele and Dawson (1979) have reported the Koala's basal metabolic rate to be only 74% of that expected for a marsupial on the basis of body mass. Milton *et al.* (1979) showed that the metabolic rate of the Howler Monkey was very similar to that predicted by Kleiber's (1961) equation. However, Müller *et al.* (1983) have suggested that basal conditions were not met in Milton *et al.*'s (1979) study since the measurements were made during the animal's active period. Müller *et al.* (1983) found that the folivorous primate *Colobus guereza* had a lower metabolic rate than expected which they claimed lent support to McNab's (1978) hypothesis. However, these experiments themselves were not performed under basal conditions since the measurements were made on sleeping, not resting animals. In addition the period of fasting (8 hours) was almost certainly too short for a post-absorptive state to be approached in an animal with an extensive foregut fermentation such as *Colobus* (Bauchop and Martucci, 1968, Bauchop, 1978).

Other groups of mammals such as the arboreal prosimians (Hildwein, 1972) and the marsupials (Hume, 1982) also seem to show a general phylogenetic reduction in metabolic rate. It may therefore be more appropriate to view the reduced metabolic rate of some arboreal folivores as a successful preadaptation rather than a specific adaptation to arboreal folivory (Degabriele and Dawson, 1979).

However, basal metabolism is only one part of the total energy expenditure or field metabolic rate. This includes the energy required for such functions as locomotion, thermoregulation, reproduction and territorial defence. Field metabolic rates have been determined for only

two arboreal folivores, the Three-toed Sloth (1.8 times the basal metabolic rate, Nagy and Montgomery, 1980) and the Howler Monkey (2.0 times basal; Nagy and Milton, 1979a). In contrast, Smith *et al.* (1982) have reported very high field metabolic rates for the arboreal (but non-folivorous) Leadbeater's Possum (5.8 times basal). This high energy expenditure was partly attributed to the effects of living in a three dimensional habitat.

Clearly, more data are needed on the energy expenditures of arboreal folivores under a wider range of conditions before generalizations can be made about their energy budgets. However, if "basal" metabolic rates are to be determined, the measurements must be made under defined conditions or else the data obtained will always be of doubtful validity.

1.3.2 **Rate of digesta passage**

Warner (1981a) in his review of digesta passage in mammals and birds, remarked upon the very long retention times shown by three species of arboreal folivore - the Two-toed Sloth (*Choloepus*), the Three-toed Sloth and the Koala. While these three species also exhibit reduced basal metabolic rates, (Section 1.3.1) part of the reason for the long retention times in the sloths is because these animals defaecate only once per week (Montgomery and Sunquist, 1978). Nonetheless, the retention times (100% excretion: 1200 hours in sloths; mean retention time for solutes, 400 hours in Koalas) are quite remarkable compared to other animals of similar body mass (Warner, 1981a).

Long retention times have been found in other arboreal folivores but do not necessarily appear to be related to the consumption of foliage diets. Wellard and Hume (1981b) and Honigmann (1941) found long retention times (65-70 h for particles and solutes) in *Trichosurus vulpecula* maintained on semi-purified diets. Unfortunately, there are too few data to evaluate the influence of the degree of folivory on digesta passage. Milton's (1981) work suggested that the folivorous Howler Monkey had a much slower passage rate than the sympatric but frugivorous Spider Monkey (*Ateles geoffroyi*). However, the passage rate was characterized by only

one aspect of marker kinetics, the time of first marker appearance and so this comparison is of very limited value.

1.3.3 **Fermentation**

The utilization of cellulose and other plant polymers by vertebrates depends on a symbiotic relationship between the organism and bacteria, protozoa and fungi (Parra, 1978; Orpin, 1981). The degradation of cellulose takes place anaerobically by microbial fermentation. Fermentation is an energy yielding process in which the oxidation and reduction of organic compounds is coupled with the transfer of electrons by NAD (nicotinamide adenine dinucleotide) resulting in the net production of ATP (adenosine triphosphate). The ATP generated by this process supplies the energy needed for metabolism and biosynthesis in the microbial cell while the gases (principally methane and carbon dioxide) and short chain fatty acids (SCFA) are excreted from the cell into the surrounding medium to be either metabolized or excreted by the host animal.

Fermentative digestion has arisen in many mammals (and other vertebrates) although there are differences in the site and extent of the fermentative regions. The variety of fermentative processes found in the arboreal folivores is a good example.

There is extensive expansion in the foregut in Old World monkeys (e.g. *Presbytis* and *Colobus)* and the Sloths (*Bradypus* and *Choloepus)* and evidence of active fermentations including the presence of cellulolytic bacteria, maintenance of a neutral or slightly acidic pH in the foregut and the production of SCFA have been reported (Bauchop and Martucci, 1968, Bauchop, 1978). All these characteristics are shared by ruminants and macropodids - two other major groups of foregut fermenters.

Expansion in the hindgut, usually the caecum and/or proximal colon, is found in prosimians (e.g. *Lepilemur*) New World primates (*Alouatta*) and folivorous marsupials (e.g. *Phascolarctos, Petauroides, Pseudocheirus* and *Trichosurus*). Evidence of active fermentations in the hindgut has recently been obtained for *Phascolarctos* (Cork and Hume, 1983)

and *Alouatta* (Milton and McBee, 1983).

Foregut fermentation involves microbial digestion of food prior to acid hydrolysis. The SCFA produced are absorbed from the foregut and microbial cells flowing out of the fermentation chamber are available for digestion in the small intestine, thus providing a valuable source of essential amino acids for the host animal. However, dietary sugars are also rapidly fermented to SCFA and because this conversion is less efficient than their direct absorption, it is a disadvantage of the foregut fermentation system. Although all foregut fermenters possess a secondary fermentation area in the hindgut, this is of minor importance compared with the foregut (e.g. Hume, 1977a). However, it is important to distinguish between the ruminant and non-ruminant foregut fermentations. In ruminants, the constriction at the reticulo-omasal orifice prevents larger food particles from leaving the rumen until they have been broken down by rumination and fermentation (Poppi *et al.*, 1980, 1981). There are no comparable structures to be found in either colobids (Chivers and Hladik, 1980) or macropodid marsupials (Hume, 1982) and it is thus likely that these species can increase digesta passage rates on higher fibre diets or at least maintain intake at a relatively higher level than can ruminants. Experimental confirmation of this idea is still scarce (Hume, 1982).

Hindgut fermentation involves acid hydrolysis prior to microbial fermentation and while this avoids the inefficiencies of the fermentation of simple sugars to SCFA, there may be disadvantages of this fermentation compared with a foregut system. It seems unlikely, for example, that microbial protein produced in the hindgut is absorbed. Slade *et al.* (1971) observed a small amount of [^{15}N] labelled lysine in the blood of horses after injection of [^{15}N] labelled bacteria into the caecum. On the other hand, Wysocki and Baker (1975) could not detect [^{14}C] labelled amino acids in portal blood after a bacterial suspension was injected into the equine caecum. Although the evidence is limited, it would seem that there is little quantitative amino acid absorption from the hindgut. The only way that the host animal could then make use of the microbial protein would be by coprophagy.

Two small folivorous mammals have been reported to be coprophagic. Hladik *et al.* (1971) have shown that *Lepilemur* ingests a special kind of faecal pellet and a similar behaviour has recently been reported in the Ringtail Possum (Chilcott and Hume, 1985). The ingestion of a special type of nutrient-rich faecal material is best termed "caecotrophy" (Hörnicke and Björnhag, 1981). It is likely that this behaviour is an important mechanism in allowing these two animals to overcome the limitations of a small body size (Section 1.2.2) and so use tree foliage as a food source.

Caecotrophy is usually found in those hindgut fermenters termed "caecum fermenters" (Hume and Warner, 1980). In caecum fermenters there is selective retention of the fluid and fine particulate digesta - the coarser particles being more rapidly eliminated. In those caecum fermenters that do not practice caecotrophy, this may benefit them by concentrating the more easily digestible nutrients in the caecum and reducing gut fill effects due to poorly digested fibrous foods. Passage rate may be increased to some extent on highly fibrous diets but this may be at the expense of water and electrolyte conservation.

Colon fermenters on the other hand are characterized by having the caecum and colon functioning as a single digestive unit. There is generally no selective retention of either digesta phase. This system is best typified by the Horse (Hume and Warner, 1980) and the Capybara (Baldizan *et al.*, 1983) and among the arboreal folivores by the Brushtail Possum (Wellard and Hume, 1981b). Janis (1976) has suggested that this system allows equids to increase the rate of passage of food on poor quality diets and so maximise the intake of easily digestible nutrients. Although this idea has not yet been adequately tested experimentally, it is likely to be the best strategy in situations where diet quality is limiting. However, when both diet quantity and quality are limiting, the "ruminant model" may well be superior.

At present there are insufficient data to detect any patterns in the occurrence of different fermentation strategies among the arboreal folivores. The sloths are among the most folivorous of all mammals and have an extensive foregut fermentation (Parra, 1978). On the other hand,

the sympatric Howler Monkey is a hindgut fermenter (Milton and McBee, 1983). Similarly, in Malaysia, the foregut fermenting colobine monkey, *Presbytis melalophos,* occurs sympatrically with the hindgut fermenting ape *Hylobates lar.* These latter two species include similar proportions of foliage in their diets (Curtin and Chivers, 1978) (although of different leaf ages and species) but there is little comparative information available on either the chemical composition of the diets or on the animals' digestive physiology on which to assess the importance of these differences. Whether the foregut fermenters need the high biological value microbial protein (Hladik, 1978) is unknown. One common suggestion (e.g. Waterman *et al.*, 1980, Janzen, 1979) is that foregut fermenters benefit from the detoxifying abilities of their microfloras. The effects of different fermentation strategies on the metabolism of plant allelochemicals will be considered in Section 1.3.5.

1.3.4 **Detoxification**

The ingestion of xenobiotics or plant allelochemicals can be tolerated if mechanisms exist for the detoxification of the compounds. Detoxification processes are aimed at the conversion of absorbed lipophilic compounds to more polar hydrophilic compounds that can be excreted in the urine or bile (Scheline, 1978).

Williams (1959) has suggested that this process is essentially biphasic - the first step (Phase I) reactions introduce into the xenobiotic, or expose within its structure, biochemically reactive groups such as -OH, -COOH, $-NH_2$ or -SH (Hirom *et al.*, 1977). This generally results in a decrease in biological activity of the xenobiotic although occasionally the reverse may occur (Scheline, 1978). The products of these reactions are then used as substrates for Phase II reactions which involve the combination of an endogenous molecule (often from carbohydrate or protein metabolism sources) with the exposed functional group of the xenobiotic.

Phase I reactions include oxidations, reductions and hydrolyses although the oxidations are by far the most important group of reactions.

While the oxidations are carried out in most body tissues, the mono-oxygenases located on the endoplasmic reticulum of the liver are the most important. These are also referred to as mixed function oxidases (MFO). It is important to note that MFO's can be induced by many xenobiotics including phenols and terpenes. Several studies (Jori *et al.*, 1969, 1970, Jori and Briatico, 1973) have shown that 1,8 cineole, a common constituent of many *Eucalyptus* essential oils (Southwell, 1978) induces MFO activity in adult and new-born rats. It would seem likely that terpenes are passed from mother to young via the milk but no evidence has been published to support this idea.

Detoxification of ingested xenobiotics therefore requires:

(1) Energy to synthesize and maintain enzyme systems;
(2) Specific nutrients as conjugates (for example, glucose, sulphate or glycine);
(3) Water to excrete the end products;

All these could be in short supply for arboreal folivores (and other mammals) and so lead to reduced intakes of particular foods since detoxification of the allelochemicals could not be achieved. While little work has been done on the detoxification processes in arboreal folivores, the problems of limited supply of water and specific nutrients have been considered in terrestial mammals. Wheldrake *et al.* (1979) showed that *Notomys alexis*, (an Australian desert murid independent of exogenous water) exhibited a much slower rate of excretion of lipophilic xenobiotics than other non-desert murids. Similarly, Maner and Gomez (1973) have highlighted the drain on sulphur amino acids for the detoxification of cyanide.

1.3.5 **Fermentation strategies and plant allelochemicals**

The relative advantages and disadvantages of various fermentation strategies for processing available primary nutrients have been considered above. While both foregut and hindgut fermentations occur among the arboreal folivores, little attention seems to have been paid to the effects

of these strategies on the metabolism of plant allelochemicals. In particular, the potential effects of different sites of absorption and detoxification have been ignored.

The first point to consider is whether the allelochemical in question is actually ingested. White *et al.* (1982) have recently shown that part of the monoterpene fraction of *Artemisia tridentata* foliage is lost during mastication by Pygmy Rabbits. If this is a widespread phenomena, then it would be an ideal way of dealing with potentially toxic compounds.

A foregut fermentation system may be less suited to feeding on compounds with high antibacterial and antifungal activity such as terpenes and certain phenols. While many authors have commented on the possibility of such compounds affecting caecal micro-organisms (e.g. Bryant and Kuropat, 1980), there is little evidence of them actually reaching the hindgut. For example, Eberhard *et al.* (1975) showed that all the components of the essential oil of *E. punctata* with high germicidal values had disappeared from Koala faecal oil. It is likely that the majority of these compounds were absorbed in the small intestine prior to reaching the hindgut (Igimi *et al.*, 1974).

Good quality dietary protein is often wasted in ruminants (and presumably other foregut fermenters) since it is subjected to microbial breakdown with the resulting ammonia being incorporated into microbial protein. While this system can be advantageous in providing essential amino acids from poor quality dietary protein or non-protein nitrogen, it is inefficient if it destroys an already adequate protein source. In domestic ruminant nutrition, dietary protein that can be protected from this degradation is termed "by-pass protein" (Kempton *et al.*, 1976b). This has been achieved by heating the protein or by treating it with formaldehyde. Tannin-protein complexes can also serve as "by-pass proteins" (Driedger and Hatfield, 1972, Barry and Manley, 1984). Tannin-protein complexes can form at rumen pH and remain stable until dissociated in the abomasum (Jones and Mangan, 1977).

The microbial population of foregut fermenters has often been described as the "first line of defence" (Reid, 1973; Freeland and Janzen, 1974). This view has been taken to the extreme by Janzen (1979) who has suggested that the "rumen" of wild animals should be seen more as a detoxification chamber than as a device for gathering proteins and calories. Freeland and Janzen (1974) have emphasized the important role of the microbes of complex rodent forestomachs (e.g. *Psammomys*) in the detoxification of oxalates. Rumen micro-organisms can degrade oxalate to formate and CO_2 (O'Halloran, 1962). Other examples of detoxification by rumen micro-organisms include the hydrolysis of the mycotoxin ochratoxin - A to the non-toxic ochratoxin - by cattle rumen (and rat caecal) microbes (Galtier and Alvinerie, 1976) and the degradation of the pyrolizidine alkaloids, heliotropin and lasiocarpine (Lannigan and Smith, 1970).

However, few authors writing in the ecological literature seem to consider the reverse situation i.e. the induction of toxicity by the microbial population. There are several common examples of these toxic conversions, including the reduction of dietary nitrate to the toxic nitrite (O'Hara *et al.*, 1975), the two-step conversion of formonentin to the oestrogen equol in sheep (Cox, 1978) and the conversion of the amino acid tryptophan to 3-methyl indole which leads to pulmonary oedema in cattle (Carlson and Dickinson, 1978).

It must be stressed that many of the micro-organisms that either degrade or synthesize toxins are inducible and that opportunities arise for synergistic interactions in the complex microbial ecosystems that make up fermentation areas. While it is not the intention here to catalogue all the types of reactions exhibited by foregut micro-organisms, it is clear that they do not always act as a "first line of defence" and that in some situations a foregut system may be at a disadvantage compared with a hindgut system as far as microbial detoxification is concerned.

Finally, the digestive strategy of an animal may influence the type of compound used in conjugation reactions. Baudinette *et al.* (1980) were unable to detect any patterns in the excretion of [^{14}C] phenol as either sulphate or glucuronide conjugates in a wide range of marsupials and

eutherians. However, Hume (1982) suggested that since foregut fermenters and carnivores do not absorb large quantities of glucose from the gut then they would rely on sulphate conjugation. Since glucose availability is not a problem for hindgut fermenters then they would be expected to excrete phenol excreted with glucuronic acid.

This predicted pattern is not displayed consistently in other similar experiments (Capel *et al.*, 1972) with some hindgut fermenters e.g. the African Elephant (Van Hoven *et al.*, 1981), showing a reliance on sulphate conjugation. In addition when Wheldrake *et al.* (1978) supplemented *Notomys alexis* with sulphate they were unable to change the ratio of phenyl-sulphate to phenyl-glucuronide conjugates.

1.4 *Eucalyptus* as a Food Resource

1.4.1 *Eucalyptus* primary nutrients

It has only been in the past few years that meaningful evaluations have been made of the nutritive value of *Eucalyptus* foliage. Prior to this, all evaluations have been made using the Proximate or Weende system of analysis. The deficiencies of this system are now well known (Van Soest and McQueen, 1973, Robertson, 1978). For example, the acid/alkali extraction used to determine crude fibre may remove up to 90% of the cell wall constituents (Robertson, 1978).

