CHAPTER ONE

Introduction: The Potoroinae, a neglected group of marsupials

AUSTRALIA'S origins in Gondwana and its long isolation from the rest of the world have allowed the evolution of an assemblage of fauna not found in other countries. An example is the superfamily Macropodoidea whose members, along with the Vombatidae, form an essentially terrestrial division within the herbivorous order Diprotodonta.

The superfamily Macropodoidea is divided into two families: Macropodidae (kangaroos and wallabies) and Potoroidae (rat-kangaroos). The Potoroidae are divided further into two sub-families: Potoroinae, containing the genera *Aepyprymnus, Caloprymnus, Bettongia* and *Potorous*; and Hypsiprymnodontinae, with the single rather ancient genus *Hypsiprymnodon.*

The *Macropodidae* are a diverse group of marsupials. Their radiation during the Pleistocene epoch, probably in response to the spreading grasslands, was so successful that many workers (for example, Moir *et al.* 1956) have compared it with the parallel radiation of the Ruminantia (Hofmann 1973; Janis 1976; Langer 1984). Indeed, macropodid species now occur in deserts and tropical rainforests, grass plains and tall eucalypt forests, on rocky outcrops and even in trees. Their diversity is reflected also in their body mass, which ranges from two to 85 kg, and in behavioural and physiological adaptations to their array of habitats. Perhaps the success of the macropodids is best described by looking at their current status. Even under massive pressure from environmental destruction, some 35 species still exist (Strahan 1983). However, a closer examination of the extant macropodids shows that the large, grazing species — *Macropus giganteus* (eastern grey kangaroo), *M. robustus spp* (wallaroos), *M rufus* (red kangaroo), *M. rufogriseus* (red-necked wallaby) and *M. agilis* (agile wallaby) — predominate; many smaller species — for example, those within the genera *Lagorchestes* (hare wallabies), *Onychogalea* (nailtail wallabies) and *Petrogale* (rock wallabies) — are extinct or rare.

The Potoroidae are closely related and superficially similar to the Macropodidae but are usually much smaller, weighing between 500 g and three kg. Until the twentieth century, potoroids had always been reasonably abundant. However, they underwent little speciation in response to the increasing aridity of the Australian continent and

thus, from the Pliocene epoch onwards, they are considered less successful than the macropodids (Flannery 1984). Indeed, describing the evolution of potoroids since the late Miocene, Flannery stated: "The potoroids in general sink into insignificance from this time onwards." Even so, the habitats of the ten extant potoroid species, prior to European colonisation, were probably as diverse as those of the macropodids. For example, *Caloprymnus campestris* (desert rat-kangaroo) was found only on the sandhills and gibber plains near Lake Eyre; *Potorous tridactylus* has always inhabited moist coastal environments; *Hypsiprymnodon moschatus* (musky rat-kangaroo) lived in the rainforests of northern Queensland. The failure of potoroids to radiate suggests that the extant species are probably remnants that have continuously retreated to suitable habitats (Flannery 1984). Because potoroids are small and often inhabit areas with thick groundcover, land clearing and the introduction of feral predators have pushed some species to extinction and endangered others.

From the preceding discussion, it appears that body size has been critical in the evolution of the modern macropodoid fauna and in its resistance to the destructive processes of the last 200 years. How is this explained?

Before answering this question it is necessary to have a basic understanding of the relationship between herbivores and their food — plant matter. Plants exist in many forms, but all higher plants may be divided, on a functional basis, into non-structural (cell contents) and structural (cell walls) components. Cell contents are digested rapidly by the animal's own enzymes; digestion of cell-wall constituents, the most abundant energy source on earth (Moir 1965), depends on microbial fermentation — a relatively slow process. This symbiosis allows the animal to obtain nourishment, in the form of microbial protein and short-chain fatty-acids, from the otherwise recalcitrant plant-cell-wall constituents. The success of the large grazing macropodids may be partly explained by their adaptation to the fermentation of plant-cell walls. However, a forestomach fermentation has been reported also in some smaller macropodoid species — for example *Setonix brachyurus*, quokka, (Moir *et al.* 1956) and *Bettongia penicillata* (Kinnear *et al.* 1979). A better explanation for the success of the large macropodids lies in the relationship in herbivores between body mass, gut size, food intake, digestibility and the rate of passage of digesta. Data on these parameters suggest that small herbivores, which have high metabolic requirements relative to body mass, are less adapted to using plant-cell-wall constituents as a source of nutrients, than are large animals. Thus, as body size decreases, so does the proportion of plant-cell-wall constituents in the preferred diet (Jarman 1974; Demment and Van Soest 1985). This is accompanied by changes in gut morphology (Hofmann 1973). From this discussion it

would be expected that potoroines and very small macropodids would have diets and digestive adaptations that are quite different from those of the larger grazing species.

Many phenomena in macropodoids appear to be related to body size. Of particular relevance to the study of digestive function is the trend towards increasing relative size of the tubiform forestomach with increasing body mass (Hume 1984) and body size-related differences in dental morphology (Sanson 1989). Other phenomena — water requirements (Nicol 1978), level of basal metabolism (White *et al.* 1988) and nitrogen requirements (Hume 1977b, 1986) — are related, to some degree, to an animal's environment. The above findings suggest that a thorough understanding of macropodoids and herbivores in general is best achieved from comparative studies of species that differ in body mass and which inhabit different environments. This theme, a recent one, occurs in many studies by Hume (for example 1974, 1977a,b, 1986) and was recognized also by Dellow (1979).

For many years, Australia's research institutions have generated information on the physiological ecology and nutrition of macropodids. Their work has been reviewed by Hume (1982) and more recently by Grigg *et al.* (1989). Much early research was of the large grazing macropodids, and was probably inspired by their threat to the pastoral industries. Thus, the smaller and most endangered macropodoid species, including the potoroids, remain largely unstudied.

This thesis attempts to redress, in a similar comparative way, our poor understanding of the nutrition, metabolism and digestive physiology of potoroine marsupials. Owing to the paucity of published information on potoroines, the study is broadly based and compares one species from each of the extant potoroine genera — *Aepyprymnus*, *Potorous* and *Bettongia*. Chapters Two and Three are general and discuss herbivory and potoroine ecology respectively. Chapters (4-11) describe experiments on nitrogen metabolism, rate of passage of digesta, microbial digestion, water metabolism and energy metabolism. The first experiments with potoroines, in particular *A. rufescens*, describe the development of a standard diet and are reported in Appendix 1. Finally, the main findings from each experiment are brought together in an overall discussion (Chapter 12). It was hoped, in this way, to provide a basis for further metabolic and nutritional studies of our smallest macropodoids.

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CHAPTER TWO

Herbivory: Problems and solutions

THIS chapter compares aspects of gastrointestinal form and function in herbivores. Its aim is not to review the subject comprehensively, but to focus on the diversity of forms within the gastrointestinal tracts of herbivores. It intends also to demonstrate that herbivory "is a bush and not a ladder" (Gould 1986) — a system in which there are many exceptions to current theories on the relationship between body size and diet.

Eisenberg (1978) limits herbivory to those species eating plants having little or no woody tissue — grasses and forbs. This thesis uses the more liberal definition of Crawley (1983) that herbivores include all animals feeding on whole plants or their parts.

All food comes from solar energy stored in the cells of photosynthetic organisms. Plant cells may be divided on a functional basis. One part contains the largely cellulosic and hemicellulosic cell walls (fibre); the other — cell contents, include much of the protein, soluble carbohydrates and secondary compounds.