Ullrey *et al.* (1981a) determined the chemical composition of eucalypt foliage fed to captive koalas in the San Diego Zoo. The foliage was divided into two groups. The leaves which were accepted, generally had higher levels of total nitrogen and phosphorus and lower levels of cell wall constituents than rejected foliage. The nitrogen levels of the mature leaves consumed were between 1.0% and 1.8% which is very low compared with the diets selected by several other herbivores (Table 1.1). Total cell walls (as NDF) varied from 22-30% but importantly, the lignin content of the leaves (9-14%) was very high compared with other herbivore diets.

Cork (1984) has made a more thorough evaluation of the nutritive value of grey gum (*Eucalyptus punctata*) foliage for Koalas. Compared with other foliages this leaf was particularly high in soluble carbohydrates and lipids although the total nitrogen content was low (1.2%) and similar to Ullrey *et al.*'s (1981a) findings. Also, while some potential structural inhibitors were present in very high concentrations (e.g. lignin, 14%) others such as silica were very low. Seasonal variation in all dietary components were not significant in Cork's (1984) study.

Cork (1984) also showed that up to 50% of the nitrogen of *E. punctata* was non-protein nitrogen and that the proportion varied between individual trees. This is consistent with the findings of Journet and Cochrane (1978) who have shown that (30-35%) of the nitrogen of *E. blakelyi* foliage was in the form of free amino acids. This may have important nutritional consequences since free amino acids are only very weakly bound by tannins (Hagerman and Butler, 1981).

In summary, eucalypt foliage would appear to be a good source of lipids and non-structural carbohydrates but perhaps limited as a food source by low nitrogen content and high amounts of lignin in the cell walls, although other structural inhibitors such as silica are present in only small amounts.

1.4.2 **Allelochemical profile**

Eucalyptus foliage contains two major groups of allelochemicals - essential oils and polyphenolics, as well as several minor groups of compounds with potential "anti-herbivore" characteristics. In this section, the occurrence and potential effects of these compounds will be considered as well as their possible primary functions.

1.4.2.1 **Essential oils**

The essential oils are the best known and most obvious of the allelochemicals present in *Eucalyptus* tissues. Essential oils are principally composed of complex mixtures of terpenoids formed from the condensation of 2 (monoterpenes) or 3 (sesquiterpenes) isoprenoid residues

(Loomis and Croteau, 1973). They generally contain no phosphorus, nitrogen or sulphur (Carlwood and Banthorpe, 1978) although naturally occurring volatile compounds can contain these elements. Eucalypt essential oils are contained in glands (Carr and Carr, 1970) covered by a tough membrane in the leaves, petioles, bark and pith of most eucalypt species (Carr and Carr, 1970). Although all eucalypt species examined possess oil glands, these are empty in some whole species (e.g. *E. obtusifolia*) or individual trees of other species (e.g. *E. gummifera*) (E. Lassak pers. comm.). While essential oils of other plant genera have been implicated as feeding repellants, attractants and cues (Schoonhoven, 1972, Levin, 1976, Rice *et al.*, 1978) only the possible repellant action of eucalypt essential oils has been considered.

Many early naturalists attempted to associate oil yield and composition with leaf choice in Koalas. Fleay (1937) suggested that cineole was necessary in some unspecified way for Koalas while Pratt (1937) claimed that Koalas in cold regions chose phellandrene-rich leaf while those in hotter northern populations chose cineole-rich leaves. This choice allegedly occurred because cineole and phellandrene had opposing thermoregulatory properties. However, Southwell (1978) has recently shown that this supposed leaf choice does not exist, while Brownlee (1940) demonstrated that phellandrene and cineole were devoid of any pyretic activity. Southwell (1978) examined the essential oils of 200 individual trees of 54 eucalypt species and found no relationship between Koala browsing and either total oil yield, cineole content or phellandrene content. Betts (1978) has suggested that the ratio of cineole to sesquiterpenes influences leaf choice by Koalas but his observations were based on only one species (*E. rudis*) and he did not actually measure the leaf cineole level.

These results are similar to those obtained with eutherian species. Connolly *et al.* (1980) found that there was no clear pattern between deer browsing and terpene composition of Douglas Fir (*Pseudotuga menzesii*) needles. Although Farrentinos *et al.* (1981) claimed that monoterpene hydrocarbons (in particular α-pinene) negatively affected feeding by tassel-eared squirrels, the coefficient of variation of the

data was greater than 50%. It is unlikely then, that a simple relationship exists between the level of herbivory and terpene yield and composition. This is probably due to the large degree of diurnal variation (Nicholas, 1973) variation between individual trees, physical forms and leaf ages in *Eucalyptus* (Berry *et al.*, 1937, McKern *et al.*, 1951, Southwell, 1973) and other genera (e.g. *Artemisia*, Welch and McArthur, 1979, Welch *et al.* 1981).

Nonetheless, there have been several studies on the effects of ingested oils. Many essential oil components are strongly bactericidal (Penfold and Grant, 1923; Low *et al.*, 1974) and this has tended to focus attention on the effects of these components on symbiotic micro-organisms. Nagy *et al.* (1964) and Nagy and Tengerdy (1968) showed that some monoterpenes from *Artemisia tridentata* inhibited the growth and activity of mule deer ruminal micro-organisms *in vitro*. Oh *et al.* (1967) showed that while oxygenated monoterpenes were strongly inhibitory to rumen microbes, both the monoterpene hydrocarbons and sesquiterpenes of Douglas Fir needles actually stimulated microbial activity. Several problems exist with these studies. Firstly, monoterpenes were added to the *in vitro* preparations at levels 4-8 times those found in *Artemesia* foliage. Secondly, although Oh *et al.* (1967) suggested that rumen micro-organisms could adapt to the monoterpenes, their "control" animals came from an area which, while lacking in Douglas Fir, was rich in a range of other monoterpene containing plants.

Similarly, Welch *et al.* (1982) have challenged Nagy and Tengerdy's (1964) and Nagy *et al.*'s (1968) interpretations of their results. They pointed out that there is no evidence of suppression of digestibility of *Artemisia* and suggested that this was because there is little interaction between rumen micro-organisms and monoterpenes. Since similar conclusions were reached independently in the present study, more extensive discussion of this later work will be made in Chapter 7.

1.4.2.2 **Polyphenolics and tannins**

The other major group of plant allelochemicals found in *Eucalyptus* are the polyphenols. Like the essential oils, these occur in some eucalypt species in sufficient quantities to warrant commercial exploitation (e.g.

E. astringens). While the total phenolic content of eucalypt foliage can vary from between 4-40% (MaCauley and Fox, 1980) only a portion of this occurs as tannins (Fox and McCauley, 1977). Indeed, tannins are only one part of the polyphenols occurring in plant tissues and the polyphenols of *Eucalyptus* foliage are so diverse that attempts have been made to use them as taxonomic aids (Hillis, 1966). However, polyphenolics are quantitatively, the most important group of angiosperm allelochemicals (Swain, 1978).

Definition and classification

Tannins are defined as substances that;

(i) are of high molecular weight (usually 300-30,000);

(ii) contain sufficient phenolic hydroxy groups to enable the formation of hydrogen bonds with other macromolecules;

(iii) form complexes that are almost undissociable at physiological pH;

(iv) cannot be degraded enzymatically

(Swain, 1978)

Tannins can be classified into four major classes. The proanthocyanidin tannins (equivalent to the condensed tannins of the older classifications) are generally of molecular weight 1000-3000 and generally oligomers of flavan-3-ols. They are usually highly water soluble and effective protein precipitants. The hydrolyzable tannins are usually of lower molecular weight (1000-1500) and contain a central carbohydrate core (usually glucose). Again they are highly water soluble and have been rated as more effective protein binders than condensed tannins. Oxytannins are not present in intact plants but are formed in response to injury. They are generally of lower molecular weight than either the proanthocyanidins or hydrolyzable tannins and less soluble and less effective protein binders. The final group is the β-tannins. This group is much less polar than the others, occurring in leaf resins of species such as *Larrea* (Rhoades and Cates, 1976). Their molecular weight usually lies in the range 300-500 although they are still effective protein precipitants.

Since tannins cannot be rigorously defined chemically, difficulties exist in discussing their general properties (Swain, 1978) and in developing methods of analysis (Tempel, 1982). Many tannins can bind and precipitate proteins and to a lesser extent, nucleic acids and polysaccharides (McLeod, 1974). These reactions are reversible and depend on pH (Jones and Mangan, 1977) the dielectric constant of the protein, (Hagerman and Butler, 1981) the molecular arrangement of the protein and the molecular weight of the tannin (Russell *et al.*, 1968), and the presence of competing substrates (Loomis and Bataille, 1966). However, protein precipitation usually only occurs above a threshold tannin concentration (Bate-Smith, 1973).

Nonetheless, in spite of all the factors affecting the nature of protein-tannin interactions, the ability of some tannins to precipitate some proteins has led to most of the observed biological effects of tannins on herbivorous animals. These effects include reduction of feed intake (Wilkins *et al.*, 1953), inhibition of microbial enzymes (Lyford *et al.*, 1967) and direct microbial toxicity (McLeod, 1974).

At present, there is much uncertainty as to the role of tannins in plant defence against herbivores. Bernays (1981) has concluded that those species of insects which do not normally feed on plants containing much tannin tend to be adversely affected, but some polyphagous species are often stimulated to feed by hydrolyzable tannins. For example, the tree locust *Anacridium melanorhodon* showed increased food digestibility when fed lettuce plus tannic acid compared with lettuce alone (Bernays *et al.*, 1980) and neither the condensed tannin quebracho nor hydrolyzable tannic acid adversely affected digestibility in any species of graminivorous Acridoidea (Bernays *et al.*, 1980, 1981).

Insects may employ several strategies to reduce any deleterious effects of ingested tannins. Species of caterpillar feeding on tannin-rich leaves usually have higher mid-gut pH than those species feeding on low tannin foliage (Berenbaum, 1980). Many protein-tannin complexes form very weakly at high pH (Jones and Mangan, 1977). Alternatively, some insect herbivores feeding on *Eucalyptus* (e.g. Chrysomelids) sequester tannins in

mid-gut cells (Bernays unpub. in Bernays, 1981).

Part of the variability in response to tannins is probably due to lack of adequate analytical techniques and the variability of tannin types. For example, tannic acid has been shown to be a mixture of four phenolic compounds, the relative proportions of which vary between samples (King and Pruden, 1970). It is thus unfortunate that tannic acid has been used as a source of tannin in some insect feeding experiments (Bernays *et al.*, 1980,) and as an analytical standard.

Much needs to be learnt concerning the distribution of eucalypt tannins and the types of reactions they undergo before conclusions can be reached regarding their role as antiherbivore defensive chemicals - if indeed this is their principal function. The involvement of eucalypt polyphenols in making available insoluble phosphorus has already been mentioned (Section 1.1.2). Another possible primary role for polyphenols including tannins may be as carbon sinks trapping excess photosynthetate (Phillips and Henshaw, 1977).

1.4.2.3 **Cyanide**

The possibilities of folivorous marsupials being poisoned by cyanide is often raised (Morris, 1953, Owen and Thomson, 1965, How, 1978). Finnemore *et al.* (1935) found the cyanogenic glycoside prunasin in the foliage of *E. cladocalyx* which is avoided by Koalas. These authors also showed the presence of benzaldehyde (a breakdown product of prunasin) in the foliage of *E. viminalis* - a species known to be eaten by Koalas although the production of cyanide in *E. viminalis* foliage was not demonstrated. In order to release cyanide, the glycoside must be brought into contact with the hydrolyzing enzymes, β-glycosidases. If these are lacking from the plant tissue (as in several *Acacia* species) then the cyanide will not be released (Conn, 1978, 1984). In addition, Southwell (1978) has shown that the particular form of *E. viminalis* studied by Finnemore *et al.* (1935) is not known to be a Koala food tree. It would seem then, that the importance of cyanide as a potentially toxic allelochemical in *Eucalyptus* has been greatly overemphasized.

1.4.2.4 **Miscellaneous**

(a) **Rutin**

Rutin is a flavonol compound (quercetin 3-rutinoside) which has therapeutic value as a strenghthening agent for blood capillaries (Humphreys, 1964). It is found in small amounts in many plant genera but occurs in commercial quantities in *E. macrorhyncha* and *E. youmanii*. *E. macrorhyncha* is an important food tree for Greater Gliders. The function of rutin in the plant is not definitely known though it has been suggested (E.V. Lassak, pers. comm.) that it serves to transport sugars in the plant. Rutin is degraded by both steer rumen and rat caecal micro-organisms to phenolic compounds (Scheline, 1978).

(b) **Saponins**

Saponins are triterpenoid glycosides which are powerful surfactants and haemolytic agents. Presumptive tests have indicated their presence in the wood of many eucalypts but there are only minor occurrences in the foliage (Simes *et al.*, 1959). Saponins are not usually readily absorbed from the gut and although they are sometimes implicated in bloat in ruminants (Reid, 1973) this is probably only a minor factor in the condition.

1.4.2.5 **Conclusion**

In spite of the dominance of *Eucalyptus* in Australia, little effort has been expended on its phytochemistry. Nonetheless, several general points must be made regarding the occurrence of allelochemicals in *Eucalyptus*.

(a) Although all of the above compounds have known toxic or digestibility-reducing potential, it cannot be assumed that these compounds act as antiherbivore defences *per se*. Primary functions are known for many of the compounds (e.g. the role of polyphenolics in the availability of phosphorus: Section 1.1.2) and for this reason the term "allelochemical" is preferred to the more common "secondary plant compound".

(b) In some cases, the allelochemical in question occurs primarily in tissues other than leaves. Therefore, if an antiherbivore role is being assessed, attention must be paid to the effects on non-folivorous animals.

(c) Finally, nothing is known of the allelochemical profile of whole plant communities. Although eucalypt leaves appear to be well defended against herbivores, this does not mean that other plants in the community are less well defended. In order to evaluate a plant's antiherbivore defences, we must know how other potential food resources are defended and how these characteristics relate to the plant's apparency (Feeney, 1976) in time and space.

1.4.3 **Digestibility of eucalypt foliage**

Several studies have been made of the digestibility of foliage diets by the Koala. Harrop and Degabriele (1976) found that in summer, dry matter digestibility of *E. punctata* foliage was 59% but in winter, this declined to 52%. Similarly, apparent nitrogen digestibility was higher in summer (38%) than in winter (24%). However, this decline in digestibility in winter was compensated for by an increased dry matter intake so that both apparently digestible dry matter and nitrogen intakes remained constant throughout the year.

In a more extensive study, Cork *et al.* (1983) determined the digestibility of the dry matter and components of the cell walls and cell contents of *E. punctata* foliage by Koalas. Overall, dry matter digestibility (54%) was similar to that measured by Harrop and Degabriele (1976). However, only 25% of the neutral detergent fibre and 26% of the acid detergent fibre was digested compared with 70% of the cell contents. Interestingly, Cork *et al.* (1983) found that Koalas apparently digested a significant proportion (18%) of the dietary lignin.

Ullrey *et al.* (1981b) using a lignin ratio technique found an average of 41% of the neutral detergent fibre of a mixture of *E. melliodora, E. robusta and E. sideroxylon* foliage was digested by Koalas.

However, since the use of lignin as an indigestible marker was not validated by Ullrey *et al.* (1981b) their results must be treated with caution in view of Cork *et al.*'s (1983) work.

In vitro estimates of the digestibility of eucalypt foliage are very much lower than the above estimates. McLeod (1973) and McDonald and Ternouth (1973), using a sheep-rumen fluid - acid pepsin technique found dry matter digestibilities of only 36-37% for *E. populnea* and *E. microtheca* leaf. The *in vitro* methods all involve the drying and grinding of the leaf and it is likely that these preparation techniques led to the loss of leaf essential oils. Essential oils have the ability to affect microbial digestion in either a positive or negative fashion (Section 1.4.2.1). Also, the size to which the leaf was ground is likely to be important in determining the extent of microbial degradation. For example, McLeod (1973) ground dried *E. populnea* foliage to pass a 1mm sieve whereas Cork and Warner (1983) have shown that 80 % of the particles in the caecum and proximal colon of the Koala are less than 109μm in diameter. Finally, it is likely that special adaptations of the Koala gut are important in explaining the higher *in vivo* dry matter digestibilities. Cork and Warner (1983) have concluded that one of the main reasons that Koalas can exist on a diet of *Eucalyptus* foliage alone, is because of the selective retention of very fine particles and fluids in the caecum and proximal colon. This allows the Koala to maintain a high rate of intake of the highly digestible non-cell wall constituents.

1.5 **The Greater Glider and Brushtail Possum as Arboreal Folivores**

1.5.1 **Distribution and life history**

The Greater Glider is found in eucalypt forests in eastern Australia from Victoria to northern Queensland. Over this range and in the eucalypt communities, it is one of the most common arboreal marsupials. There is considerable intraspecific variation in body size. However, in south-east Australia, the Greater Glider has a head-body length of 35-40cm and an adult body mass of 1100-1400 grams (How, 1978). Populations from

Queensland tend to be smaller and lighter, though with much larger ears (McKay, 1984). Greater Gliders are strictly nocturnal, sheltering in hollow tree limbs during the day.

Tyndale-Biscoe and Smith (1969a) and Griffith (1973) have reported densities of 1 animal per 1.2-1.4 ha in tall eucalypt forest communities in N.S.W. The Greater Glider is polyoestrus. In southern N.S.W. breeding is confined to about a 3 month period in late autumn. The adult and sub-juvenile sex ratio is about 60% female but only 60-70% of adult females breed in any year. If male juvenile mortality is density dependent, and if adult Greater Gliders are monogamous, then these two factors could operate to regulate population size (How, 1978).

On the other hand, the Brushtail Possum is the most widespread of the Australian arboreal marsupials, being found in most tree communities from tall sclerophyll forests to woodlands and areas of permanent water in semi-arid regions. It is, however, replaced by *Trichosurus arnhemensis* in northern Australia and by *T. caninus* in wetter sclerophyll areas and rainforests of eastern Australia, south of Brisbane (Winter, 1979). *T. vulpecula* has also been introduced to New Zealand where it has become extremely damaging to the native (non-eucalypt) tree communities (Meads, 1976). Densities vary from about 2.5 animals per ha in woodland and dry sclerophyll forests in Australia (Dunnet, 1964) up to 8 animals per ha in parts of New Zealand (Kean and Pracy, 1953).

T. vulpecula usually breeds twice each year with the main peak (about 90% of adult females breeding) in autumn and a secondary peak (50% of adult females breeding) in spring. Mortality is high particularly among independent dispersing young and only about 25% of these reach 1 year of age. The high fecundity and high survival rate of dependent young maximise the potential population recruitment although density-dependent factors seem to determine the actual recruitment (How, 1978).

1.5.2 **Degree of arboreality and folivory**

The Greater Glider is one of the most strictly arboreal and strictly folivorous of the arboreal folivores. Eisenberg (1978) in his

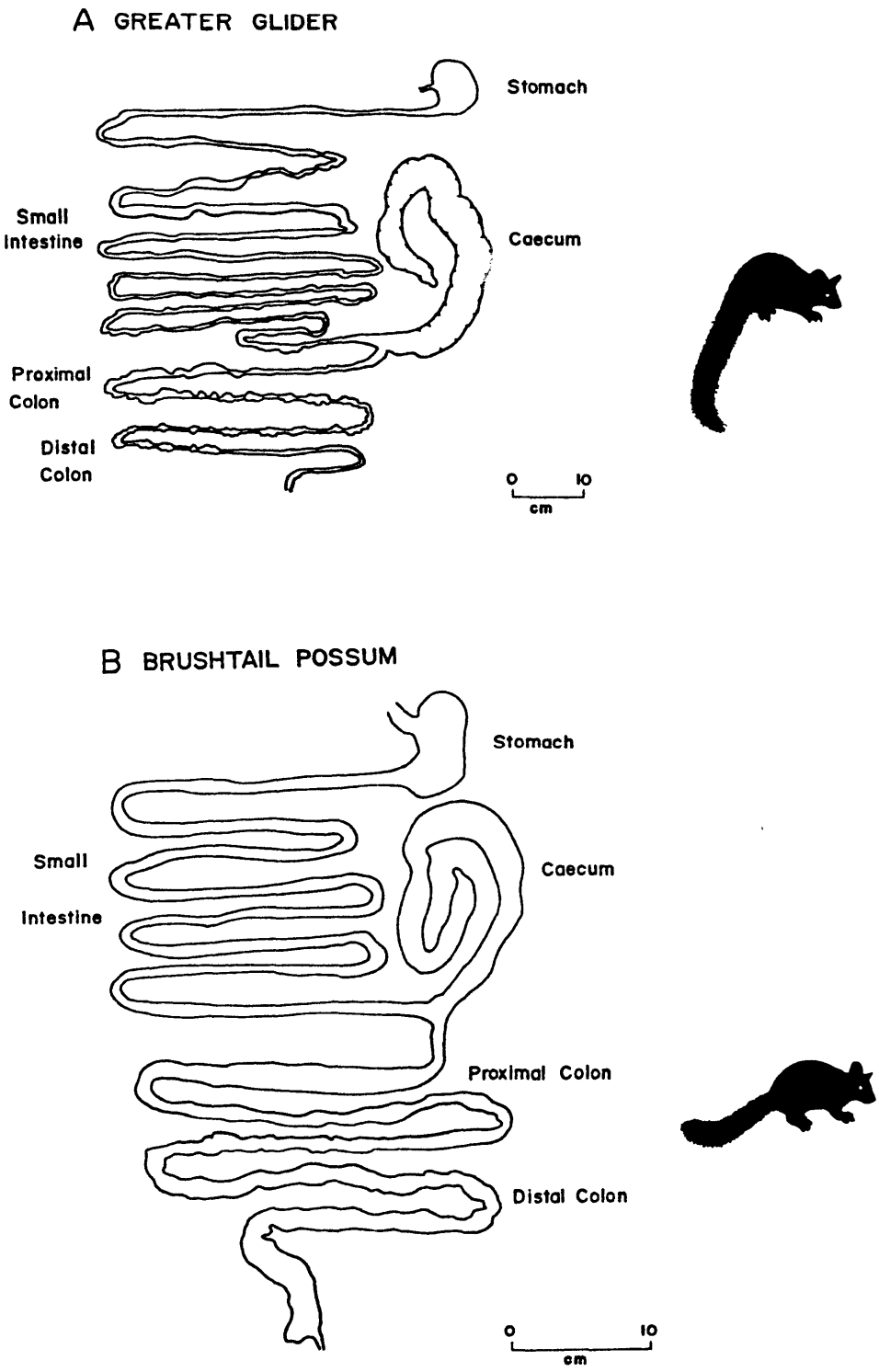

FIGURE 1.1: *The alimentary tract of (A) the Greater Glider and (B) the Brushtail Possum.*

classification of arboreal folivores has rated the Greater Glider at 4.5 on a 5 point scale for both degree of arboreality and degree of herbivory. Although the Koala and the sloths were rated higher, this is probably only due to a lack of information about the Greater Glider. Koalas descend to the ground regularly to move from tree to tree. The Greater Glider rarely moves on the ground and indeed the species appears extremely ungainly and awkward if captive animals are placed on the ground (personal observation). However, Robertshaw (1976) reported that Greater Gliders comprised up to 2% of the diet of dingoes (*Canis familiaris dingo*) in north-eastern N.S.W. although these instances may represent sick or injured animals. In addition to being totally herbivorous, the Greater Glider is almost totally folivorous (Marples, 1973).

The Brushtail Possum on the other hand, was rated by Eisenberg (1978) as not as completely arboreal and folivorous as the Greater Glider (3.5/5.0 for arboreality and 4.0/5.0 for herbivory). While the rating for arboreality is probably valid, the Brushtail Possum is, in nearly all situations, totally herbivorous although there are occasionally reports of insects being accidently or opportunistically ingested (Winter, 1976).

1.5.3 **Structure of the digestive tract**

On the basis of their gut morphology both the Greater Glider and the Brushtail Possum can be classified as hindgut fermenters (Hume and Warner, 1980). An active fermentation has been demonstrated in the caecum of the Greater Glider (Cork and Hume, 1978) and while no similar study has been made in the Brushtail Possum, the production of short chain fatty acids from fermentation of cellulose can be inferred from the work of Taylor (1972). The Brushtail Possum (Figure 1.1B) has a simple stomach lined principally with fundic gland mucosa with minor areas of pyloric glandular mucosa and squamous epithelium (Hume, 1982). The small intestine is well developed although it is the enlargement of the caecum and proximal colon which is most striking. Both these organs are smooth.

The Greater Glider (Fig 1.1A) too has a simple stomach although the small intestine comprises a greater proportion of the total gut length

in this species than in the Brushtail Possum (Smith, 1980). While there is no enlargement of the proximal colon, the caecum of the Greater Glider is heavily sacculated like that of the Ringtail Possum (Harrop and Hume, 1980). The Greater Glider caecum however, lacks the muscle band (taenia) which is found running along the length of the Ringtail Possum caecum (Harrop and Hume, 1980). Although the Koala caecum is often described as the "greatest instance of caecal development among the mammalia" (MacKenzie, 1918) the caecum of the Greater Glider is actually larger as a percentage of the total gut length (Smith, 1980).

Anatomically, at least, the Brushtail Possum must be considered to be a colon fermenter in the scheme of Hume and Warner (1980). In this classification, the colon and the caecum are effectively one functional unit. The Greater Glider on the other hand would be classed as a caecum fermenter.

1.5.4 **Diet**

In most situations, the Greater Glider feeds almost exclusively on the foliage of eucalypts. Troughton (1965) reported the species to be "strictly vegetarian" eating the tender leaf tips and blossoms of certain eucalypt species. While no detailed studies of the diet have been made, Marples (1973) examined the stomach contents of 135 Gliders collected in Southern NSW. He found them to contain finely masticated eucalypt leaf with minor amounts of bud and bark debris. The leaves of a wide range of eucalypt species have been reported as being eaten. These include *E. pauciflora, E. radiata, E. viminalis and E. dalrympleana* in southern N.S.W. (Smith, 1965); *E. radiata and E. eleophora* in Victoria (Fleay, 1933) and *E. gummifera, E. saligna, E. obliqua, E. fastigata, E. nitens, E. andrewsii, E. radiata and E. pauciflora* in northern N.S.W. (Griffith, 1973). Whether these records are for young or mature foliage is not known. Troughton (1965) suggested that the leaves of the narrow-leaved peppermint (*E. radiata*) are most favoured although this may simply be an indicator of floristic diversity (Braithwaite *et al.*, 1983).

In addition, several non-eucalypt species are reported to be eaten. These include *Casuarina* spp (Troughton, 1965) *Acacia melanoxylon*, and *Pomaderris aspera* (Griffith, 1973). With so little information it is difficult to draw conclusions regarding the diet of the Greater Glider. How (1978) has concluded that it is almost completely folivorous, consuming the dominant eucalypts of the particular association. However, there is the possibility that seasonal preferences for particular species or plant parts (e.g. petioles) might be important.

While there have been many ecological studies of the Brushtail Possum, few have been concerned with diet and feeding. Freeland and Winter (1975) found that Brushtail Possums in open woodland near Brisbane spent about 66% of their feeding time consuming mature *Eucalyptus* foliage. They also found that the selected species were used disproportionately compared with their overall abundance but that not all trees of those species were palatable. The remaining 33% of feeding time was spent eating plants from the herb layer and flowers or foliage of non-eucalypt trees and shrubs. Owen and Thomson (1965) found that mature eucalypt leaf was the major item in stomach contents of Brushtail Possums from tall, wet forest in Victoria. However, only 20% of stomachs contained understorey species. This is in contrast to the diet selected by the Mountain Possum in the same area where 90% of stomachs contained understorey species including fungi and lichens.

In forested areas of New Zealand, the Brushtail Possum feeds selectively on the leaves of only a few of the available tree species, severely defoliating and killing some trees. In pasture areas however, up to 50% of the stomach contents may consist of grasses and herbs (Harvie, 1973).

The apparent reliance on the herb-layer for some of the diet of the Brushtail Possum led Freeland and Winter (1975) to ask why this animal descends to the ground to feed (where presumably search times are greater and the risk of predation higher) when there is an apparently abundant eucalypt leaf resource. They suggested it was due to the presence of toxic allelochemicals in the leaves but little evidence was reported in support of this view.

1.5.5 **Energetics**

Although the Brushtail Possum has been the subject of more physiological investigations than any other marsupial species, few have been concerned with energetics. The standard metabolic rate of the Brushtail Possum has been reported by Dawson and Hulbert (1970) as 2.09 $W \cdot kgW^{-0.75}$ with a body temperature of 36.2°C. While no values for the metabolic rate of the Greater Glider have been published, Morrison, Harvey and Morris (unpub. in Robinson and Morrison, 1957) found an "excellent insulating layer - a fine long fur - and a reduced metabolic rate in this very gentle species".

Free living animals however, need energy for locomotion, reproduction and thermoregulation and these may be important parts of the total energy expenditure. No studies have been published concerning any of these activities for the Greater Glider. However, some attention has been given to temperature regulation in the Brushtail Possum. Dawson (1969) showed that the main avenue of heat loss at high ambient temperatures was by panting; licking was of only minor importance. Bell *et al.* (1983) however, suggested that at exercise, the Brushtail Possum relied more on heat loss mediated by cutaneous evaporation than from respiratory routes.

1.5.6 **Response to Primary Nutrients**

1.5.6.1 **Early experiments**

In the late 1930's and early 1940's Honigmann conducted a series of experiments in which he measured digesta passage rate, nitrogen retention and fibre digestibility in Brushtail Possums fed a diet of carrots, bananas and meal worms. The rate of passage of stained wheat grains was much longer than in a number of primates of similar body mass examined at the same time; the minimum passage time in Brushtails being 10 hours and the maximum 96 hours. Further, Honigmann found that nitrogen balance was consistently positive even at a dietary nitrogen level of 1.7% N and that the digestibility of the carrot and banana crude fibre was 80%.

These three results have generally been confirmed by later workers.

1.5.6.2 **Digesta passage time**

Gilmore (1970) found that plastic chips, chromic oxide and *Eucalyptus* leaf cuticle were all retained for a much greater length of time in Brushtail Possums than would be expected on the basis of body mass. Although there were significant differences between the flow characteristics of the markers, the time for total marker clearance was between 80 and 100 hours.

Wellard and Hume (1981b) used a more satisfactory double marker system comprising ^{51}Cr-EDTA, a solute marker and ^{103}Ru-P, a particulate marker to measure the mean retention time in animals fed diets of varying NDF content. These diets were semi-purified and based on honey and grain by-products. There was no apparent selective retention of either marker in the whole gut - the mean retention time (MRT) of the ^{51}Cr-EDTA marker on diets containing 41% NDF was 64 hours and of the ^{103}Ru-P marker, 71 hours. Defaecation patterns of animals fed low fibre (17% NDF) diets were too erratic to allow MRT's to be calculated. This is in contrast to the results of Cork and Warner (1983) who measured the MRT of the same markers in Koalas fed *E. punctata* foliage. They found the MRT of ^{51}Cr-EDTA was 198 hours compared with only 93 hours for the ^{103}Ru-P marker. The ^{51}Cr-EDTA was apparently selectively retained in the caecum and proximal colon. Cork and Warner (1983) suggested that this might benefit the Koala since the clearance of fibre from the hindgut could proceed without a corresponding increase in the clearance of the more digestible solutes and fine particles.

While nothing is known of the pattern of digesta flow in Brushtail Possums fed diets of *Eucalyptus* leaves, it is unlikely that there would be separation between the two digesta phases, although the absolute retention times of the markers may be different from those found by Wellard and Hume (1981b).

1.5.6.3 **Nitrogen metabolism**

Wellard and Hume (1981a), using the semi-purified diets described above, measured nitrogen retention in Brushtail Possums over a wide range of dietary nitrogen intakes. Casein was added to the diets to vary the nitrogen level. The maintenance nitrogen requirement (MNR) was estimated as 0.2g of dietary nitrogen per $kgW^{-0.75} \cdot d^{-1}$. These values are very low by eutherian standards and generally lower than values recorded for a range of marsupials (Hume, 1982). The MNR was however inversely related to the NDF content of the diet. Wellard and Hume (1981a) attributed this effect to greater endogenous faecal nitrogen losses on the higher fibre diets. The much higher estimate of Fitzgerald et al. (1981), viz. 1.1 $g \cdot kgW^{-0.75} \cdot d^{-1}$ was based on the assumption that nitrogen balance can be equated with short-term changes in body mass and is therefore likely to be in error.

Again, little is known of the performance of Brushtail Possums fed foliage diets. Fitzgerald (1978) found much lower apparent nitrogen digestibilities in Brushtails fed leaves compared with the commercial diets described above at similar nitrogen intakes. Koalas are able to remain in positive nitrogen balance solely on the foliage of *Eucalyptus punctata* (Harrop and Degabriele, 1976, Cork, 1981).

1.5.6.4 **Fibre digestibility**

Wellard and Hume (1981b) also measured the apparent digestibility of the neutral detergent fibre of the semi-purified diets described above. They found that the Brushtail Possum could digest about 55% of the NDF on both high (41% NDF) and low (17% NDF) fibre diets. The semi-purified nature and fine particle size of the fibre source of these diets could partly explain these high values. Interestingly, caecectomy had no effect on fibre digestibility but the mean retention times of the two digesta markers were approximately doubled. This suggests that the caecum and colon of this species function as a single unit and confirms that the Brushtail Possum can be classified as a "colon fermenter". In contrast,

Fitzgerald *et al.* (1981) found apparent digestibilities of cellulose and hemicellulose of 30% and 70% respectively on the artificial diets described above. Since different fibre analysis systems were used, these results are not directly comparable with those of Wellard and Hume (1981b).

1.5.7 **Response to allelochemicals**

There have been no studies published on the response of Greater Gliders to dietary allelochemicals. While there have been several fragmentary studies with the Brushtail Possum, many of these have been aimed at elucidating detoxification mechanisms in the Koala - the Brushtail Possum being used as a "model".