To handle the vast array of vascular plants which they eat, herbivores have adopted many strategies. Some select plant parts for the highly digestible cell contents, and discard the fibre or pass it through the gut. Others use an alternative strategy: they metabolise the cell walls — the earth's most abundant energy source. Herbivores of both strategies must avoid or detoxify the numerous secondary plant compounds.

Hofmann (1973) divides ruminant herbivores into three broad categories: concentrate selectors (CS), intermediate feeders (IM) and bulk and roughage eaters (GR). A similar system defined by Eisenberg (1976) and expanded by Langer (1988) rates six classes of herbivores (one = omnivory; six = GR). These categories may be used for all herbivores, although it may sometimes be difficult to separate concentrate selectors from omnivores (Langer 1988). Both classifications are loosely guided by animal size.

2.1 Diet, body size, gut capacity and metabolic requirements

Smaller herbivores usually select a more nutritious diet than do larger herbivores. The trend has been reported for several mammalian groups — for example, primates

(Harvey and Clutton-Brock 1981) and baboons (Demment 1983); ruminants (Hofmann 1973) and antelopes (Jarman 1974) and macropodoids (Sanson 1978; Hume 1982). The relationship between diet and body size has a physiological basis. As body size changes, so do total metabolic requirements and gut capacity. Clearly, large animals eat more food and produce more heat. More relevant, though, is the data of Kleiber (1932), which showed that maintenance requirements are proportional to the three-quarter power of body size. The consequence of this less-than-unity constant is that small homeothermic animals have higher maintenance costs per unit body mass. Because total metabolic requirements are often a simple multiple of basal requirements (for example, Green 1989), the energy costs of free existence are also relatively higher in small animals. It might be expected that smaller animals would need, comparably, a larger gut — but they do not. Parra (1973 cited by Parra 1978) showed that as the body size of both ruminant and non-ruminant herbivores increases, so does the relative capacity of their gut as a percentage of body mass. However, Demment (1982) argues that this conclusion is an artefact of the measurement techniques used: rather gut capacity and body mass increase isometrically. In both cases the ratio of metabolic requirements to gut capacity increases as body size decreases.

Whether it is a ruminant, non-ruminant foregut or hindgut fermenter, the small herbivore must display physiological and/or behavioural adaptations to maintain nutritional status, as discussed below.

1) *The animal may speed the digestion process.* Parra (1978) suggested that, for any feedstuff, fermentation rates have limits which are already approached.

2) *The animal may increase digestibility.* This option requires longer retention of digesta in a gut that, presumably, is already filled to capacity.

3) *The animal may eat more of the same diet.* As food intake increases, retention time in the gut decreases and, as a result, so does cell-wall digestibility. No matter how much the animal eats, digestibility of cell contents is more or less maintained. In theory, it is feasible for a herbivore, given sufficient time, to satisfy energy balance with a high intake of cell contents, rather than through fermentation products. Janis (1976) suggested that equids use this strategy. As small herbivores require faster throughputs of digesta than do large ones such as horses, this strategy becomes less appealing with diminishing body mass.

4) *The animal may select a more concentrated diet.* A small animal may take this approach when there is an unlimited supply of quality forage containing soluble nutrients.

2.2 Gut structure: how herbivores obtain nourishment

All animals, from single-cell organisms to primates, have similar biochemical characteristics and nutritional requirements (Moir 1968). Because no animals are autotrophic, the nutrient source represents the prime variable separating different organisms. To obtain nourishment in their vast range of nutritional niches, animals have adapted profoundly both in their digestive systems and in their methods of gathering food. Thus, many harvest and select with the lips, the tongue, the dental apparatus, the hard palate and, in some animals, with the forepaws. Once lubricated with saliva, the food is processed to a varying degree by the masticatory apparatus. The resulting bolus passes via the oesophagus to the stomach. According to the functional anatomy of the stomach, quite different processes may take place. All these processes concern the degradation of ingesta, the absorption of nutrients and the regulation of digesta passage. Food passes from the stomach to the small intestine, where enzymatic digestion and absorption occur, then to the large intestine where structural carbohydrates may be further digested and where absorption occurs, particularly of water and electrolytes.

Herbivores that rely on fermentation of plant polysaccharides have evolved digestive systems to counter the physical and chemical defences evolved by higher plants. Expanded foreguts and hindguts are examples.

The complexity of the fermentation ecosystem indicates the ecological advantages conferred upon the host by microbial synergism (Moir 1968). Apart from making available an otherwise unobtainable energy source, microbial metabolism has other far-reaching benefits. These include an exchange of dietary amino acids, for those of microbial origin, and microbial detoxification of plant-inhibitory substances. Regardless of digestive strategy, efficient fermentation has reasonably static requirements. These are: comminution of plant food by teeth that grind and puncture; slow passage of digesta through an anaerobic fermentation chamber set apart from the acid-secreting region; buffering capacity to ensure a near-neutral pH; a stable osmotic pressure; temperature regulation; supply of substrate at an adequate rate; and removal of end-products. The fact that similar developments occur in entirely different phyletic lines — for example, Ruminantia, Tylopoda, Macropodoidea — supports the notion of Gould and Lewontin (1979) that evolutionary constraints are the major determinants of genotype.

Different mammalian taxa have developed their own subtle techniques for improving their fermentation systems. Among these, in the physical processes, are ways to retain digesta and separate particles; the chemical processes include absorption and regulation of osmotic potential. The following sections discuss the means by which

various mammalian herbivores achieve these processes and hence accommodate a microbial fermentation in their gut. The special problems faced by small herbivores, such as potoroines, are central to the discussion. Also, because the forestomach appeared to be relatively less important, and the hindgut probably more important, in fermentative digestion in potoroine marsupials than in macropodids (Hume and Carlisle 1985; Frappell and Rose 1986), PGF and HGF strategies are compared.

2.2.1 Mastication

Both PGF and HGF herbivores rely on the mechanical disruption of ingested plant materials as a necessary first step in the sequence of processes which furnish their metabolic needs. Many PGF receive a significant proportion of their requirements through microbial fermentation, the rate of which depends partially on the ingesta surface area to volume ratio (McLeod and Minson 1969) and on the availability of microbial attachment sites (Latham *et al.* 1978). Both are provided through mastication, which reduces particle size and damages plant-cell walls, a process repeated many times in ruminating PGF.

The mechanical disruption of plant-cell walls is critical in HGF. In the absence of cellulases, it represents the major process for releasing cell contents proximal to the intestinal absorption sites. Rate of passage through the gut is a function of particle size and therefore depends also on mastication. Small particles are selectively removed from the foregut of many PGF, including ruminants (Sutherland 1988), and selectively retained for fermentation in the caecum of many HGF (Björnhag 1987). Lanyon and Sanson (1986) showed that the stomach digesta of *Phascolarctos cinereus*, koalas, with very worn teeth include a higher proportion of large particles. Gipps and Sanson (1984) reported that *Pseudocheirus peregrinus*, common ringtail possums, with worn teeth had lower dry-matter digestibilities. Furthermore, in the same species, Pahl (1985) showed that tooth wear shortens life and hence affects population parameters.

Because the masticatory apparatus responds evolutionarily to the mechanical properties of foods (Kay and Hylander 1978), it is a useful tool for understanding dietary adaptations (Lucas and Luke 1984). The most comprehensive studies of the functional morphology of teeth have been conducted with primates (Kay 1978; Rosenberger and Kinzey 1976; Maier 1984). Their research showed that species eating different proportions of fruit, leaves and insects have different molar structure. For their body size, frugivores have small teeth with poorly-developed shearing, crushing and grinding features on their molars. Leaf-eating species tend to have larger teeth with

well-developed mechanical features. Similar functional differences have been recognized in macropodoids (Sanson 1989).

Unlike damage in most other animal tissues, that in teeth is permanent. Herbivores, particularly grazers, face considerable problems imposed by excessive tooth wear. Maintenance of efficiency depends on how teeth wear down, which in turn depends on the morphology of teeth, the manner in which they occlude and the physical nature of the diet. Some marsupials — phalangerids, potoroids and various macropodids, for example — which presumably eat less-abrasive diets — do not replace worn teeth. Other herbivores, such as ruminants, manatees and some macropodids, have overcome tooth wear by developing continuously-growing molars, or by molar progression.

The compensatory strategies open to an animal with worn teeth are discussed by McArthur and Sanson (1988). The animal may: 1) chew more; 2) tolerate less processing; or 3) select food that requires less processing. All three strategies may disadvantage the animal and reduce its fitness, emphasizing the importance of dentition in the ecology of the animal.

2.2.2 Salivary glands

In PGF, the relatively steady foregut pH of about 6.5 is largely due to the rapid absorption of short-chain fatty-acids (SCFA). However, the copious secretion of saliva buffered to pH 7-8 is important also in maintaining the foregut pH (Kay 1966). The large size of salivary glands and their voluminous secretions — contributing 70-90% of foregut water — reflect their physiological importance as regulators of the water-to-dry-matter ratio of the foregut (Kay 1966). Indeed, Kay (1966) and Carr (1984) regarded the provision of an appropriate liquid environment for foregut function as the primary role of the parotid glands. By drawing on its own body fluids for this water and for salts also, the foregut-fermenting herbivore has circumvented the seasonal deficiencies that may be a feature of its natural environment.

The rumino-reticulum itself is not a secretory organ although similar organs in other foregut fermenters — for example, in Tylopoda — have extensive glandular epithelium capable of producing an alkaline secretion (Eckerlin and Stevens 1973). This led Waring *et al.* (1966), Kuhn (1964) and Hoppe *et al.* (1974) to propose that saliva might be less important in macropodids, colobid monkeys and *Camelus spp*, respectively. Kuhn went even further, and suggested that the absence of rumination in colobids reduces the need for saliva. The same could be said for macropodids. This theory is supported by ruminant research. Kay (1960) showed that the development of parotid glands is concomitant with the initial consumption of fibrous food and the onset

of rumination; cattle fed concentrate diets produced less saliva presumably because they ruminate less (Bailey and Balch 1961).

An animal's diet may directly affect its saliva production. Unfortunately, there are few functional studies of salivary glands and most comparisons of salivary function can be based only on gland size. This is a reasonable approach because parotid gland size and flow rate are correlated, and parotid mass generally increases with the digestibility of the diet (Kay 1987). However, this approach ignores the differences in saliva composition recognized by Hofmann (1989). This aside, in free-living ruminants, the total salivary-gland mass as a percentage of body mass (on average of all species investigated) is 0.36 in CS, 0.26 in IM and 0.18 in GR (Hofmann 1989). Domesticated ruminants, which are predominantly grazers — for example, sheep and cattle, — have relatively small parotid glands (Kay *et al.* 1980). Glands of a similar size, structure and function are found also in Tylopoda (Hoppe *et al.* 1974). As expected, from their highly digestible diets, parotid glands are relatively large in folivorous primates, including colobid monkeys (Hill 1952), *Alouatta spp* (Bauchop and Martucci 1968) and spider monkeys (Bauchop 1977). The large grazing macropodids, too, have large salivary glands (MacKenzie and Owen 1919; Forbes and Tribe 1969). The parotid glands of *Macropus* rufus (red) and *M. giganteus* and *M. fuliginosus* (grey kangaroos) are histologically similar to those of sheep (Tribe and Peel 1963), but are twice the size — 1.07 versus 0.63 g per gland per kg body mass for kangaroos and sheep respectively (Porter 1981 cited by Beale 1984). The composition of saliva is essentially the same in sheep, cattle and *M. rufus*. Furthermore, the study by Beale (1984) showed that the maximum flows from the parotid glands of sheep and kangaroos were very similar on a secretion rate/gland mass basis. These findings are contrary to those expected, because the grazing macropodids studied often eat a poor-quality diet, they have a lower metabolic rate than do eutherians and a lower buffering capacity in their foreguts (Waring *et al.* 1966). Presumably, the large macropodid parotid glands are related to aspects of foregut physiology other than buffering. One possibility is that the large secretion of saliva is necessary to regulate the water content of the digesta in the tubiform forestomach.

The preceding discussion makes it clear that many factors determine salivary-gland morphology and function. Saliva is not just a buffer. Other roles include the regulation of gut water, a defence against plant secondary compounds, and possibly in conjunction with the gastric sulcus, a means by which soluble nutrients can bypass the fermentation region (Hofmann 1989).

2.2.3 Gut capacity

The large storage capacity of herbivores, a feature commensurate with their need to retain digesta, is the prominent gastrointestinal structure separating herbivores from carnivores and omnivores. As cited earlier (Section 2.1), Parra (1973 cited by Parra 1978) and Demment (1982) have each analysed the available data on gut size and the volume of fermentation contents in herbivores.

Gut capacity data show considerable variation both within species and herbivore taxa. This is largely explained by inherent weaknesses in the methods used to obtain the data. For example, the original technique of filling the empty gut with water to a standard pressure relies on knowing normal gut distension. This, and other weaknesses of the method, were discussed by Warner and Flatt (1965) and Demment (1982). The alternative — measuring gut contents directly — is flawed by the need to assume a certain level of gut fill (Demment 1982). This is difficult because, for example, the concentrate-selecting ruminants maintain a lower level of gut fill than do grazers (Hofmann 1989).

Despite the variance, the linear relationship between fermentation contents and body mass is still significant, and does not indicate any major difference in mass of fermentation contents between pre- and post-gastric fermenters (Langer 1988). The mass of fermentation contents is typically about 12-15% of body mass.

2.2.4 Gastric sulcus

A gastric sulcus resembling that which is so well documented in domestic ruminants is found with varying degrees of complexity in other PGF. For example, it is well developed in colobids and edentates. It is less developed in the Tylopoda (Moir 1968), which is surprising, considering their phyletic affinity with the Ruminantia.

When present, the sulcus in macropodids runs along the lesser curvature, from the oesophagus to the distal tubiform stomach. The sulcus is well developed in the genera *Macropus* (except adult *M. giganteus*), *Onychogalea, Lagorchestes, Dendrolagus* and *Wallabia*, but is ill-defined in *Petrogale*. It is absent in *Thylogale*. Presumably, the function of the sulcus is similar in all PGF: to prevent the fermentation of milk (Black 1970). The nutritional significance is even greater if the speculations of Black and Sharkey (1970), Langer *et al.* (1980), Dellow (1982), and Hofmann (1989) prove correct — that, in adults, the sulcus facilitates fluid movement past the site of microbial fermentation. Thus, soluble nutrients may escape fermentation and be digested enzymatically. Hume and Warner (1980) suggested that the development of a sulcus in the tubiform forestomach of macropodoids is a recent phenomenon, because it is

generally present in grazers but absent from the phylogenetically older species — potoroine marsupials and some browsing species. This is somewhat contradictory, because a sulcus might be most beneficial in species adapted to high-quality diets. Often these are small animals.