1.5.7.1 **Essential oils**

Hinks and Bolliger (1957a) reported that urinary excretion of glucuronic acid by Brushtail Possums was trebled when the diet was changed from fruit and vegetables or Moreton Bay Fig leaves (*Ficus macrophylla*) to the foliage of *Eucalyptus acaciaeformis*. The amount of glucuronic acid excreted was about $0.6g \cdot d^{-1}$ or $0.36g \cdot kgW^{-0.75} \cdot d^{-1}$ on the eucalypt diet. In a later paper, Hinks and Bolliger (1957b) confirmed this figure and also reported that Koalas excreted about 1-2g of glucuronic acid per day ($0.42g \cdot kgW^{-0.75} \cdot d^{-1}$). More recently Eberhard *et al.* (1975) have reported Koalas to excrete about 3g of glucuronic acid/day. It is likely that the increase in glucuronic acid output by the Brushtail Possum when fed *Eucalyptus* leaf is the end product of the detoxification of eucalypt essential oils, (Section 1.3.4) since a similar response can be elicited by feeding cineole (Cleland, 1946) or borneol (Hinks and Bolliger, 1957a), two terpenes commonly found in *Eucalyptus* foliage (Southwell, 1978).

Cork (1981) has calculated that the excretion of 3 g/day of glucuronic acid by a Koala could amount to about 20% of its fasting glucose entry rate. A similar calculation based on the data of Hinks and Bolliger (1957a) and using Ballard *et al.*'s (1969) relationship between body mass and fasting glucose entry rate in eutherians (and after correcting for the

Brushtail Possum's lower metabolic rate) yields a somewhat lower result. Only 8% of the Brushtail's estimated fasting glucose entry rate would be consumed in the production of glucuronic acid for detoxification. Nevertheless, this is still a substantial amount but there are no data in Hinks and Bolliger's (1957a,b) work to suggest that the two species were receiving an equivalent terpene load.

More recently, Southwell *et al.* (1980) have conducted a series of experiments aimed at elucidating the detoxification pathways of cineole, α-pinene, β-pinene and p-cymene in the Brushtail Possum. Only traces of the ingested compounds were found in the faeces even at a dosage of 5.0 $ml \cdot d^{-1}$, suggesting efficient absorption or transformation mechanisms. The results showed that there was wide variation in the mode of detoxification of absorbed terpenes between the Koala and Brushtail Possum. Several novel urinary metabolites of cineole were isolated (Flynn and Southwell, 1979).

1.5.7.2 **Other**

(a) **Salicin**

The damage that introduced Brushtail Possums cause to native and exotic forests in New Zealand has already been mentioned. One of the plants that is often attacked is *Populus* spp. However, Edwards (1978) has found that the palatability of *Populus* clones was correlated with the salicin content of foliage. Salicin is a phenolic glucoside which is readily hydrolysed by a wide variety of intestinal bacteria to saligenin which is in turn converted to salicylic acid and then excreted in the urine either unchanged or as the glycine conjugate (Scheline, 1978).

(b) **Sodium monofluoroacetate**

Monofluoroacetates occur naturally in several plant genera in parts of Western Australia (notably *Oxolobium* and *Gastrolobium*). Sodium monofluoroacetate (Compound 1080) is also used as the principal vertebrate poison in Australia. King *et al.*, (1978) and Mead *et al.*, (1979) have shown that Brushtail Possums from areas containing these plants are much more resistant to the effects of 1080 than animals from other regions. Ingested fluoroacetate is generally converted to fluorocitrate which blocks

the TCA cycle. Adapted Brushtails are able to defluorinate fluoroacetate by a glutathione dependent enzyme mechanism (Mead *et al.*, 1979).

1.6 **Recapitulation**

This review has confirmed the need for a broad approach to the study of the digestion and metabolism of foliage diets by arboreal marsupials. The adaptation of *Eucalyptus* to low nutrient soils and relatively dry environments may be reflected in the composition of the foliage i.e. low protein content, a high level of structural tissues and large amounts of carbon-based allelochemicals. While these features may adversely affect the nutritional quality of the leaves for herbivores, it is unlikely that they have evolved specifically as anti-herbivore defences.

The literature reviewed also highlights the interdependence of body size, metabolic rate and digestive capacity. It was proposed that the effects of secondary plant compounds on herbivores are likely to depend on the nature of the digestive process, with major differences expected between foregut and hindgut fermenters.

Although there is little information available on the chemical composition of the diet of either the Greater Glider or Brushtail Possum, it is clear that the Brushtail feeds on plants in the herb layer as well as tree foliage. Consequently there have been suggestions that the utilization of *Eucalyptus* foliage by Brushtails is limited by their inability to deal with the fibre and /or the leaf allelochemicals. On the other hand, the Greater Glider appears to be more strictly folivorous, suggesting that it is an exception to the general inverse relationship between body size and diet quality.

Chapter 2

GENERAL MATERIALS AND METHODS

2.1 **Animals**

Greater Gliders were captured during forest logging operations in northern N.S.W. All animals came from New England Blackbutt forest (Forest Type 161: Forestry Commission of N.S.W., 1965) in the Styx River, Nundle, Nowendoc and Chaelundi State Forests. Brushtail Possums were caught in wire box traps (Wellard, 1979) set at the base of den trees in open woodland dominated by *E. melliodora, E. viminalis* and *E. caliginosa*.

The majority of Greater Gliders collected were mature females but both sexes were used in the feeding experiments, whereas only mature male Brushtail Possums were captured and used in experiments.

2.2 **Animal Husbandry**

2.2.1 **Holding enclosures**

Both species were held in large outdoor enclosures when not being used in experiments. The enclosure holding the Greater Gliders measured 9m x 3m x 4m. The Brushtail Possums' enclosure was of similar length and breadth, but only 2.5m high. The back section of both enclosures was shielded against the weather. A variety of tree trunks and branches were arranged to provide runways and hollow logs served as daytime retreats. Plastic buckets to hold foliage were suspended under the runways and drinking containers were fastened to the branches.

2.2.2 **Maintenance feeding**

Greater Gliders were initially offered leaves from a wide range of eucalypt species. In order of apparent preference, these were *E. radiata,*

E. andrewsii, E. nicholii, E. saligna, E. viminalis, E. obliqua, E. laevopinea, E. microcorys and *E. cameronii*. Since *E. radiata* was the most favoured and one of the most easily collected species near Armidale, it formed the bulk of the Greater Gliders' diet. Occasionally *E. andrewsii* foliage was offered to provide some dietary diversity but *E. radiata* foliage was used in all feeding experiments.

It soon became apparent that it would not be possible to maintain Brushtail Possums exclusively on eucalypt foliage for the duration of the study. The only species eaten in common with the Greater Gliders were *E. saligna* and *E. viminalis* although different leaf parts were consumed by the two possum species. In addition, only minor amounts (10-20 g) were eaten and animals showed selectivity to the level of individual trees. Observations were made of Brushtail Possums feeding on *E. melliodora* foliage in Hillgrove Creek State Forest, but leaves collected from these particular trees were only sparingly eaten in the animal house. Nonetheless, *E. melliodora* foliage seemed to offer the best hope of obtaining a staple food source. Therefore, a large number ($\simeq$ 150) of samples were collected from individual trees of this species and the preferences of captive animals noted. Eventually, a small number of trees were identified as "acceptable", although this placed a severe limitation on the range of possible experiments.

During non-experimental periods, Brushtail Possums were fed apples, carrots, bread, and a variety of other fruits and vegetables. Some leaves were always available. These included *Angophora floribunda, E. melliodora, E. bauerana, E. viminalis, E. saligna* and *E. caliginosa*. The biases that may have been introduced by these feeding techniques are discussed in Chapter 4.

2.2.3 **Collection of foliage**

E. radiata foliage was collected weekly in the Styx River State Forest. Cut branches were packed in plastic bags and stored at 5° C in the dark, standing in buckets of water. Leaves treated in this way remained acceptable to Greater Gliders for up to 10 days. Leaves were cut from

individual trees only once. *E. melliodora* foliage was collected from those trees previously deemed to be acceptable and treated as outlined above. However, it was necessary to cut foliage from the same trees regularly.

2.2.4 **Disease and management**

Occasionally, large numbers of mites were seen around the eyelids of Greater Gliders. These were controlled by a light application of a 1:200 aqueous solution of Malathion (Cyanamid: Sydney). Ticks (*Ixodes tasmani*) attached to the ears of newly captured animals were easily removed with tweezers. All Greater Gliders examined carried a tapeworm in the small intestine and a large number of nematodes in the caecum and proximal colon. Many of these were excreted in the faeces.

Six Greater Gliders died in the course of the study. In only one case was a definite cause of death established (cardiac arrest). The other animals died after a long period of very gradual weight loss. No pathological evidence was apparent *post mortem*. All *post mortem* examinations were performed by the N.S.W. Department of Agriculture Regional Veterinary Laboratory, Armidale.

Few managememt problems were encountered with the Brushtail Possums. Again, tapeworms were occasionally seen in the small intestine at slaughter. However, the susceptibility of Brushtail Possums to haemorrhagic enteritis following rapid diet changes (Wellard, 1979) dictated that the introduction of new animals to eucalypt diets be done gradually.

2.3 **Experimental Conditions**

2.3.1 **Rooms and cages**

The main experimental room was maintained on a 12 h/12 h light/dark cycle. The lights were connected to a dimmer which increased or decreased light intensity over forty minutes to simulate dusk and dawn.

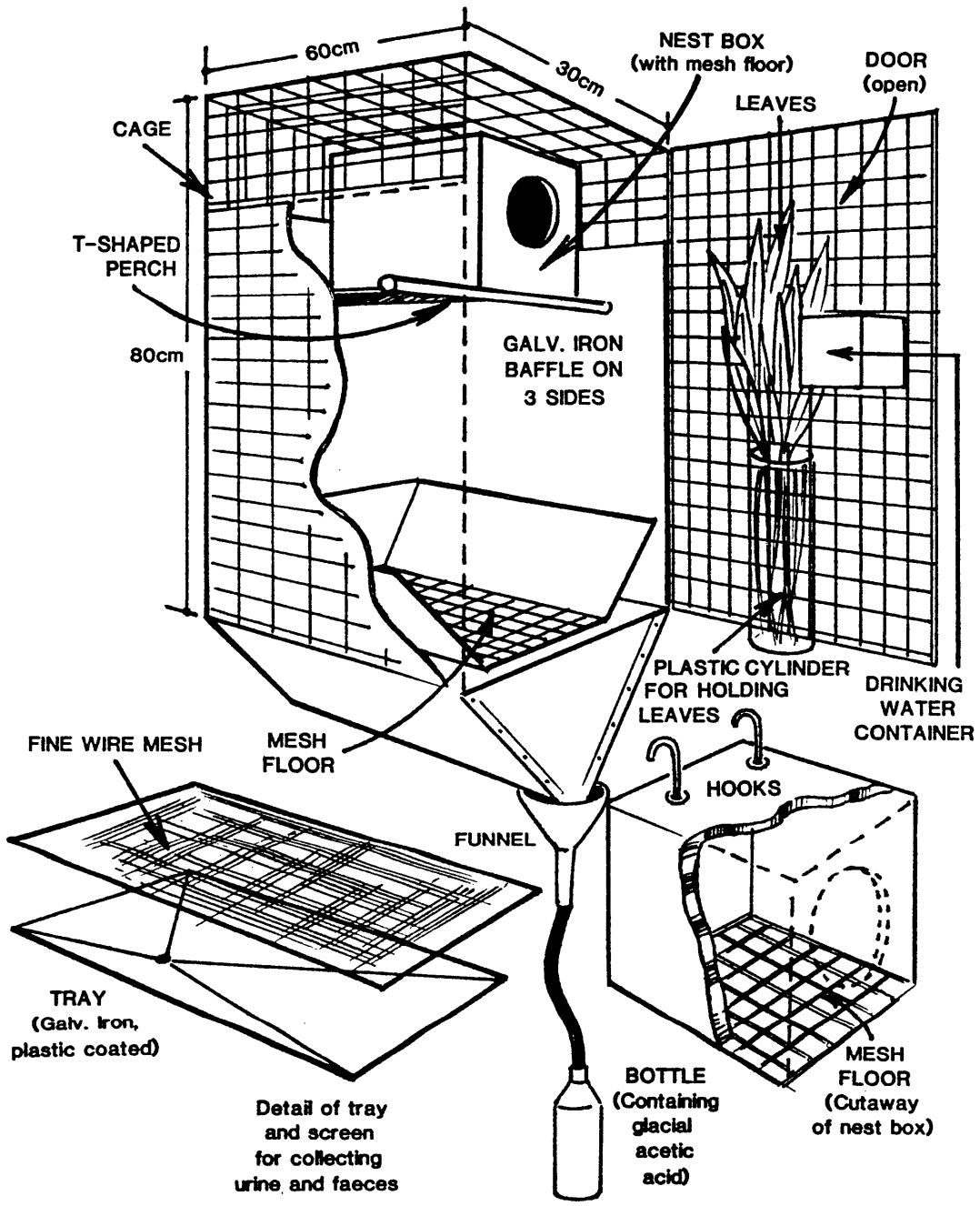

FIGURE 2.1: *Metabolism cages and collection apparatus used in feeding experiments.*

The room air temperature was maintained at 20 ± 3° C with a reverse cycle air conditioner and relative humidity varied between 30 and 70%. Calorimetric measurements were made in a thermostatically controlled room which kept the air temperature at 20 ± 0.5° C.

Greater Gliders were held in lightweight wire mesh metabolism cages (60 x 30 x 80 cm). Each cage was equipped with a nest box, perch, drinking water container and plastic cylinder to hold foliage (see Figure 2.1). Urine and faeces were collected on a screen and plastic coated tray. The urine flowed into a plastic bottle containing sufficient glacial acetic acid to maintain the urine below pH3. This system recovered 92-95% of the urinary NH_3, urea and total nitrogen and so measured volumes were corrected accordingly.

Brushtail Possums were housed in metabolism cages of similar construction to those used for the Greater Gliders but only 60cm high. The Brushtails did not use the nest boxes initially provided and so these were withdrawn. The feeding, watering and collection apparatus was identical to that provided for the Greater Gliders.

All animals were weighed weekly during experiments and every 2-4 weeks during non-experimental periods. Animals were weighed in a hessian bag on a Mettler Top Pan Balance to the nearest gram.

2.3.2 **Input/output measurements**

Greater Gliders were not active during the light phase. Brushtail Possums also rested during the light phase but occasionally defaecated near midday. By making collections in the mid-afternoon (1400-1700 h), end point errors in determining faecal output were avoided.

The following routine was adopted during experiments. Firstly, drinking water residues and evaporation controls were measured. Faecal pellets were collected, weighed, bulked for each animal and stored at -10° C. Urinary volume was measured, bulked for each animal and stored at -10° C. Samples of the feed residues were taken for determination of dry

matter content and chemical composition. Leaves which had fallen to the floor of the cage were collected, dried and added to the dry matter residue. These leaves were not used for the chemical analysis of feed residues in case they had been contaminated by urine. Greater Gliders dropped only a few leaves, although the large quantity dropped by one Brushtail Possum necessitated a separate determination of the chemical composition. However, there were no differences between these leaves and those left on the stems.

At the end of each experiment, the dry matter content of the faeces was determined as described below. Another portion was oven dried to constant mass at 55° C for chemical analysis. Samples of the feed offered and feed residues were freeze dried for later chemical analysis.

Finally, fresh foliage was offered to each animal. Small twigs were removed from the branches such that there was sufficient for each animal plus some for diet sampling and some as a control. One bunch of leaves was stripped, mixed, and samples taken for immediate determination of the dry matter content. Another sample was stored at -10° C for later chemical analysis. The change in mass of the control bunch of leaves over 24 h was never more than ±1% and so corrections to dry matter were not necessary.

2.4 **Analytical**

The following lists the chemical techniques used in this study. Many samples of leaf and faeces were stored in a dry condition prior to analysis, but a separate determination of the residual moisture was made when these samples were analysed.

2.4.1 **Dry matter**

The dry matter content of feed, feed residues and faeces samples was determined by drying a portion at 105° C to constant mass in a forced

draught oven. Urine samples were dried under reduced pressure after freezing in liquid nitrogen.

2.4.2 **Ash and acid-insoluble ash**

The ash content of feed, feed refusals and faeces was determined by combusting samples in a muffle furnace at 500° C for 3 hours. Acid-insoluble ash content was determined according to Van Keulen and Young, (1977). Ashed samples of feed and faeces, were washed in 4N HCl, filtered through Whatman No. 42 ashless filter papers and re-ashed at 500° C for 3 h.

2.4.3 **Gross energy**

Gross energy content of feed, faeces and urine samples were determined using a Gallenkamp Adiabatic Bomb Calorimeter standardized with benzoic acid. Feed, feed residues and faeces samples were compressed into small pellets and ignited. Urine samples (previously acidified) were placed in a crucible with a small amount of cotton wool, frozen in liquid nitrogen and dried under reduced pressure. This combination was ignited in the calorimeter with a correction being made for the energy content of the cotton wool.

2.4.4 **Nitrogen compounds**

(a) Total nitrogen content of feed, faeces and urine was determined by the Kjeldahl technique of Ivan *et al.* (1974) using selenium as a catalyst. Blanks and standards were run regularly to check recoveries. These were always between 99.0% and 100.5%.

(b) Non-dietary faecal nitrogen was determined by the method of Mason (1969) using 1% sodium lauryl sulphate. The neutral-detergent fibre (NDF) content of the faecal samples was determined and the total nitrogen content of the NDF residues determined as above. Neutral-detergent residues were more convenient to use than acid-detergent residues although both solutions

extracted similar amounts of nitrogen from sheep (Mason, 1969) and Koala (Cork, 1981) faeces.