2.2.5 Epithelia

In the PGF multilocular stomach, the fermentation chamber is typically lined by a non-glandular, non-mucus producing, keratinized, squamous-type epithelium. Among the exceptions are the Tylopoda (Moir 1968), colobids, bradypodids and hyraxes (Bauchop 1977) and macropodids (Langer *et al.* 1980), which have a variable lining of cardiac epithelia, capable of producing a mucous fluid moderately rich in bicarbonate (Eckerlin and Stevens 1973). This, according to Moir (1968), is an evolutionary legacy resulting from the gradual replacement of cardiac epithelia by squamous epithelia, in response to the mechanical stimulation of bulky roughage. Stratified squamous epithelium is not restricted to species with pre-gastric fermentation. The notion implies a relationship between diet quality and epithelial type. This is supported by the presence of stratified epithelia and cardiac glands over some 35% of the unilocular stomach in perissodactyls (Moir 1968). Assuming that an animal's diet is partly determined by its body size, this translates to a relationship between body size and epithelial morphology. Hume and Warner (1980) concluded, however, that more information on food preference and gastric histology of a wide range of species is necessary to confirm this speculation.

In some PGF, notably the Ruminantia and Hippopotamidae, the stomach surface area is increased by papillation, a feature somewhat related to diet. Concentrate selectors have more extensive papillation than roughage eaters (Hofmann 1973; Langer 1974). Furthermore, the situation is dynamic. A decline in diet quality causes a reduction in papillary surface area (Hofmann 1989). Presumably, the sudden release of SCFA from the rapid fermentation of a concentrated diet has a stimulatory effect upon mucosal mitosis (Sakata and Engelhardt 1983). The absence of papillae in most other PGF stomachs remains a mystery that Moir (1968) associates with the frequency and intensity of stomach contractions. For example, the Tylopoda, whose foregut resembles those of many ruminants, lack papillae. But Vallenas (1965) in studies of *Lama guanicoe pacos*, alpaca, observed 3-5 times as many stomach contractions as in sheep.

The lack of papillae substantially reduces the absorptive surface area of the stomach. If the absorption of SCFA from non-papillated stomachs proceeds at a similar rate to that from papillated stomachs, there must be absorption mechanisms independent of surface area. Moir (1968) and Rübsamen and Engelhardt (1978) suggested that

cardiac glands might augment absorption in the Tylopoda. The same role could be allocated to the cardiac glands in potoroine marsupials and various macropodids. However, Gemmell and Engelhardt (1977) asserted that we have insufficient knowledge to relate epithelial structure to absorptive function in a general way. Therefore, in the arrangement of forestomach epithelia, functional differences between macropodid species remain unexplained.

2.2.6 Separation of digesta phases

Microbial synergisms in animals are characterised by a voluminous fermentation vat. In PGF mammals, this lies either "in-line" between the cardia and pylorus (Hippopotamidae, Macropodidae, Tayassuidae) or is offset (Bradypodidae, Colobidae, Pecora, Potoroinae, Tragulina, Tylopoda). Presumably the latter allows better retention of a portion of the digesta (Hungate 1976). The usual explanation is that an offset gastric region facilitates increased microbial intervention. However, this is probably an oversimplification. Offset gastric regions are not only found in PGF that eat low-quality food (Pecora and Tylopoda) but also in those selecting a higher-quality diet (Potoroinae, Colobidae, Tragulina, Bradypodidae). Presumably, the latter diets need less microbial involvement (Langer 1988).

In harmony with a multilocular stomach, most foregut fermenters use mechanisms for separating coarse and fine particles. This is indicated by the oral-aboral particle size gradients found in some ruminants (Trudell-Moore and White 1983; Sutherland 1988), Tylopoda (Langer 1973 cited by Langer 1984) and peccaries (Langer 1979). Studies of macropodids suggest a similar particle separation. Dellow (1982) showed that fluid and presumably fine particles are squeezed through the coarse particles in the tubiform forestomach of *Macropus giganteus*, *M. eugenii*, tammar wallaby, and *Thylogale thetis*, red-necked pademelon.

In studies with ruminants, Grau (1955) and Schels (1956, cited by Langer 1984) advanced hypotheses to explain retention and separation. They emphasized that the size and density of particles and the physical form of the reticular surface are major determinants. More recently, Langer (1984) stated that the reticulo-omasal orifice contributes importantly to the form of digesta entering the omasum. However, the endoscopic studies of McBride *et al.* (1984) tend to refute Langer's view, because particulate separation occurs proximal to this point. Only recently has significant progress been made to explain retention and separation in the reticulo-rumen of domestic stock. Sutherland's (1988) research is worthy of particular note. In a series of experiments with lucerne-fed sheep, he recognized two crucial separation mechanisms and mentioned the possibility of others. He identified selective retention of certain

particles within the raft of the rumen. This retention, he stated, results from the mixing cycles of the rumino-reticulum, the unmixing of particles themselves and interactions between particles. All these factors depend on particle size, shape and density. Sutherland noted also a reticular-settling mechanism through which the sediment becomes remarkably free of large particles. Reid (1984) described how the sediment is then conveyed to the reticulo-omasal orifice. Other processes that may be important include filtration by unguliform papillae and omasal separation (Ehrlein 1980; Weston and Cantle 1984).

Particle separation depends on a complex series of interactions (Sutherland 1988). Two factors are essential: nutrients in the foregut must be kept in balance, and the passage of solids demands an outflow of liquid. Only recently have these factors been identified in domestic ruminants. Thus, it is not surprising that only superficial explanations exist for retention and separation in non-domestic PGF. For example, Langer (1974) proposed that stomach-folds aid particle separation in the Hippopotamidae, and the distinct stomach musculature may perform a similar function in the Colobidae (Langer 1984) and Macropodinae (Dellow 1982). Langer (1984) pointed to the small apertures between stomach compartments in Tylopoda, Tayassuidae, Bradypodidae, Colobidae and Potoroinae. Also, he suggested that the eversion of glandular stomach compartments in camelids may affect the transit of digesta. The previously-mentioned relationship between saliva production, the gastric sulcus and digesta transit in concentrate-selecting ruminants (Hofmann 1989), adds another dimension to the study of digesta retention.

2.2.7 The lower tract

Descriptions of the alimentary tracts of herbivores concentrate on the fermentation zone, and generally assume that the small intestine has a similar form and function as in unilocular species. To some extent, this is true. Stevens (1980) remarked that "gross structural characteristics of the mammalian small intestine show little species variation". Nevertheless, there seems tremendous scope for altering mucosal surface area by changing intestinal length, diameter, villus type, mucosal folding and thickness of muscle layers in response to diet. In studies of macropodids Osawa (1987) showed that the animal's nutritional pattern influenced the size and morphology of the small intestinal villi and the length of the caecum and large intestine. This view was supported by Schieck and Millar (1985), who reported that the mass and length of the small intestine did not reflect the diets of small mammals. Instead, the mass and length of the caecum and colon was the best dietary indicator. A similar conclusion was drawn by Hofmann (1989) with respect to ruminants. Hindgut fermentation is still very

important in CS and IM grades. This is reflected in a number of measurements. First, the total length of the intestine is 12-15 times body length in CS but 25-30 times body length in GR. However, in CS the hindgut is relatively larger, both in length and in volume relative to that of the rumino-reticulum, than in GR. Furthermore, the hindgut is a particularly dynamic organ in CS. When forage quality declines, the volume of the caecum and proximal colon may increase up to five-fold (Hofmann 1989).

2.3 The consequences of microbial metabolism

The benefits to the host of microbial metabolism arise through catabolism of carbohydrates to SCFA, proteolysis and deamination, vitamin synthesis and lipid hydrolysis and hydrogenation. By combining selective retention and rumination, ruminants — particularly the GR species — maximise exposure of digesta to microbial processes. Coincidentally, ruminants provide several indices by which to evaluate the extent of microbial modification of ingesta.

PGF mammals receive little glucose directly, because the microbial metabolism of hexoses is almost complete. Consequently, hepatic enzymes — glucokinase and the less-specific hexokinases, which convert glucose to glucose-6-phosphate directly — are usually of low activity. Instead, liver function is focused on gluconeogenesis (Barker 1961; Moir 1968; Van Soest 1982).

Blood glucose concentrations in suckling ruminants are similar to those in unilocular animals (<100 $mg.dl^{-1}$) but, with the onset of fermentation, these levels fall to about 70 $mg.dl^{-1}$. This suggests some relationship with foregut function. Ergo, blood-glucose levels, which are low in some other PGF — for example, macropodids (Waring *et al.* 1966) — have been used as an index of foregut microbial function. However, levels are high in Tylopoda (Moir 1968) and intermediate in colobid monkeys (Bauchop 1977), indicating a complex situation. Again, there is litle known about variation in blood-glucose levels with body mass in different mammalian groups.

It should be emphasized that blood-glucose requirements, are similar in all taxa studied (Van Soest 1982). There is evidence that Ruminantia, wherever possible, conserve glucose by converting SCFA and other metabolites. In adult ruminants this is demonstrated by negligible levels of cytosol enzymes. These enzymes — specifically ATP-citrate-lyase and NADP-malate-dehydrogenase — convert glucose to fatty acids. Both enzymes are more abundant in suckling animals (Van Soest 1982).

Foregut fermenters seem tolerant also of the hypoglycaemic state induced by intravenous administration of insulin, while hindgut fermenters become comatose. However, there are insufficient data to relate this phenomenon to foregut fermentation

alone, and studies with other PGF taxa, particularly Tylopoda, and of taxa with species covering a wide size range would be useful.

The presence of trans-acids in the depot fats of *M. rufus*, *M. giganteus*, *Camelus dromedarius* and *Hippopotamus amphibius* support the notion that microbial modification of dietary constituents is extensive in many PGF. The depot fats of these non-ruminant PGF — for example, *Setonix brachyurus*, quokka, and *M. eugenii* (Hartman *et al.* 1955) contain also considerable amounts of di- and poly-unsaturated fatty acids, implying that exposure to microbial processes is less extensive than in domestic ruminants. Calaby (1958) proposed that this is due to faster digesta passage in macropodids. However, the same results might come from less extensive trituration of food particles, as evidenced by a greater proportion of large particles in the faeces of *Macropus robustus robustus*, eastern wallaroo, and *M. r. erubescens*, euro, compared with sheep (Freudenberger *et al.* 1989).

A consequence of intensive microbial activity in the forestomach is the mass of microbial nucleotides arriving at the small intestine. PGF have seemingly responded to this by evolving high pancreatic ribonuclease activity. A relationship between gut structure and ribonuclease activity is apparent in the data of Barnard (1969). He found that PGF, such as macropodids and domestic ruminants, have higher concentrations of RNA than do HGF that also use some forestomach fermentation. The latter, in turn, have higher concentrations than are found in strict HGF. Again, there are exceptions. For example, *H. amphibius* has very low levels of pancreatic ribonuclease (Moir 1968). To draw any conclusions requires studies of other PGF, notably Tylopoda, colobines and possibly some of the concentrate-selecting ruminants.

Hume (1982) cited work by A.C. Wilson *et al.* (pers. comm.), who found high levels of lysozyme in the ruminant abomasum. Lysozyme is an enzyme responsible for the cleavage of bacterial cell-wall polysaccharides. The action presumably allows utilisation of cell contents. Not surprisingly, activity is low in the stomachs of non-ruminant eutherians, but Hume did not state whether these included non-ruminant PGF.

2.4 Hindgut versus foregut fermentation strategies

The preceding sections raise the question of why small herbivores have evolved both pre- and post-gastric fermentation strategies, causing debate about which is the "superior" or more highly evolved system. One argument is that foregut fermentation is superior to hindgut fermentation because it produces higher fibre digestibility (Moir 1968; Schneider and Flatt 1975). This has been confirmed in several comparative studies — for example, (Ingalls *et al.* 1966; Slade and Hintz 1969; Keys *et al.* 1969;

Hintz *et al.* 1973) — between ruminants and hindgut fermenters. However, much of this difference in digestibility is explained by differences in body size and the associated variables of passage rate and diet. So, Janis (1976) argued that there are small differences only in digestibility between foregut and hindgut fermenters of similar size. This aside, *Microtus pennsylvanicus*, meadow voles, (small murid rodents with highly developed caeca) were shown by Keys and Van Soest (1970) to digest between 20 and 40% of the cell walls in alfalfa, bromegrass and orchardgrass. By comparison, typical values for sheep and horses are 45-70% and 35-50% respectively; the much larger grazing, hindgut fermenting *Hydrochoerus hydrochaeris*, capybara, has the digestive potential of sheep (Parra 1978).

Other workers, for example Bauchop (1977) took the view that the dominance expressed by the Artiodactyla within eutherian herbivores, and the Macropodidae within the metatherian herbivores, demonstrates the advantages conferred upon foregut fermenters.

The preceding comparisons serve only to promote an already futile debate, in which animals are regarded as entities in isolation from their environments, or assessments are based on species spanning a narrow degree of herbivory. For example, while ruminants represent a particular gastrointestinal modification, there is within this, and all herbivore groups, a wide range of adaptations relative to feeding strategies. There is little doubt that fermentation regions have evolved through host-microbe associations and the ecological advantages they confer on the host (Moir 1968). Instead of arguing that one digestive system is superior to another, we should be considering the merits conferred by each system in specific environments.

A more balanced view of digestive strategy was Langer's (1986) examination of PGF and HGF in tropical forests. His study is certainly open to criticism: 1) it is confined to families whose mean body weight exceeds 3 kg; 2) the term "tropical forest" is obscure; 3) the study includes some animals — such as *A. rufescens* — which do not live in tropical regions. For all that, Langer's study exposes trends that refute the presumed dominance of foregut fermentation. For instance, there are as many foregut- and hindgut-fermenting herbivores in the tropical forests of the Australian, Ethiopian and Oriental zoogeographical regions; hindgut fermentation dominates the Neotropical areas. Secondly, animals with hindgut fermentation tend to inhabit the canopy, and select a higher-quality diet than the foregut-fermenting dominators of the forest floor.

How do Langer's conclusions compare with our understanding of PGF and HG digestive strategies? A comparison is difficult because there are several architectures of each digestive strategy. So let us digress by first considering the fundamental questions of PGF versus HG digestion, and the variations about each strategy, before examining ecological trends within each group.

There are many similarities in the actual digestive processes, both biological and chemical, between PGF and HGF (Parra 1978; Stevens 1980). For example, gut capacity, microbial numbers and the microbial environment are similar regardless of digestive strategy. There are also important differences. The substrate presented to the hindgut microbes is usually richer in cell-wall material than that entering the foregut. This supports a bacterial population whose products contain high molar proportions of acetate. Also, protozoa, common inhabitants of the foregut, are rare in the hindgut.

In both foregut and hindgut fermenters, nitrogen and hence water are conserved by recycling urea from the blood to the fermentation chambers, where it is available for microbial metabolism. Pregastric fermentation, in which the major proteolytic sites are distal to the fermentation zone, provides greater opportunity for utilisation of the protein synthesized by microbial metabolism. Hence, the animal has a non-dietary source of quality protein. Among others, Kinnear *et al.* (1979) regarded this as a major role of PGF in some species. Of course, nutrient costs are associated with microbial protein production. The process requires degradation of ingested protein, followed by synthesis of microbial protein, a futile cycle when diets contain protein of high biological value.