(c) The urea-nitrogen content of urine and plasma samples was determined by an automated diacetyl-monoxime method (Marsh *et al.* 1965). Urease methods were unsuitable for use with urine samples possibly due to interference by benzoic acid.

(d) The ammonia-nitrogen content of urine was determined by steam distillation after the addition of a saturated sodium tetraborate solution using the apparatus described by Ivan *et al.* (1974).

(e) The creatinine-nitrogen content of urine samples was determined by an automated version of the classical method of Folin and Wu (1919). Urine samples upon which creatinine determinations were to be made were frozen in liquid nitrogen 15-20 seconds after excretion.

(f) The allantoin-nitrogen content of urine samples was determined manually by the method of Young and Conway (1942). Potassium allantoate was used as a standard, and this was prepared following the method described by Young and Conway (1942).

(g) The uric acid content of urine samples was determined using an Auto Analyser version of the method described by Brown (1945).

2.4.5 **Crude lipid**

The crude lipid content of leaf samples was determined on dry, ground leaves by a modification of the method of Folch *et al.* (1956) using 2:1 chloroform/methanol as the extractant. Solvents were removed from the lipid phase by rotary evaporation at 35° C and residual lipid determined gravimetrically.

2.4.6 **Total non-structural carbohydrates**

Total non-structural carbohydrates were extracted from dried,

ground leaf with dilute (0.2N) H_2SO_4 (Smith *et al.*, 1964). The solutions were filtered and then neutralised with NaOH. An aliquot was analysed for glucose by the dinitrosalicylic acid technique according to the methods of Luchsinger and Cornesky (1962).

2.4.7 **Dietary fibre**

Neutral-detergent fibre, acid-detergent fibre and acid lignin were determined using the techniques described by Goering and Van Soest (1970). Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were determind on separate samples of feed and faeces. Acid lignin (AL) was determined on the acid-detergent fibre residue. "Cellulose" and "hemicellulose" were taken to be the difference between ADF and AL and NDF and ADF respectively. The possible errors involved in these methods are discussed in Chapter 4. Several variations to Goering and Van Soest's (1970) techniques have recently been recommended (Robertson, 1978; Mould and Robbins, 1981; Van Soest, 1982). Some of these, in particular the effects of adding sodium sulphite to the neutral-detergent extractions and of pre-extraction of samples with neutral-detergent solution prior to determination of ADF, were tested. The results are presented in Chapter 4.

2.4.8 **Phenolic compounds**

(a) A polyphenolic standard was prepared from both *E. radiata* and *E. melliodora* foliage by the methods of Dement and Mooney (1974). Fox and MacCauley (1977) found that this technique isolated pure polyphenolic compounds from eucalypt foliage.

(b) Extracts were made of leaves, faeces and digesta samples with hot 50% aqueous methanol (Swain, 1979). The total phenolic content of this extract was determined with the Folin-Ciocalteu reagent (Folin and Ciocalteu, 1919). It was recognized that this method would also detect other -OH groups such as those of the amino acids tyrosine and tryptophan.

(c) Leucoanthocyanidins are the most common condensed tannin in eucalypt leaves (Hillis, 1966). A portion of the 50% methanolic extract

was heated at 95° C for 2 h with 5% butanolic HCl (Dement and Mooney, 1974). The spectrum of the resulting red product was recorded between 500 and 600 nm. The maximum absorbance of the *E. radiata* extracts occurred at 545 nm and of *E. melliodora* at 547 nm. A standard curve was produced after dissolving the material prepared as described in Section 2.4.8(a) in 50% aqueous methanol.

(d) The ability of the leaf extracts to precipitate protein was determined by a modification (Jones *et al.* 1976) of the method of Bate-Smith (1973). Fresh ovine blood was haemolysed in tris-HCl buffer (0.1 M, pH 6.5; 1 part blood to 50 parts buffer). The methanol was removed from the leaf extracts by rotary evaporation at 30° C and replaced with buffer. Aliquots of this solutions were added to 2 ml of the haemolysed blood, chilled in ice for 10 min and centrifuged at 12000 g at 4° C for 10 min. The absorbance of the supernatant was determined at 578 nm. Standard curves were prepared from the isolated polyphenolic material. An example is shown in Chapter 6.

2.4.9 **Essential oils**

(a) Essential oils were extracted from leaves, faeces and gut contents by steam distillation with cohobation in all glass apparatus (Hughes, 1970). Eucalypt leaves were distilled (in duplicate) for 8-12 h depending on species. Gut samples were distilled for 12-24 h, but only single distillations were carried out. Since drying led to large losses of terpenes, all foliage samples were distilled when the material was fresh. All oil samples were stored in air-tight, glass perfume bottles over sodium sulphate at -20° C.

(b) Analytical gas-liquid chromatography (GLC) was carried out on a Perkin-Elmer 900 instrument using a quartz-silica SCOT column (50 m x 0.5 mm id) coated with FFAP and with helium as the carrier gas. A Hewlett Packard 3370A Integrator was used to determine the peak areas.

(c) Combined GLC-mass spectrometry was performed on a Shimadzu GC6-AMP instrument with a SCOT column coated with FFAP (70 m x 0.5 mm id) and

programmed from 80° C at 2° C/min. This system was connected to an AEI-MS12 mass spectrometer via an all-glass straight split. Mass spectra were recorded at 70 eV ionising voltage with an ion source temperature of 150° C. Spectra were recorded on a VG Digispec Display data system which produced standard bar graphs for direct comparison with published spectra.

2.5 **Statistical**

Analysis of variance procedures (Snedecor and Cochran, 1967) were used to test the significance of the difference between means of more than two experimental periods or treatments. Throughout this work, these differences are represented by the following code; where means bear more than one of the superscripts a,b,c,d,e,f,g or h, those which share none in common, are significantly different at the 5% level ($P<0.05$).

Comparisons between two means were made using Student's t test. Where intakes between treatments or periods were different, other dependent parameters were compared by analysis of covariance (Snedecor and Cochran, 1967).

Regression equations were calculated by the method of least squares and are expressed with the correlation coefficient (r) and the residual standard deviation (RSD). Non-linear regression models were fitted on the basis of a significant second order coefficient. In order to improve the predictive value of regression equations for Brushtail Possum data, points from treatment 4 (- PEG) in Chapter 6 were included along with those from the main feeding experiments. These points are marked with triangles to distinguish them from the main data body for Brushtails.

To facilitate the comparison of data between species of different body mass metabolic parameters have been expressed in terms of metabolic body mass ($kgW^{-0.75}$).

Chapter 3

THE RATE AND PATTERN OF DIGESTA FLOW THROUGH THE GUT OF THE GREATER GLIDER AND BRUSHTAIL POSSUM

3.1 Introduction

Many early studies of the utilization of high fibre diets by herbivores implied that the most appropriate strategy to adopt would be to maximise the digestion of plant cell wall constituents (e.g., McIntosh, 1966). However, it is now generally recognized that this is not always true. For example, Janis (1976) suggested that equines could increase their intake of soluble nutrients when pasture quality (but not quantity), was a limiting factor for ruminants. Recent work by Dierenfeld *et al.* (1982) suggests that this strategy is adopted in the extreme by the Giant Panda. In both cases instanced, maximal fibre digestibility is sacrificed for increased intake of total digestible nutrients.

The utilization of high fibre diets by small herbivores may present particular problems due to their high mass-specific energy requirements (Kleiber, 1961, Parra, 1978). However, several small herbivores possess mechanisms by which the more indigestible parts of the diet are separated from the rest of the digesta and eliminated comparatively rapidly (Björnhag, 1972). Mechanisms such as these could also be expected to be found in small herbivores such as the Greater Glider and Brushtail Possum.

Several studies of digesta passage in arboreal folivores n to (Hentgbomery and Sunquist, 1978, Milton, 1981, Cork and Warner, 1983) have total amount excreted over the subsequent two weeks. In both species the level of marker in faeces was < 0.2% of the peak level at the end of the two-week collection period.

3.2.3 Collection of faeces and urine

Total collections of faeces and urine were made for approximately 340 hours in all experiments. Collections of faeces were made two hourly for the first 72 hours after dosing and at 0600, 1800 and 2000 hours for

the next seven days. Faeces were collected at 0600 and 1800 hours for the final four days. All pellets were weighed and stored at -10° C. Urine was collected daily and bulked for each animal.

3.2.4 **Analysis of markers**

Faecal samples were packed to a constant height in tared plastic scintillation tubes and assayed using the dual channel method of Tan *et al.* (1971). An equivalent volume of urine was treated similarly. Background radiation was estimated from tubes packed with unlabelled faeces or urine. All ^{51}Cr-EDTA counts were corrected for ^{103}Ru-P activity in the ^{51}Cr-EDTA channel and all samples from each animal were counted within 12 hours of each other to avoid errors due to radioactive decay. After assaying the radioactivity, tubes of faeces were dried in a forced draught oven at 50° C for 7 days and reweighed to determine their dry mass. Results were expressed as counts per minute per gram of faecal dry matter.

3.2.5 **Statistical**

The mean retention time (MRT) of each marker was calculated from the formula:

$$\frac{\sum M\,T}{\sum M}$$

where M is the amount of marker excreted in each time interval and T is the time in hours since dosing (Blaxter *et al.*, 1956).

The 5, 50, 95 and 99% excretion times were estimated from the plot of percentage dose remaining against time. An equation of the form Y =

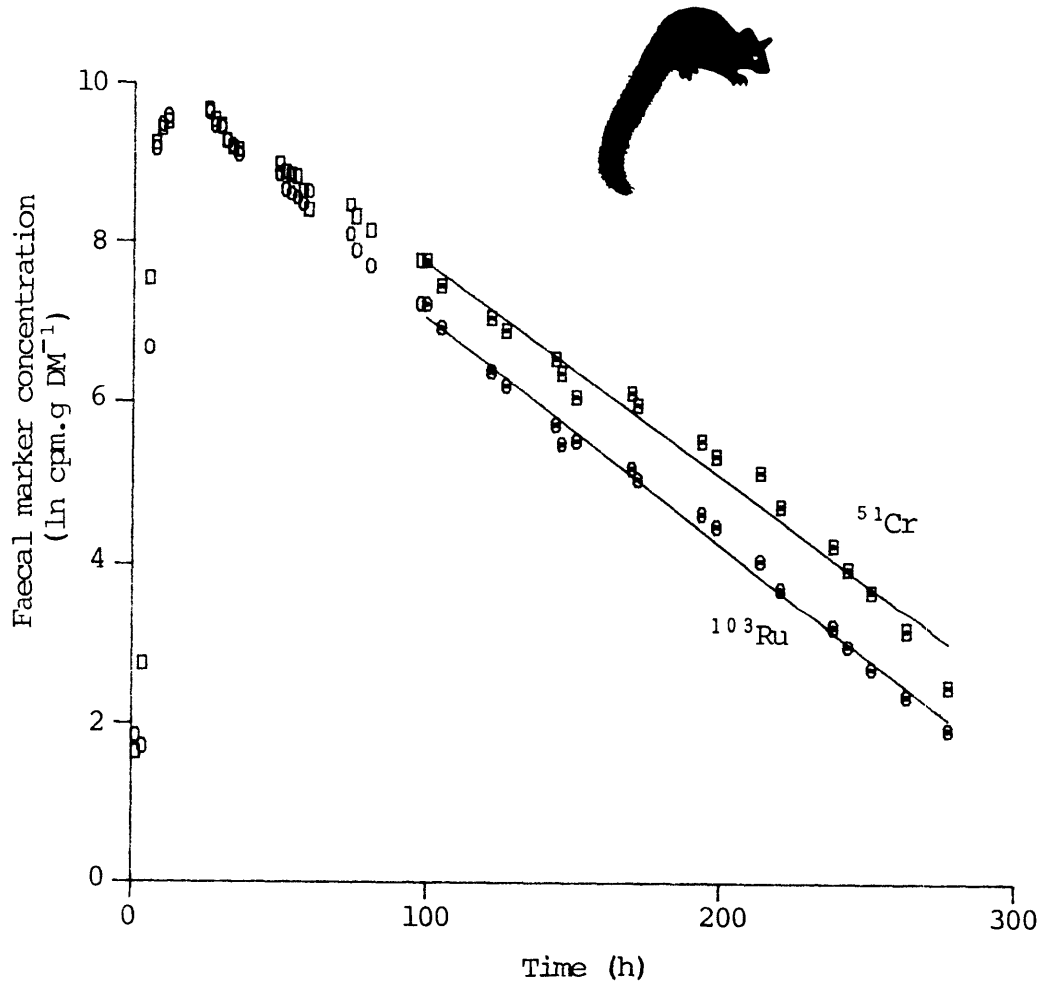

FIGURE 3.1a: *Change in faecal concentration of ^{51}Cr and ^{103}Ru with time in one Greater Glider following an oral dose of ^{51}Cr-EDTA and ^{103}Ru-P.*

Regression equations fitted to curves (>100h):
(a) ^{51}Cr: y = 10.38 - 0.026x, r = 0.986 (P<0.001)
RSD = 0.247
(b) ^{103}Ru: y = 9.86 - 0.028x, r= 0.997 (P<0.001)
RSD = 0.137

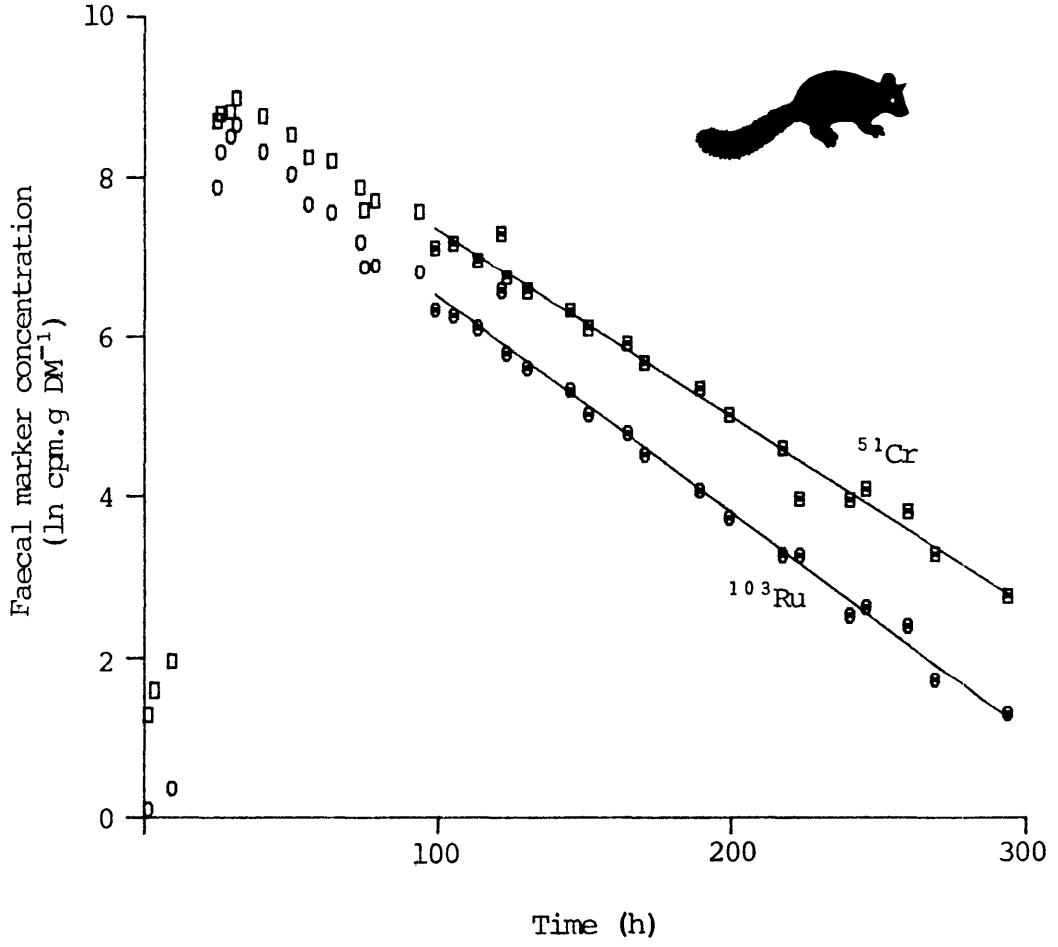

FIGURE 3.1b: *Change in faecal concentration of ^{51}Cr and ^{103}Ru with time in one Brushtail Possum following and oral dose of ^{51}Cr-EDTA and ^{103}Ru-P.*

Regression Equations fitted to curves (>100h):
(a) ^{51}Cr: y = 9.69 - 0.024x, r = 0.991 (P<0.001), RSD = 0.193
(b) ^{103}Ru: y = 9.22 - 0.027x, r = 0.993 (P<0.001), RSD = 0.198

TABLE 3.1a: *Measures of the retention of single, oral doses of ^{51}Cr-EDTA and ^{103}RU-P estimated by faecal collection in Greater Gliders (values expressed in hours)*

Experiment	Animal	Body weight (kg)	Marker and excretion time											
			^{51}Cr-EDTA						^{103}Ru-P					
			5%	50%	95%	99%	MRT[1]	1/k[2]	5%	50%	95%	99%	MRT[1]	1/k[2]
A	G1	1.050	6	31	118	180	52	36	4	21	102	169	54	27
B	G1	1.080	8	34	140	193	55	37	9	34	112	171	52	32
A	G2	0.860	5	29	115	176	43	35	2	20	96	149	36	28
B	G2	0.855	8	27	99	148	42	31	7	22	77	116	37	22
A	G3	0.940	4	25	128	200	38	50	5	19	107	167	38	40
B	G3	0.910	8	32	120	182	48	38	7	27	100	151	42	31
A	G4	1.300	7	35	132	200	49	43	8	30	109	165	48	35
B	G4	1.310	12	41	143	215	62	42	12	35	121	180	53	35
A	G5	1.090	9	36	135	204	51	46	9	32	113	170	49	37
B	G5	1.070	10	39	145	219	57	38	7	30	115	174	50	31

[1] Mean retention time

[2] k = slope of final linear portion of plot of ln[conc] v. time

TABLE 3.1b: *Measures of the retention of single, oral doses of ^{51}Cr-EDTA and ^{103}RU-P estimated by faecal collection in Brushtail Possums (values expressed in hours)*

Experiment	Animal	Body weight (kg)	Marker and excretion time											
			^{51}Cr-EDTA						^{103}Ru-P					
			5%	50%	95%	99%	MRT[1]	1/k[2]	5%	50%	95%	99%	MRT[1]	1/k[2]
A	BT1	1.770	8	32	121	183	50	38	8	32	117	178	50	33
	BT2	2.340	11	30	97	144	45	30	12	31	97	144	47	28
B	BT2	2.380	12	29	90	132	46	26	14	32	95	139	48	28
	BT3	2.360	14	33	100	147	60	23	19	40	115	168	51	23
C	BT4	2.697	14	40	135	202	58	44	14	36	117	174	54	36
	BT6	1.925	12	33	111	165	53	33	13	33	107	158	51	32
	BT7	2.952	3	26	110	170	42	34	2	23	99	153	40	28

[1] Mean retention time

[2] k = slope of final linear portion of plot of ln[conc] v. time

A Diet: mixed foliage

B Diet: mixed foliage

C Diet: *E. melliodora*

ae^{-kt} was fitted to this plot and solved for Y = 0.95, 0.50, 0.05 and 0.01. 1/k (the fractional turnover rate of the major compartment (Grovum and Williams, 1973)) was determined from the slope of the final linear portion of the plot of $\log_e$ marker concentration against time.

3.2.6 **Distribution of digesta particle sizes**

Samples of digesta were collected from the stomach, hindgut and rectum of three Greater Gliders and three Brushtail Possums slaughtered in conjunction with experiments described in Chapter 7. The particle size fractionation was carried out by Dr D.W. Dellow, DSIR Division of Applied Biochemistry, New Zealand, by the method of Evans *et al.* (1973). The dry matter recovered on each sieve (and the residual) was expressed as a proportion of the total dry matter recovered. No account was taken of the soluble dry matter.

3.3 **Results**

3.3.1 **Marker behaviour**

In all experiments with Greater Gliders, only 2-3% of the total faecal ^{51}Cr-EDTA counts and less than 0.2% of the total ^{103}Ru-P counts were detected in the urine. There was a similar (1-3%) amount of ^{51}Cr-EDTA counts in the urine of the Brushtail Possums but less than 0.1% of the ^{103}Ru-P counts when this species was fed solely on *E. melliodora*. No estimate of marker absorption was made when the Brushtail Possums were fed the mixed foliage diet.

3.3.2 **Marker excretion and retention times**

Figures 3.1a and 3.1b show the pattern of faecal excretion of ^{103}Ru-P and ^{51}Cr-EDTA in Greater Gliders and Brushtail Possums respectively. There were no significant differences between any parameters of digesta passage in the Brushtail Possums when they were fed the mixed foliage diet or when they were fed *E. melliodora* foliage only.

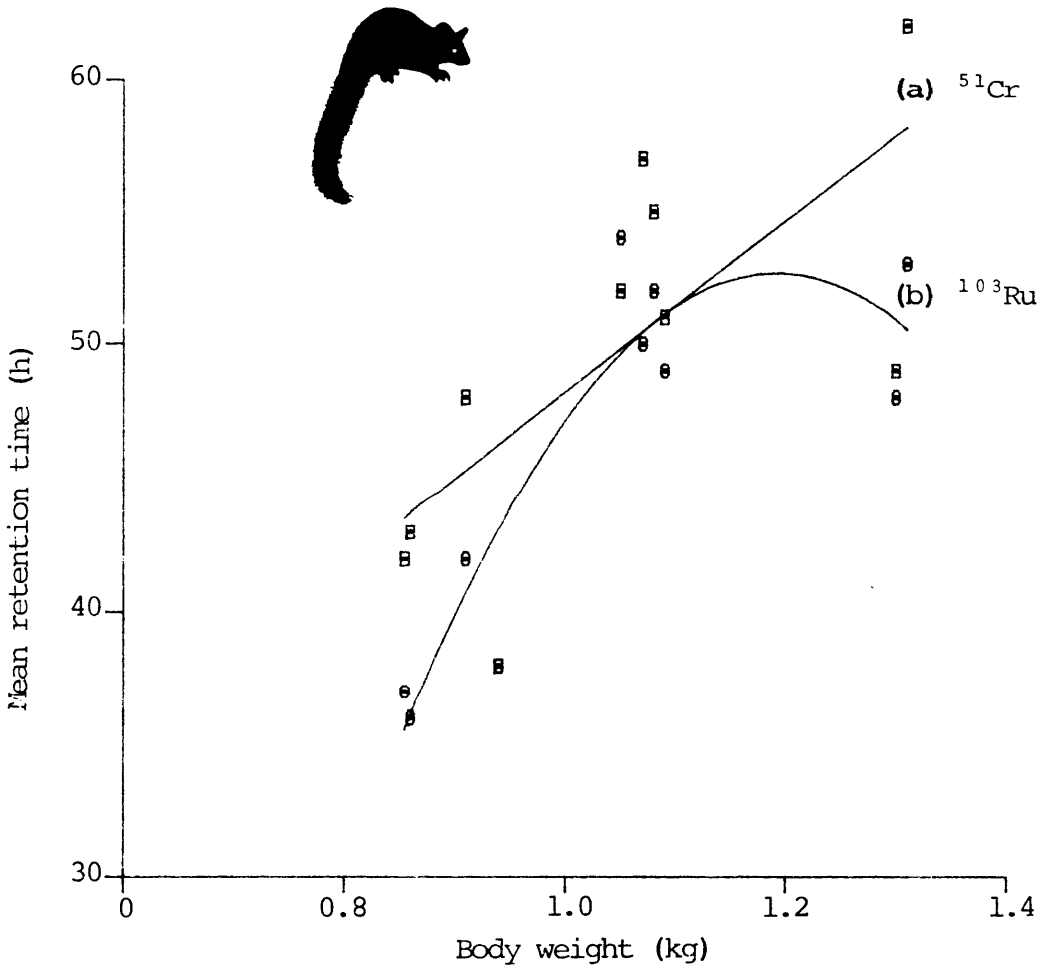

FIGURE 3.2: *Relationships between body weight of Greater Gliders and -*
(a) ^{51}Cr mean retention time; and
(b) ^{103}Ru mean retention time.

Regression equations:
(a) ^{51}Cr: $y = 16.0 + 32.2x$, $r = 0.672$ ($P<0.05$), RSD = 5.44
(b) ^{103}Ru: $y = -162.3 + 361.1x - 151.6x^2$, $r = 0.893$ ($P<0.001$), RSD = 3.15

TABLE 3.2a: *Proportions (% of recovered DM) of digesta from various gut segments of Greater Gliders, retained on sieves of different sizes (values are Mean ± SE of 3 animals)*

Gut segment	Sieve size					
	> 1 mm	0.50 mm	0.25 mm	0.125 mm	0.075 mm	< 0.075 mm
Stomach	0.4 ± 0.3[a]	2.9 ± 3.1[a]	21.4 ± 2.8[b]	29.1 ± 2.9[b]	16.0 ± 0.9[a]	30.1 ± 1.9[b]
Caecum (distal)	1.3 ± 0.6[a]	2.1 ± 1.1[a]	8.3 ± 2.0[a]	19.4 ± 6.6[a]	19.8 ± 2.7[a]	49.2 ± 9.4[a]
Caecum (proximal)	1.0 ± 0.8[a]	0.7 ± 0.5[a]	11.7 ± 4.2[a]	12.9 ± 0.0[a]	27.1 ± 10.3[a]	46.5 ± 11.9[a]
Rectum	0.6 ± 0.6[a]	1.8 ± 0.8[a]	21.5 ± 7.4[b]	37.2 ± 1.4[b]	23.1 ± 6.6[a]	15.8 ± 3.8[c]

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

TABLE 3.2b: *Proportions (% of recovered DM) of digesta from various gut segments of Brushtail Possums, retained on sieves of different sizes (values are Means ± SE of 3 animals)*

Gut segment	Sieve size					
	> 1 mm	0.50 mm	0.25 mm	0.125 mm	0.075 mm	< 0.075 mm
Stomach	6.1 ± 1.7^{a}	9.0 ± 1.1^{c}	26.4 ± 0.4^{d}	24.3 ± 1.6^{a}	13.6 ± 3.0^{d}	19.8 ± 3.9^{a}
Caecum (distal)	3.9 ± 0.9^{a}	4.2 ± 0.6^{a}	12.7 ± 0.6^{a}	29.6 ± 4.2^{a}	27.2 ± 5.7^{ab}	22.2 ± 10.3^{a}
Caecum (proximal)	2.8 ± 1.0^{a}	3.0 ± 0.9^{b}	15.7 ± 1.6^{ab}	22.5 ± 1.2^{a}	31.4 ± 2.4^{b}	24.7 ± 3.9^{a}
Proximal colon (distal)	3.9 ± 0.3^{a}	3.8 ± 0.8^{a}	12.2 ± 2.3^{a}	24.0 ± 7.1^{a}	24.9 ± 0.9^{ac}	31.0 ± 6.3^{a}
Proximal colon (proximal)	2.9 ± 0.5^{a}	3.8 ± 0.8^{ab}	18.0 ± 2.7^{bc}	23.1 ± 3.3^{a}	26.3 ± 2.5^{a}	25.9 ± 2.9^{a}
Rectum	4.6 ± 2.1^{a}	4.4 ± 0.9^{a}	20.5 ± 3.7^{c}	31.0 ± 4.1^{a}	20.1 ± 3.4^{c}	19.5 ± 1.4^{a}

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

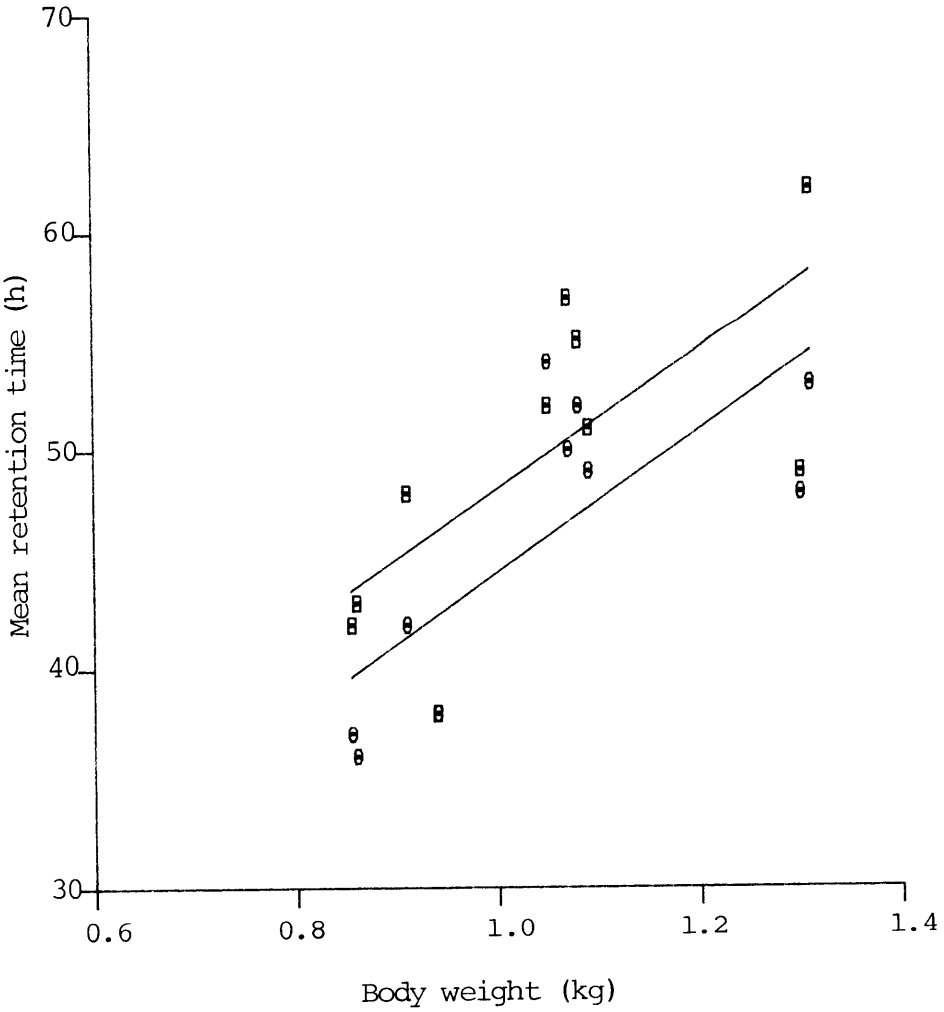

FIGURE 1.2: *Relationships between body weight of Greater Gliders and -*
(a) ^{51}Cr (□) mean retention time; and
(b) ^{103}Ru (○) mean retention time.

Regression equations:
(a) ^{51}Cr: y = 16.0 + 32.2x, r = 0.672 (P<0.05), RSD = 5.44
(b) ^{103}Ru: y = 11.7 + 32.6x, r = 0.726 (P<0.01), RSD = 4.80

Retention times in both species were long. Table 3.1a shows individual animal data for mean retention time, 5%, 50%, 95%, 99% excretion times and the fractional turnover rate for each marker in the Greater Gliders. Table 3.1b shows the same measurements for the Brushtail Possums. There were no significant differences in the MRT, 5%, 50%, 95% and 99% excretion or fractional turnover times between the two markers in the Brushtail Possum. However, the fractional turnover rate of both ^{51}Cr-EDTA and ^{103}Ru-P was greater ($P<0.001$) than the respective MRT's. On the other hand, there was no significant difference in either the 5% excretion times or the MRT's of the two markers in the Greater Gliders. However, there was a trend towards greater separation of the two markers with increasing proportional excretion times. Thus, while the 5% excretion times were similar, the 50% ($P<0.05$), 95% ($P<0.01$) and 99% ($P<0.01$) excretion times for ^{51}Cr-EDTA were greater than those for ^{103}Ru-P. The fractional turnover rate of both markers was greater ($P<0.01$) than their respective MRT's.

There was considerable between animal variation in retention times in the Greater Glider. However much of this could be explained by differences in body mass (Figure 3.2).

3.3.3 **Particle size distribution**

Tables 3.2a and 3.2b give details of the distribution of digesta particle sizes in the gut of the Greater Glider and Brushtail Possum respectively. There were more fine (<0.075 mm) particles in the stomach and caecum of the Greater Gliders than in the stomach, caecum and proximal colon of the Brushtail Possums. Although the faeces of each species contained similar proportions of fine particles, those of the Brushtail Possum contained more coarse (>0.50 mm) material. In fact, there was more coarse digesta particles in all digesta segments of the Brushtail Possums than in the Greater Gliders. There was a greater ($P<0.001$) proportion of fine (<0.075mm) particles in the caecum of the Greater Glider compared with the faeces, but the proportion of fine particles was similar in all digesta samples from the Brushtail Possum.

3.4 **Discussion**

In the last few years, a wide range of materials ranging from glass and plastics to rare earth elements has been used as digesta markers. Several recent reviews have considered the properties of these materials and their applicability to different experimental situations (Uden *et al.* 1980, Ellis *et al.*, 1982, Dixon *et al.*, 1983b, Teeter and Owens, 1983). No marker yet devised has proved to be "ideal". The characteristics of an ideal marker have been discussed by Kotb and Luckey (1972), Engelhardt (1974) and Faichney (1975). These can be summarized as:

a) It must not be absorbed from the gastrointestinal tract (GIT);

b) It must not affect or be affected by the GIT or by the microbial population;

c) It must be physically similar to, or intimately associated with the material it is to mark;

d) It must not interfere with the analysis of other digesta components.