Several workers (Slade and Robinson 1970; Ulyatt *et al.* 1975) have shown that some amino-acid absorption occurs in the hindgut, but the nutritional significance of this uptake is probably small (Rérat 1978), because free amino acids are scarce in fermentation chambers, and absorption occurs by diffusion.

As with microbial protein, vitamins contained in microbial cells are probably more accessible to the PGF (Moir 1968), although Sorrel *et al.* (1971) measured uptake of vitamin B12 from the HG.

With respect to carbohydrate metabolism, the relative advantages of PGF and HGF depend on the balance between structural and non-structural dietary carbohydrates and on body size. Animals metabolise SCFA with less efficiency than their precursors, sugars and starches (Blaxter 1962). Also, in producing heat and fermentation gases, they lose energy-yielding nutrients (Blaxter 1962; Preston and Leng 1987).

In summary, HGF benefits herbivores consuming diets rich in soluble carbohydrates and high-quality protein — that is, those in which plant-cell contents predominate. PGF is probably advantageous in herbivores consuming diets based on structural carbohydrates. These are diets where nutrient release requires microbial intervention, where vitamins are limited, where protein quality and quantity is low and where the diets contain toxic substances. This generally agrees with Langer's (1986) findings. By following the theory developed in Section 2.1 we can expect those herbivores with PGF to be generally larger than those with HGF, another presumption supported by Langer's (1986) study.

The situation is more complex, however, because PGF- and HGF-type herbivores have developed adaptations to cope with the impediments in each basic system. Hofmann (1973, 1989) describes several variations of ruminant PGF that he suggests are diet-related. Digestion is maximised in the Ruminantia, at least in domestic species, because fine particles are selectively passed from the reticulum to the omasum and then to the abomasum. When food quality declines and soluble substrates become limiting, the microbial populations may be unable to hydrolyse the cell-wall material. In this situation, restricted digesta passage through the reticulo-omasal orifice inhibits the animal from eating. This situation is somewhat averted by rumination, an adaptation for reducing digesta particle size that is unique to the Ruminantia and Tylopoda. Herbivores, in which particle size does not limit digesta passage, may cope with abundant poor-quality food by maintaining intake at the expense of digestibility. This was shown in *M. r. robustus* by Hollis (1983).

Fast passage and reduced digestibility is the strategy used by the colonic fermenters, one of the specialized HGF groups recognized by Hume and Warner (1980). Among this group are the Equidae (Björnhag *et al.* 1984), *Ailuropoda melanoleuca*, giant panda, the Proboscidae and possibly some metatherians (for example, the Vombatidae). All have a characteristic integration of colonic and caecal function. Janis (1976) advanced a hypothesis explaining the evolutionary significance of HGF in equids. She suggested that horses deal with poor-quality diets by increasing digesta passage. This allows a concomitant rise in food intake. Thus, nutrient requirements are met through maintaining ingestion of soluble nutrients at the expense of cell-wall digestion. Unfortunately, little experimental work has been done to test this theory. However, one recent paper provides some useful data (Izraely *et al.* 1989; Table 2.1). These researchers fed *Equus asinis africanus*, African donkey, and goats either alfalfa hay (47% NDF; 22% crude protein) or wheat straw (77% NDF; 2.8% crude protein).

When alfalfa was fed, the intake of NDF by *E. a. africanus* was double that of goats. Izraely *et al.* (1989) do not provide food intake data, but it is clear that both species ate less when fed wheat straw. As expected, the drop in intake by *E. a. africanus* was much less than that observed in goats. *Equus asinis africanus* achieved this by maintaining a high rate of passage, which goats were unable to do. The expected drop in NDF digestibility in *E. a. africanus* fed wheat straw did not occur. The most plausible explanation for this is that, in *E. a. africanus*, NDF intake and mean retention time were similar on both diets.

Table 2.1 Neutral detergent fibre (NDF) intake and digestibility and the mean retention time of digesta in donkeys and goats*

	Donkeys		Goats	
	alfalfa hay	**wheat straw**	**alfalfa hay**	**wheat straw**
NDF intake ($g.kg^{-0.75}.d^{-1}$)	40[a]	47[a]	23[b]	12[c]
NDF digestibility (%)	54[a]	51[a]	62[b]	59[b]
Mean retention time (h)	38[a]	36[a]	48[b]	70[c]

* Cell-wall constituents were determined as crude fibre in the goat experiments. Adapted from Izraely *et al.* (1989).

The study reported above makes it clear that the theory advanced by Janis (1976) requires stringent testing in comparative studies of a range of PGF and colonic fermenters. Such a study should include species covering a wide span of body size and feeding types.

A problem with digestive strategies that rely on fast digesta passage is the possibility that micro-organisms and other nitrogen-rich fine particles may be washed from the digestive tract. Björnhag *et al.* (1984) examined this dilemma by feeding a poor-quality straw to horses. They identified a separation mechanism at the junction of the proximal and distal colons which extracts fluid and fine particles, and then shunts them, by retrograde movements, towards the proximal colon. There is selective retention also of large particles in the proximal colon (Argenzio *et al.* 1974). The selective retention of all particles explains why there is a 2h difference only in mean retention time between ^{51}Cr-EDTA and ^{103}Ru-P in the horse (Orton *et al.* 1985). *Phascolarctos cinereus*, a species with a slow rate of passage compared to the horse, selectively retains only the fine particle-solute phase. This results in a much longer mean retention time for ^{51}Cr-EDTA than ^{103}Ru-P (Cork and Warner 1983).

It is interesting, although difficult, to compare colon fermenters with non-ruminant PGF herbivores with a colon-like foregut — for example, grazing macropodids. The PGF can maximise the metabolism of microbial products, in particular protein. By comparison, the HGF can use ingested plant solubles without incurring the costs of fermentation. The HGF benefit also through the pre-treatment of cell walls with acid pepsin (Parra 1978). Cell walls passing to the HG of a PGF have been exposed also to acid pepsin, but are presumably less digestible because they have already evaded digestion in the FG.

The high mass-specific nutrient requirements of small HGF herbivores demand fast processing of digesta. Does this mean that they cannot tolerate a poor-quality diet?

Apparently not. Several — for example, *Petauroides volans*, greater glider, and *Pseudocheirus peregrinus*, — eat *Eucalyptus spp* foliage. Others — for example, rabbits, have invaded arid regions where often only fibrous diets are available. Instead, the small HGF have anatomical and physiological adaptations centred about a large caecum. These provide selective retention of fluid and fine particles while voiding the larger, less digestible material relatively fast. Thus, high rates of passage and fermentation are maintained. However, important nutrients are still excreted in the faeces because of the limited digestion and absorption facilities distal to the fermentation site. The small HGF has overcome faecal losses by reingesting a proportion of the faecal material (caecotrophy). This group, which Hume and Warner (1980) labelled caecum fermenters, are the main subject of the reviews by Hörnicke and Björnhag (1980) and Björnhag (1987). They discuss several herbivore groups whose species possess this retention mechanism. Included are myomorph, caviomorph and hystricomorph rodents; lagomorphs; and metatherians such as *P. peregrinus*.

The retention mechanism has a basic design, but the complexity varies between species. Bacteria and other fine, nutrient-rich particles are trapped by mucus and transported, by retrograde motion, to the caecum. The separation activity continues throughout the active foraging period. During the resting period, the separation stops and caecotrophs are formed. These are transported through the colon and ingested directly from the anus.

Selective retention of nutrient-rich digesta, even in the absence of caecotrophy, still has some advantages for the animal. It may provide fermentation products — notably SCFA (Sperber 1968) — and decreases gut fill (Björnhag and Sperber 1977). It decreases also the loss of nitrogen in the faeces, and maintains a high concentration of microbes in the caecum, thus maximising digestive activity.

2.5 Conclusions: why herbivores represent "a bush and not a ladder"

There is little doubt that body size, through its influence on metabolic requirements, is often an important determinant of an animal's herbivory rating. Indeed, Langer (1988) found a positive correlation between body size and herbivory rating ($r = 0.265$, $n = 128$, $P<0.01$). Thus, small herbivore species often have a low herbivory rating (CS) and large species a high rating (GR). However, there are many exceptions: *Taurotragus oryx* (eland - 800 kg), *Giraffa camelopardalis* (giraffe - 750 kg), *Alces alces* (moose - 420 kg) and *Bison bonasus* (European bison - 900 kg) are all large CS or IM species; *Ourebia oribi* (oribi - 15 kg), *Antidorcas marsupialis* (springbok - 30 kg), *Gazella spekei* (Speke's gazelle - 15 kg), *Pseudocheirus peregrinus* (0.9 kg),

Petauroides volans (1.3 kg) and *Phascolarctos cinereus* (9 kg) all have high herbivory ratings. How do we explain these deviations from the classical body-size/herbivory-rating model? As pointed out in the current chapter, there is tremendous diversity of both form and function within the gastrointestinal tracts of herbivores. There is similar variation in other anatomic, metabolic and behavioural features of herbivores. This fact is well illustrated by the long neck of *Giraffa camelopardalis* and the bipedal stance taken by feeding *Litocranius walleri* (gerenuk), which allows both large herbivores to select concentrates; by the ability of many species to store nutrients, or to migrate when conditions become unfavourable (Hofmann 1989); by variation in total energy expenditure (Blaxter 1972), and by synergism between populations of different species (de Boer and Prins 1989). This diversity provides ways by which species can occupy niches contrary to those expected from consideration of body size alone.

Much of the current study is concerned with investigating the gastrointestinal and metabolic processes enhancing the survival chances of potoroine marsupials. The species, their ecology and the current state of knowledge of their nutrition, digestive physiology and metabolism are discussed in Chapter 3.

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CHAPTER THREE

The ecology and digestive physiology of potoroid marsupials

3.1 The evolution of potoroid marsupials

THE evolution and taxonomy of the Potoroidae has been reviewed recently by Flannery (1989). Section 3.1 is a brief overview of his findings.

In contrast to the macropodids, potoroids radiated early. Indeed, Miocene fossil deposits — dated at about 30 million years — are characterized by a diverse assemblage of potoroid species. It seems that three potoroid subfamilies — Propelopinae, Palaeopotoroinae and Hypsiprymnodontinae — differentiated early. All had a plesiomorphic molar, and probably a unilocular stomach. In the mid-Miocene, the hypsiprymnodon-like species gave rise to the generalist Potoroinae, and the more specialized browsing Bulungamayinae. Despite this Miocene radiation, the potoroid dominance was relatively shortlived. By the late Pliocene, potoroids were an insignificant part of the Australian fauna, eclipsed by a spectacular macropodid radiation possibly initiated by the spread of grasslands. Thus, only two potoroid subfamilies — Hypsiprymnodontinae and Potoroinae — containing 10 species, survived to modern times (Table 3.1); 15 taxa in three subfamilies — Propelopinae, Palaeopotoroinae and Bulungamayinae — became extinct. The latter subfamilies varied far more than the extant ones. For example, the Propelopinae weighed as much as 70 kg, and may have been partly carnivorous.

Of particular interest in Flannery's work is his taxonomy of recent potoroine species. In particular, he suggested close affiliation between *Caloprymnus* and *Aepyprymnus*.

3.2 Distribution and habitat

Changes caused by Europeans such as land clearing, the introduction of feral predators and competitors, and hunting for sport and government bounties — led to the demise of potoroine marsupials, described most eloquently by Finlayson (1958):

"In the modern wreck of this remarkable group of mammals, the plan of its unfolding into the vast territories, which it formerly occupied, is but dimly to be seen."

and Troughton (1943), referring to *P. tridactylus*:

> "It was once common in the swampy coastal brushes of Victoria and South Australia, but depredation by foxes have rendered the old nature-book term of 'Common Rat-Kangaroo' a mere mockery for all of its kind on the mainland."

Assuming that species evolve in response to the environment, the above excerpts make it clear that to acquire any appreciation of potoroid biology, particularly nutritional and physiological adaptations, we must first examine the distribution and habitat use of potoroines in the lands they occupied prior to European influence.

3.2.1 Distribution

The distributions of potoroids have been reviewed by Seebeck and Rose (1988) and Seebeck *et al.* (1989). The following section relies heavily on these works.

Prior to European influence, potoroids were widely distributed throughout Australia but mostly below latitude 20°S. Four species inhabited the arid and semi-arid regions; six were found in the wetter regions of the east and south-east (Fig 3.1). It seems that *H. moschatus*, *P. platyops*, *B. tropica* and *C. campestris* have always had restricted distributions; it is difficult to comment on *P. longipes* because it is a recent discovery; but the other five species were once widespread.

Since white colonisation, *P. platyops* and *C. campestris* have probably become extinct; *B. leseur* occupies remnants of its former range; *B. gaimardi*, though common in Tasmania, is extinct on the mainland. *P. tridactylus* remains relatively widespread and common within parts of its former range — especially Victoria — although it has disappeared from Western Australia and some Bass Strait islands and is becoming rare in New South Wales.

Aepyprymnus rufescens, also regarded as common, has been adversely affected by European settlement. The species was once abundant in Queensland and New South Wales and even extended into Victoria. Gould (1863) claimed it to be almost universally dispersed over New South Wales, both on the coastal lowlands and the interior side of the mountain ranges. Nevertheless, after white settlement their abundance was shortlived, for in the 1880s the Pasture Protection Boards of Tamworth and surrounding areas declared them agricultural pests and offered bounties on "Kangaroo-Rats" for over two decades. Thousands were killed (for example 78,938 recorded by Tamworth Pasture Protection Board in 1892). At Drake, in northern New South Wales, where *A. rufescens* is now considered common, an hour's spotlighting will reveal an average of no more than two or three. Can we really regard them as common?

Table 3.1 Modern species of Potoroidae and their present status. (After Seebeck et al. 1989)

Species	Common name	Present status
Sub-family Hypsiprymnodontinae		
Hypsiprymnodon moschatus	musky rat-kangaroo	uncommon
Sub-family Potoroinae		
Potorous platyops	broad-faced potoroo	extinct
P. tridactylus	long-nosed potoroo	common
P. longipes	long-footed potoroo	rare
Bettongia leseur	burrowing bettong	rare
B. penicillata	brush-tailed bettong	rare
B. tropica	Queensland bettong	unknown
B. gaimardi	Tasmanian bettong	common
Aepyprymnus rufescens	rufous bettong	common
Caloprymnus campestris	desert rat-kangaroo	extinct