Not all of these criteria were tested in the present study. The amount of both markers appearing in the urine of the two species was similar to that absorbed by the Koala (Cork and Warner, 1983), Ringtail Possum (Chilcott, 1982), Tammar Wallaby (Warner, 1981b) and several domestic herbivores (Warner, 1981a). However, during the study of the absorption of essential oils from the gut of the Greater Glider (Chapter 7), evidence was obtained suggesting that Cr-EDTA may not be completely inert with feeds high in essential oils. Whether this was due to the disruption of the Cr-EDTA complex or by oxidation of oil components (Chapter 7) is unknown. However, Steele and Clapperton (1982) have warned of the possibility of interference with long-chain fatty acid analyses by the presence of chromic oxide. Although the specific activity of the ^{51}Cr-EDTA was reduced by dilution with Cr-EDTA in this part of the study,

TABLE 3.3: *Rate of passage of digesta markers in herbivores*

Species	Diet	Marker	Phase[1]	Excretion %	Excretion Time (h)	MRT (h)	Reference
EUTHERIAN - Foregut fermenters							
Sheep	Lucerne hay	^{51}Cr-EDTA	S	50	21	-	Dellow (1982)
		^{103}Ru-P	P	50	25	-	Dellow (1982)
Sloth (*Bradypus*)	Foliage (free ranging)	Glass beads	P	95	1200	-	Montgomery and Sunquist (1978)
- Hindgut fermenters							
Horse	Oat hay and concentrates	^{51}Cr-EDTA	S	-	-	22	R.K. Orton (pers. comm.)
		Ru-P	P	-	-	24	R.K. Orton (pers. comm.)
Howler Monkey	Fruit and leaves	Plastic ribbon	P	95	24-72	-	Milton (1981)
MARSUPIAL - Foregut fermenters							
Grey Kangaroo	Lucerne hay	^{51}Cr-EDTA	S	50	14	-	Dellow (1982)
		^{103}Ru-P	P	50	30	-	Dellow (1982)
Tammar Wallaby	Lucerne hay	^{51}Cr-EDTA	S	50	15	-	Dellow (1982)
		^{103}Ru-P	P	50	24	-	Dellow (1982)
Red-necked Pademelon	Lucerne hay	^{51}Cr-EDTA	S	50	12	-	Dellow (1982)
		^{103}Ru-P	P	50	23	-	Dellow (1982)
- Hindgut fermenters							
Koala	*E. punctata*	^{51}Cr-EDTA	S	95	589	198	Cork and Warner (1983)
		^{103}Ru-P	P	95	281	93	Cork and Warner (1983)
Ringtail Possum	*E. andrewsii*	^{51}Cr-EDTA	S	95	159	63	Chilcott and Hume (1985)
		^{103}Ru-P	P	95	79	35	Chilcott and Hume (1985)
Brushtail Possum	*E. melliodora*	^{51}Cr-EDTA	S	95	109	51	Present study
		^{103}Ru-P	P	95	107	49	Present study
Greater Glider	*E. radiata*	^{51}Cr-EDTA	S	95	128	50	Present study
		^{103}Ru-P	P	95	105	46	Present study
		^{51}Cr-mordant[2]	P	95	42	23	Present study

[1] S = solutes; P = particles

[2] > 1.0 mm < 0.5 mm

the concentration of Cr was still several orders of magnitude less than that used in the initial essential oil experiments (Section 7.2.1).

The nature of the ^{103}Ru-P marker may also have reduced some of the differences expected in these experiments. The characteristics of ^{103}Ru-P attachment will be further discussed later, but the consensus of several recent studies (Faichney and Griffiths, 1978, Dixon *et al.*, 1983a,b, Dixon and Milligan, 1984) seems to be that ^{103}Ru-P exists in dynamic equilibrium between all available binding sites.

The differences in particle size distributions in the various gut segments, between the two species, are consistent with the results of studies of dental morphology by Gipps (1980). It was shown that the Greater Glider and Ringtail Possum have the capacity to finely comminute their food by cutting and controlled compression, and shearing stresses. In contrast, the Brushtail Possum (and Mountain Possum) employed less a cutting action but more a coarse grinding. This resulted in a much finer mean particle size in the stomach of the two Pseudocheirids than in the stomach of the Phalangerids in Gipps' (1980) study, although the differences observed may have been confounded by different diets. While further particle breakdown occurs lower in the gastrointestinal tract, the data in Tables 3.2a and 3.2b show that the initial mastication is of primary importance in establishing a large fine particle pool. The creation of a large surface area should allow greater bacterial colonization and subsequently greater fibre digestibility in the caecum (Akin, 1979, Akin and Barton, 1983, Chapter 4).

Warner (1981a) reviewed the literature available on digesta passage in a wide range of mammals and birds. Some data of particular relevance to the present study are presented in Table 3.3. While large foregut fermenters such as cattle and bison generally have the longest absolute retention times, the arboreal marsupials, given their small body size, are also notable for long retention times. There is some evidence that long retention times are a feature of other arboreal folivores as well. Montgomery and Sunquist (1978) found that in Three-toed Sloths, total excretion times for a dose of glass beads was about 1200

TABLE 3.4: *Summary of digesta passage studies in Brushtail Possums*

Diet	Marker	T^1_{MIN}	T^2_{PK}	T^3_{MAX}	MRT[4]	Reference
Mixed fruit and vegetables	Wheat grains stained with methylene blue	10	-	96	-	Honigmann (1941)
Carrots, pellets, fresh greens	Chromic oxide	25	40	86	-	Gilmore (1970)
	Plastic chips	8	16	80	-	Gilmore (1970)
	Eucalypt cuticle	38	74	99	-	Gilmore (1970)
Pellets, bread, fruit	Plastic chips (0.5-3 x 1.5 mm)	24	-	312	-	Taylor (1972)
Apples	Plastic chips (2 x 2 mm) (SG = 1.07)	-	-		96	Lintermans (1979)
E. viminalis leaf[5]	Plastic chips (2 x 2 mm) (SG = 1.07)	-	-	-	69	Lintermans (1979)
Eucalypt and *Acacia* leaf[5]	^{51}Cr-EDTA/^{103}Ru-P	8/6	-	150/144	68/63	A.Smith and J.Biggins (unpublished)
Apples	^{51}Cr-EDTA/^{103}Ru-P	8/6	-	154/192	58/66	A.Smith and J.Biggins (unpublished)
Apple and leaf[5]	^{51}Cr-EDTA/^{103}Ru-P	8/6	-	120/144	38/47	A.Smith and J. Biggins (unpublished)
Semi-purified (honey and grain byproducts)	^{51}Cr-EDTA/^{103}Ru-P	-	-	-	64/71	Wellard and Hume (1981b)
Eucalypt foliage	^{51}Cr-EDTA/^{103}Ru-P	11/12	26/24	-	51/49	Present study

[1] Time of first appearance of marker

[2] Time of peak marker excretion

[3] Time of last appearance of marker

[4] Mean retention time

[5] Energy intake less than basal requirements

hours. Honigmann (1941) found similar long retention times in the Two-toed Sloth. Milton (1981) found that the time of first appearance of plastic ribbon in the faeces of the Howler Monkey was about five times as long as in the frugivorous Spider Monkey. However, interpretation of the sloth data are complicated by storage of faeces in the rectum for up to 7 days (Montgomery and Sunquist, 1978). This, and the use of inappropriate markers such as plastic strips (Milton, 1981) and glass beads, means that at present, much of the data on the eutherian arboreal folivores is not comparable with the marsupial data. Clearly, more extensive and careful work is needed before generalizations can be made.

There have probably been more studies of digesta passage in the Brushtail Possum than in any other non-domesticated herbivore. These studies are summarized in Table 3.4. However, many of the studies have used inappropriate markers. For example, the plastic chips used by Gilmore (1970) and Taylor (1972) are far larger than the biggest particles likely to be encountered in the Brushtail Possum gut. There is also no evidence that these markers were associated with the particulate digesta phase since no information was given on their specific gravity. A second problem is that those studies which have tried to examine the effects of foliage diets on digesta retention in Brushtail Possums, have used diets which were very poorly accepted by the animals, supplying in most cases less than their basal energy requirements. Blaxter *et al.* (1956) have shown that prolonged sub-maintenance feeding in sheep leads to longer MRT's.

The MRTs recorded for foliage diets in this study are similar to values recorded by Wellard and Hume (1981b) for semi-purified diets of similar NDF content. In addition, Wellard and Hume's (1981b) study and that of Fitzgerald (pers. comm.) have shown no significant differences between the MRTs of ^{51}Cr-EDTA and ^{103}Ru-P and this has been interpreted as evidence that there is no preferential retention of fluids and fine particles in the Brushtail Possum gut (Wellard and Hume, 1981b, Hume, 1982). However, preferential retention of fine particles need not necessarily result in different MRTs for these two markers. In the present study, the similarity in MRTs of each marker was supported by the lack of

significant differences in the distribution of fine digesta particles between different gut segments.

There has been only one previous attempt to measure digesta retention times in the Greater Glider. G.D. Sanson (pers. comm.) used the same marker system applied here, but faecal collections were only made for 100 hours and at this stage, the final marker concentrations were still 10-15% of peak values and MRTs could not be accurately calculated. Nonetheless, peak appearance times seemed similar to those in the present study.

The finding of selective retention of very fine particles in the caecum of the Greater Glider (Table 3.2a) does not appear to be consistent with the data on marker retention. The MRTs of ^{51}Cr-EDTA and ^{103}Ru-P were not significantly different, although the trend was towards slightly longer ^{51}Cr-EDTA MRTs. Using similar techniques, Cork and Warner (1983) showed that retention of fine particles in the caecum and proximal colon of the Koala is accompanied by a significantly longer retention of the fluid marker. Chilcott and Hume (1985) showed that ^{51}Cr-EDTA is retained twice as long as ^{103}Ru-P in Ringtail Possums, although a higher concentration of fine particles in the caecum compared with the rectum was not demonstrated.However, Ringtail Possums are caecotrophic and reingestion was not prevented during Chilcott and Hume's (1985) experiments. This would have the effect of increasing the apparent retention time of both the ^{51}Cr-EDTA and the ^{103}Ru-P markers.

The most likely explanations of the patterns observed in the Greater Glider result from the non-ideal behaviour of the two digesta markers. ^{51}Cr-EDTA has been shown to bind to particulate matter in the rumen of sheep (Warner, 1969) and cattle (Dixon and Milligan, unpub. in Dixon *et al.* 1983b). If this occurred in the gut of the Greater Glider, then it could explain the lack of differences between the ^{51}Cr-EDTA and ^{103}Ru-P MRTs. However, the magnitude of the attachment in the rumen (1-3% of marker dose) would seem to be insufficient to explain these results.

A more likely explanation involves the migration of ^{103}Ru-P between particles in the gut (Tan *et al.*, 1971, Faichney and Griffiths, 1978, Dixon and Milligan, 1984). This migration results in the concentration of ^{103}Ru-P on fine particles being greater than that on coarse particles (Cork, 1981, Dixon *et al.*, 1983b, Egan *et al.*, 1983, Dixon and Milligan, 1984) and suggests that ^{103}Ru-P attachment to particulate matter is essentially a surface area effect. Since the majority of particles in the caecum of the Greater Glider were less than 0.125 mm, it seems likely that the majority of ^{103}Ru-P was associated with these particles. If this material was flowing with the fluid digesta phase (Björnhag, 1972, 1981), there would have been no apparent marker separation in the Greater Glider. As a corollary to this, prevention of caecotrophy in the Ringtail Possum should result in very similar ^{51}Cr-EDTA and ^{103}Ru-P MRTs.

The recent development of heavy metal (Cr and Au) plant cell wall mordants as digesta markers (Uden *et al.*, 1980) has enabled the examination of the kinetics of specific particle pools (e.g., Pond *et al.*, 1981). It was considered feasible to use this technique to label large particles (0.5 mm) that had already passed through the gut of the Greater Glider and feed these back to the animals. If the arguments developed above are correct, this should result in a particulate MRT much shorter than that of ^{103}Ru-P.

P A R T B

3.5 **Materials and Methods**

3.5.1 **Preparation of particles**

Although it had been originally intended to use particles recovered from the faeces of the Greater Glider, it proved impossible to dissociate the faecal pellets by either soaking them in water or by boiling in neutral detergent solution. Hence, undigested plant particles were used. *E. radiata* leaf was ground to pass a 2 mm screen and dry sieved to obtain those particles <1.0 mm >0.5 mm. These particles were refluxed with

neutral detergent solution for 3 hours and washed under running water for 3 days. After several extractions with acetone, the material was dried overnight at 50° C.

3.5.2 **Preparation of mordants**

The particles were mordanted with chromium by a modification of the method of Uden *et al.* (1980). Three volumes of a solution of $Na_2Cr_2O_7 2H_2O$ containing 13% of the fibre mass as Cr and one volume of a solution of $Na_2\ ^{51}Cr_2O_7 2H_2O$ (9.25-18.5 GBq/mg Cr: Amersham International) were added to the particles. This material was refluxed for 24 hours, washed thoroughly to remove excess Cr and suspended in tap water. One volume of a solution of ascorbic acid (half the original fibre mass) was added and the preparation left to stand for 3 hours. The fibre was then thoroughly washed, dried overnight at 50° C and dry sieved to exclude any particles <0.5 mm.

3.5.3 **Administration of markers**

The mordanted particles were administered to five Greater Gliders by stomach tube to prevent them being chewed. At 1630 hours (1-1½ hours before feeding) the animal was sedated with "Ketalar" (Parke-Davis: 0.5 $ml.kgW^{-1}$) and the tube (0.8 mm OD) lubricated with glycerine and passed down the oesophagus into the stomach. The mordanted particles were flushed through the tube with 3-5 ml distilled water. This was followed with 2 ml of a solution of ^{103}Ru-P (about 0.56 MBq) and a further 2-3 ml of water. The sedation lasted between 20 and 30 minutes and all animals appeared and acted normally after this time.

3.5.4 **Analysis**

Faeces and urine samples were collected as previously described (Section 3.2.3) for 300 hours after dosing. Faeces were packed to a constant height in glass scintillation vials. An equivalent volume of urine was treated similarly. All calculations were as previously described (Section 3.2.5). The two components of the ^{51}Cr-mordant excretion curve

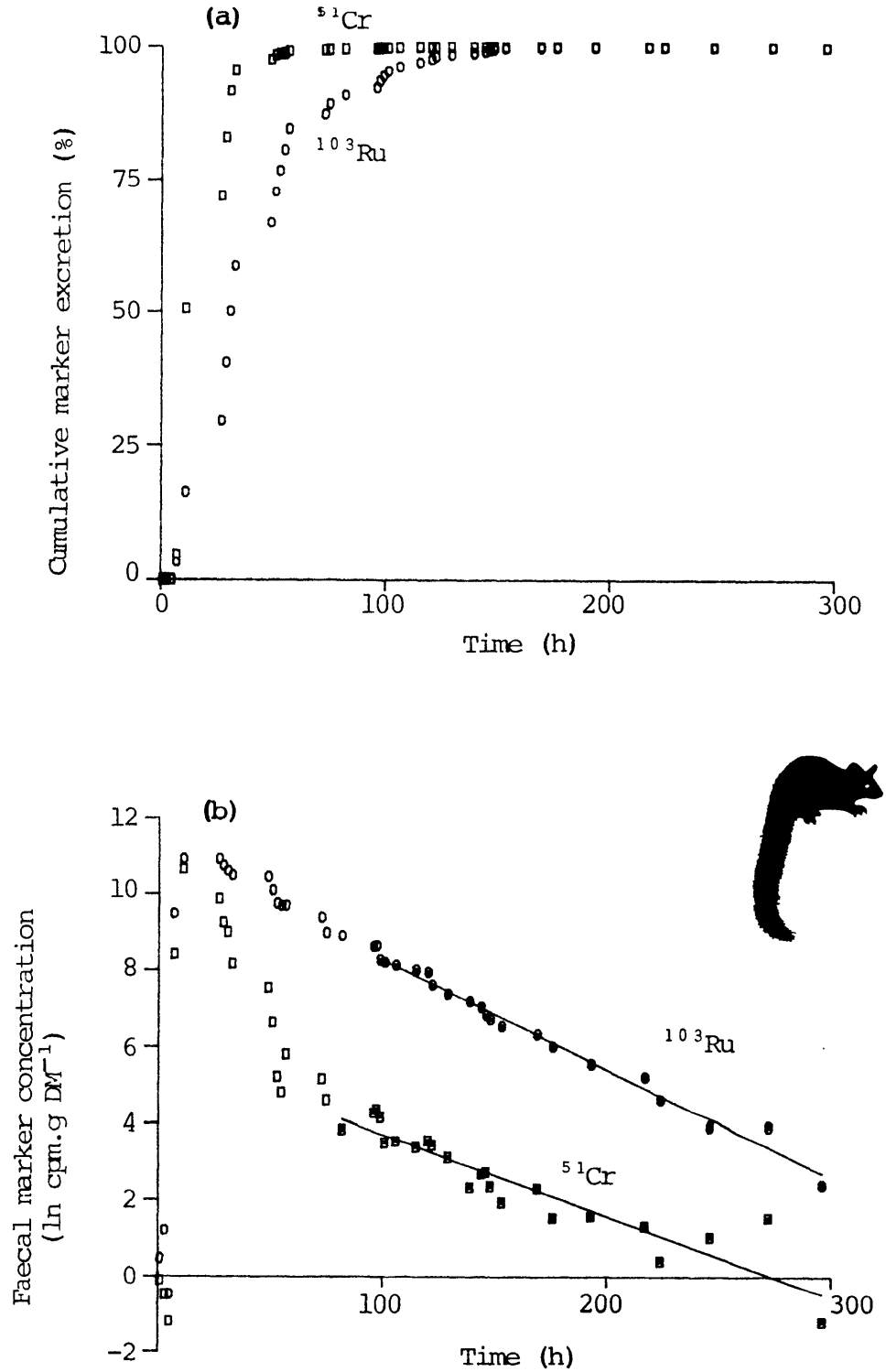

FIGURE 3.3: *(a) Cumulative faecal excretion and -*
(b) Change in faecal concentration of ^{51}Cr and ^{103}Ru with time in one Greater Glider following an oral dose of ^{51}Cr-mordanted cell walls and ^{103}Ru-P.

(b) Regression equations fitted to curves (>100h):
(i) ^{51}Cr: y = 5.84 - 0.021x, r = 0.927 (P<0.001), RSD = 0.515
(ii) ^{103}Ru: y = 11.12 - 0.028x, r = 0.993 (P<0.001) RSD = 0.201

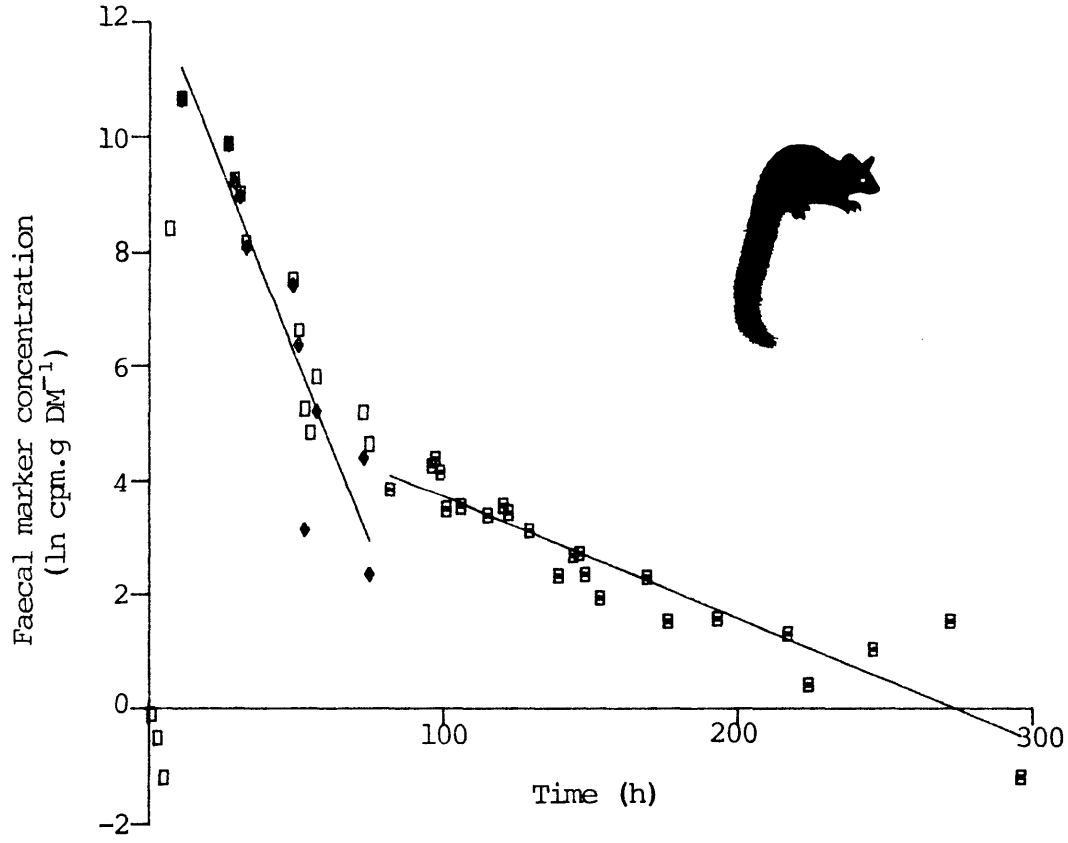

FIGURE 3.4: *Change in faecal concentration of ^{51}Cr with time in one Greater Glider following an oral dose of ^{51}Cr-mordanted cell walls (<1.0mm > 0.5mm).*

Regression equations:
(i) y = 12.63 - 0.129x, r = 0.916 (P<0.001), RSD = 1.123
(Diamonds are points for regression of component 1 (line) corrected for influence of component 2.)
(ii) y = 5.84 - 0.021x, r = 0.927 (P<0.001), RSD = 0.515
(Regression for component 2: as per (a) in Figure 3.3)

TABLE 3.5: *Measures of the retention of single, oral doses of a ^{51}Cr-mordant of E. radiata cell walls (≥0.5 mm <1.0 mm) and ^{103}Ru-P estimated by faecal collection in Greater Gliders (values expressed in hours)*

Animal	Body weight (kg)	Marker and excretion time												
		^{51}Cr-cell wall mordant (0.5 mm)							^{103}Ru-P					
		5%	50%	95%	99%	MRT[1]	$1/k_1$[2b]	$1/k_2$[2a]	5%	60%	95%	99%	MRT[1]	$1/k_1$[2a]
G6	1.177	5	13	43	64	29	9	54	12	40	131	196	57	43
G7	1.246	6	17	57	84	25	12	42	5	35	141	215	53	41
G8	0.967	7	14	41	59	25	7	44	11	36	127	190	53	40
G5	1.144	4	10	30	44	18	6	39	8	27	93	140	40	32
G9	1.181	4	11	39	59	20	7	38	10	31	106	158	46	34

[1] Mean retention time

[2] k = slope of final linear portion of plot of ln[conc] v. time

[a] $1/k_2$ (^{51}Cr-Mordant) is not significantly different from $1/k_1$ (^{103}Ru-P)

[b] $1/k_1$ (^{51}Cr-Mordant) is significantly different ($P < 0.001$) from $1/k_2$

were separated by a graphical curve peeling technique (Rescigno and Segre, 1966).

3.6 **Results**

3.6.1 **Marker behaviour**

Only 0.1% of the ^{51}Cr-cell wall mordant marker appeared in the urine. Homogenization of faecal samples, followed by high speed centrifugation showed that negligible counts were present in the supernatant. This eliminated the posibility that the marker occurred in a soluble form in the faeces. These two pieces of evidence suggested that the ^{51}Cr remained attached to the plant particles during their passage through the gut. Less than 0.1% of the ^{103}Ru-P was detected in the urine.

3.6.2 **Excretion of markers**

Curves of marker excretion versus time for both particulate markers are shown in Figure 3.3 Although the terminal portion of the log transformed ^{103}Ru-P excretion curve was linear ($r = 0.993$), the ^{51}Cr-cell wall mordant excretion curve was curvilinear, with two distinct components (Figure 3.4). The first accounted for about 99.4% of the dose and the slope of this component was higher ($P<0.001$) than the slope of the second component. The second component suggested a minor pool turning over slowly. The slope of this second component was not significantly different from that of the terminal portion of the ^{103}Ru-P excretion curve.

The computed MRT of the ^{51}Cr-cell wall mordant (Table 3.5) was less than half ($P<0.001$) that recorded for the ^{103}Ru-P marker. Although the 5% excretion times were similar, there was increasing divergence between the two markers at 50%, 95% and 99% with the ^{51}Cr-cell wall mordant being excreted more rapidly.

3.7 **Discussion**

Although heavy metal mordants have been used to label feedstuffs for the last 10 years, many of the early preparations were only poorly recovered after passage through the gastrointestinal tract (Martz *et al.*, 1974). However, recent improvements to the mordanting technique (Uden *et al.*, 1980) have resulted in almost complete (>98%) recoveries of the mordanted metal ion.

Ellis *et al.* (1982) in a recent review of particulate digesta markers concluded that chromium mordants are by far the most tenaciously bound, and hence unquestionable, particulate flow markers. However, this advantage may be offset in some situations by several problems. Firstly, the addition of a large amount (10-13%) of chromium to the digesta particles will result in an increase in the specific gravity of the labelled particles relative to the rest of the digesta. To some extent, this is unavoidable since one of the strengths of this technique is that the mordanted material is essentially indigestible.

A second problem may be that if digestion effects a reduction in particle size, then the chromium-mordanted material may behave differently from the particles they are designed to mimic. On the other hand, this may be less of a problem in a hindgut fermenter such as the Greater Glider, in which the majority of particle breakdown occurs during mastication, as opposed to the ruminant system.

The MRT of the ^{103}Ru marker and the shape of the excretion curve were similar to those in the earlier experiments. However, the excretion curves of the Cr-mordant marker suggested at least two distinct components (Figure 3.4). The first component seemed to represent a pool which was turning over very rapidly. It is, however, unlikely that this represented large particles bypassing the caecum. Bjornhag (1972) showed that, in rabbits, all coarse particles entered the caecum and mixed with the caecal contents before passing into the proximal and distal colons. The magnitude of the retention times for the chromium mordanted material also suggest that large particles did not simply bypass the caecum.

The second component of the curve could have represented particles which, having entered the proximal colon, were refluxed back into the caecum before eventually passing into the distal colon. Although the mordanted particles were sieved to ensure that they were all in the range 0.5-1.0 mm before dosing, it proved impossible to dissociate the faeces and so check that the marked particles had not been broken down. However, even though it is possible that some particles were broken down (or fine particles missed in sieving), this second slower component of the faecal excretion represented only about 0.6% of the total dose and so it is unlikely to have altered the interpretations of these results.

The similarity between the fractional outflow rates of the ^{103}Ru-P and this second component of the chromium mordant suggests that both these curves represent the same fine particle pool. Cork and Warner (1983) have recently proposed that particulate digesta flow in the Koala could be explained by postulating digesta pools in parallel rather than in series as most models of digesta flow in mammals assume (Warner, 1981a). This interpretation was based on deviations from predicted faecal marker concentrations of those observed near the peak marker excretion and from the observation that the MRT of ^{103}Ru-P was often less than the estimated retention time in the major pool (1/k). In both the Brushtail Possum and the Greater Glider, MRT values were (with only one exception, (G3, Exp. 1) Part A) always greater than the derived 1/k values. This suggests that retention of markers in secondary pools was more important in these species than in Koalas. Alternatively, it may reflect a greater bias of ^{103}Ru for fine particles in the Greater Glider and Brushtail Possum than in the Koala. Nonetheless, the excretion curves for the ^{51}Cr-mordant in Greater Gliders clearly show two pools turning over at greatly different rates, consistent with the hypothesis that coarse particles are excreted more rapidly whilst fine particles are preferentially retained.

In the following section, some consideration will be given to the mechanisms and advantages of digesta separation in the hindgut.

3.8 **Mechanisms and Advantages of Selective Digesta Retention**

A separation of fine and coarse particles has been demonstrated in the hindgut of several species of birds and mammals (Björnhag and Sperber, 1977, Björnhag, 1981). However, in the absence of retrograde transport, flow in organs such as the caecum and proximal colon of the Brushtail Possum should be "tubular", with a faster passage of liquid digesta than particles. This is the situation found in the tubiform macropod stomach (Dellow, 1979). However, if retrograde transport does not occur in the Brushtail Possum (as the data on marker passage and particle distribution suggest), then the fluid digesta should flow more rapidly than the particulate fraction. It may be that limited retrograde transport does occur in the Brushtail hindgut but radiographic studies would be required to test this idea.

Two complementary mechanisms have been proposed to explain the separation of coarse and fine particles in the caecum of the rabbit. These are antiperistaltic movements in the colon and the secretion of fluid into the proximal colon. The antiperistaltic movements probably force digesta back towards the caecum, while the secretion of fluid into the proximal colon may effect the retrograde transport of fine particles (Björnhag, 1972, 1981).

There is a gradient of electrical and mechanical activity during caecotrophe formation in the proximal colon of the rabbit which may lead to the mechanical separation of solid and liquid digesta (Ruckebusch and Hörnicke, 1977). More recently, Ehrlein *et al.* (1982) have demonstrated a complex pattern of motor activity in the rabbit proximal colon which produced a displacement of faecal material in both an oral and distal direction. While there are no data available on the motility of the colon of either the Brushtail Possum or the Greater Glider, some degree of antiperistalsis has been demonstrated in the hindgut of sheep, rats, guinea pigs and recently horses (Sellers and Georgi, 1982) and it would be surprising not to find this more widespread.

There is more information available on the second proposed mechanism, the secretion of fluid into the proximal colon. Rübsamen *et al.* (1983) showed that water and Na^+Cl^- was secreted into the proximal colon of the Greater Glider, but that in contrast, there was a net absorption of these substances from the proximal colon of the Brushtail Possum. In similar experiments, Clauss (1978) showed that this fluid secretion (which Björnhag (1972) had predicted) also occurred in the rabbit proximal colon. Björnhag (1981) suggested, on the basis of radiographic evidence, that water secreted into the proximal colon was responsible for "rinsing" the fine particles from the coarse material and re-establishing them in a fluid phase for transport back to the caecum.

In horses, there is a large net absorption of water from the proximal colon rather than the secretion seen in the rabbit or Greater Glider. There is, however, a slight net secretion of water into the distal colon of the horse (Argenzio *et al.* 1974). In birds (geese: Clemens *et al.* 1975; turkeys and guinea fowl: Björnhag and Sjöblom, 1977) particle separation in the hindgut seems to be achieved by retrograde transport of urine from the cloaca to the caeca (Björnhag and Sperber, 1977). However, by analogy with the rabbit, the major region of the hindgut effecting retrograde transport would be in the proximal colon. It would seem then that antiperistaltic movements can occur in the hindgut without bringing about a major separation of fine and coarse particles. Similarly, the secretion of fluid into the proximal colon of the Greater Glider is not linked to the practice of caecotrophy as in the rabbit.

Nonetheless, the similarity in the pattern of particle distribution and hindgut secretion/absorption in the rabbit and Greater Glider suggests that similar particle separation mechanisms are involved. To date, the results of studies of separation in the hindgut suggest that fluid secretion into the proximal colon is an essential mechanism to bring about particle differentiation. Absorption of water brought back to the caecum by antiperistaltic movements and its subsequent secretion into the proximal colon constitutes an important internal water recycling mechanism.

The selective retention of solutes and fine particles in the hindgut has been seen to provide several advantages (Björnhag and Sjöblom, 1977, Björnhag, 1972).

The most often quoted (Cork and Warner, 1983, Chilcott and Hume , 1985) is the opportunity for the animal to pass the coarser particles through the system more rapidly than the fine particles and so reduce the gut filling effect of a highly fibrous diet. This is seen as particularly important for small herbivores who have high mass-specific energy requirements. While there is no doubt that the larger particles are eliminated, there is little evidence to confirm that these particles are, in fact, more highly fibrous. Björnhag and Sjöblom (1977) suggested that since fibrous materials occurred in relatively large "parcels" in the plant (e.g. vascular bundles), then the larger particles would *per se* be more fibrous. Although total cell wall content of ruminant faecal particles usually decreases with decreasing particle size, the proportion of lignin in this fibrous material shows a U-shaped distribution with decreasing particle size (Van Soest, 1982).

Although ruminant data may not provide a good comparison, there appears to be no similar work with hindgut fermenters. Nonetheless, even supposing that the retained particles were no less fibrous, bacterial attachment at broken surfaces should be relatively greater (Akin, 1979, Akin and Barton, 1983). This alone should provide the potential for greater fibre digestion.

Since retrograde transport is lacking, or of limited effect in the Brushtail Possum, this could partly explain why this species does not feed exclusively on eucalypt material in the wild. The intake of dry matter by the Brushtail Possum when fed exclusively on *E. melliodora* foliage is less than that of Greater Gliders and Koalas at similar dietary NDF and lignin contents (Chapter 4). Whether this represents a limit to the rate at which coarse fibrous material can be eliminated cannot, at present, be judged.

Much of the small particle pool that is refluxed back into the caecum consists of bacteria and their retention is seen as the other major

benefit of retrograde transport (Björnhag, 1972). This retention should reduce faecal nitrogen losses (Sperber, 1968) and Björnhag (1972) has suggested that it would provide a greater concentration of micro-organisms for microbial digestion. While it is unlikely that microbial concentration could be increased, the rate of microbial cell turnover could be significantly reduced. Again, little data is available, but the limited information presented by Slade (1971) suggests little difference in the rate of microbial cell production between rabbits and equines.

Clearly, more detailed and extensive investigations need to be carried out before the full significance of retrograde transport can be assessed. However, the preferential retention of fine particles in the hindgut appears to be an important adaptation for coping with high fibre diets.

3.9 Summary

The retention time of ^{51}Cr-EDTA and ^{103}Ru-P in the gut of both the Greater Glider and the Brushtail Possum was long compared with other herbivores fed fibrous diets. There was no apparent separation between the two markers. Analysis of the distribution of digesta particle size from different parts of the gut of the Brushtail Possum confirmed this finding. In contrast, there was a preferential retention of fine particles in the caecum of the Greater Glider. Further experiments in the Greater Glider showed that coarse particles labelled with ^{51}Cr-mordants had MRTs less than half that of ^{103}Ru-P. It was concluded that most ^{103}Ru-P attached to fine particles and since the fine particles and digesta solutes were refluxed back to the caecum together, separation between ^{51}Cr-EDTA and ^{103}Ru-P was not observed. Selective retention of fine particles in Greater Gliders was suggested to be partly responsible for the higher dry matter intakes of this species compared with the Brushtail Possums.