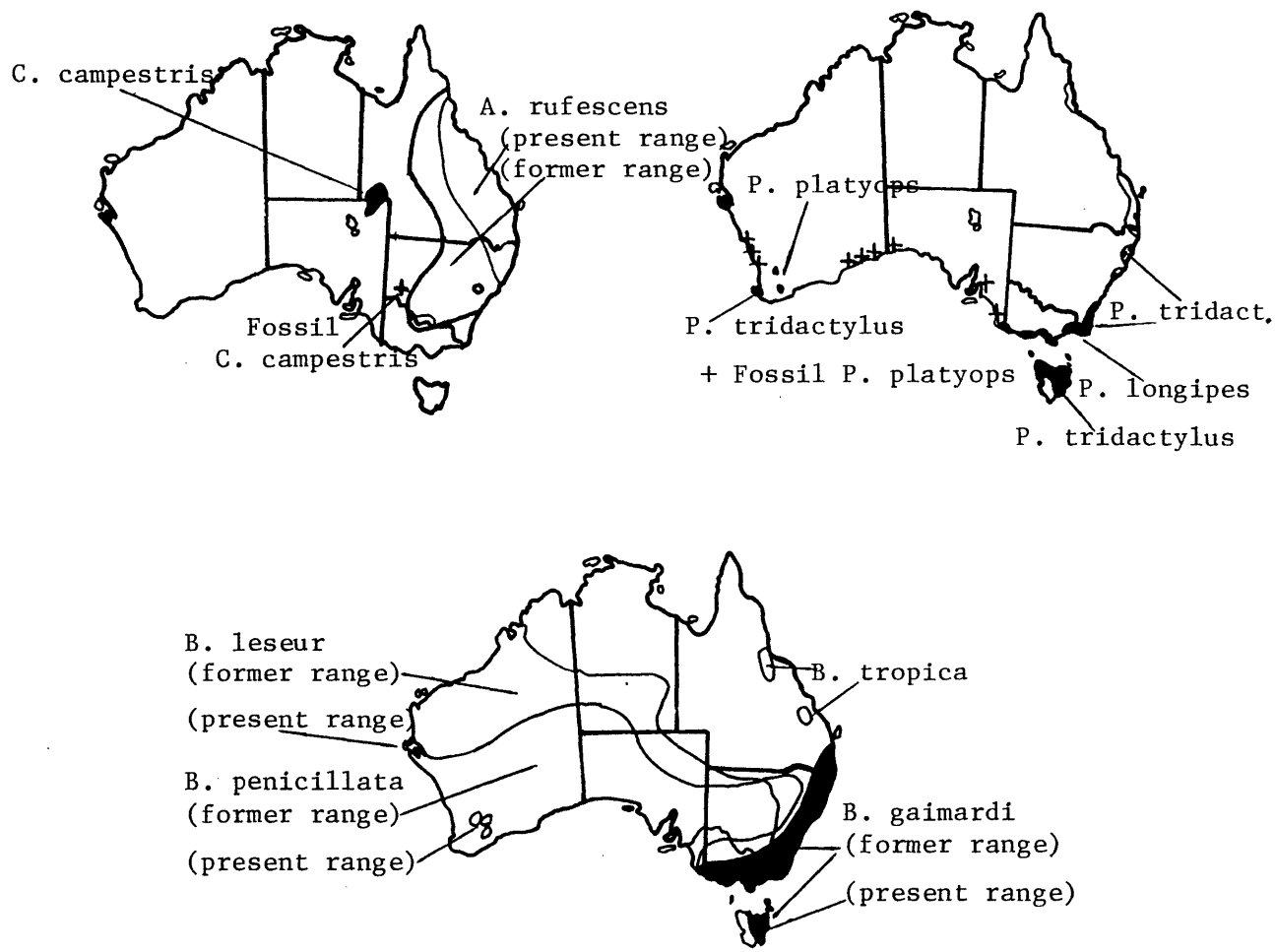

Fig 3.1 Distribution of modern potoroids. (a) Aepyprymnus rufescens and Caloprymnus campestris (b) Potorous spp and Hypsiprymnodon moschatus; (c) Bettongia spp. (After Seebeck et al. 1989)

3.2.2 Habitat

Although potoroine marsupials were formerly distributed throughout areas encompassing great ecological diversity, all these regions had a ground-cover of shrubs or tussock grasses. This Section covering potoroine habitats attempts to describe the situation at the time of European influence. Again the information relies heavily on the review of Seebeck *et al.* (1989).

Potorous

Potorous tridactylus

In southern Australia, the habitats of the small, quadripedal *P. tridactylus* range from woodland with dry-heath understorey, through coastal scrub and mixed open forest, to wet heath. Troughton (1943) remarked on the preference for low damp locations with dense herbage. This view is supported by the more recent studies of Heinsohn (1968) and Kitchener (1967) in Tasmania; Emison *et al.* (1975), Menkhorst *et al.* (1982) and Seebeck (1981) in Victoria; and Johnston (1973) and Newsome *et al.* (1974) in southern New South Wales. Little is known about the habitat requirements of *P. tridactylus* in northern New South Wales, although Schlager (1981) searched the records and identified two distinct habitats. The first is a coastal habitat relying on the cover provided by the thick heath. The other occurs on the eastern escarpment where *P. tridactylus* makes use of closed forest and adjoining disturbed areas.

Potorous longipes

Potorous longipes is a recent discovery (Seebeck and Johnson 1980), with few fossil or sub-fossil records, and thus no comment can be directed to former habitat use. The species now inhabitats several areas from coastal to hinterland sites which vary markedly in terms of climate, topography and flora. Nevertheless, a recurring feature in the studies of Chesterfield *et al.* (1983), Horrocks *et al.* (1984), Scotts and Seebeck (1989) and Seebeck and Johnston (1980) is the presence of a dense under-storey. It is possible that the distributions and habitats of *P. longipes* and *P. tridactylus* overlap in north-eastern Victoria and/or south-eastern NSW.

Potorous platyops

The broad-faced potoroo passed into history before much was known of its ecology. It seems never to have occurred in forested areas Kitchener (1983) and the only information about its habitat is a statement that Kitchener attributes to John Gilbert:

" ... all I could glean of its habits was that it was killed in a thicket surrounding one of the salt lagoons in the interior".

Muir *et al.* (1979) cited by Seebeck *et al.* (1989) suggest that these thickets may provide up to 70% canopy cover.

Bettongia

In contrast to *Potorous* spp, the *Bettongia* seek more open habitats.

Bettongia leseur

Describing the habitat of *B. leseur* in central Australia Finlayson (1958) stated:

"With the exception of rocky hills and ranges and dense thickets, it colonises most types of country; grassy and herbaceous loam flats within the major ranges, open mulga and ironwood parks skirting the ranges and penetrates deep into true sandridge areas as well."

Similarly Dahl (1926) observed *B. leseur* inhabiting wooded regions with undulating sandhills in Western Australia's northwest. He described one area:

"clad with spinifex ... a few green bushes ... and two or three stunted trees."

In contrast, near Adelaide, *B. leseur* was common on the coastal plain where it chose open forest or woodland, both with a herbaceous understorey.

Bettongia leseur is now confined to a few Western Australian islands, where it inhabits the hummock grasslands (Seebeck *et al.* 1989). The same authors suggest that habitat selection is influenced by its habit of constructing complex burrow systems, housing 20-50 individuals (Ward 1909). However, burrow location is flexible, for these have been reported in several materials ranging from sandhills (Dahl 1926), firm, loamy soil (Burbidge 1983), outcrops of friable rock (Finlayson 1958), limestone rises (Giles 1889), or under capping layers of stone (Ride and Tyndale-Biscoe 1962).

Bettongia penicillata

Finlayson (1958), remarking on distribution records of *B. penicillata* stated:

"The sites which furnish the above records present ecological contrasts of an extreme kind."

These included desert adjoining spinifex plains, coastal dunes and high plateaux. *Bettongia penicillata* seems less selective than *B. leseur* in habitat selection, and this may well reflect its nest-building behaviour. For example, *B. penicillata* occurred in the mallee and stringy-bark ranges of South Australia from which *B. leseur* was absent. Morton (1861) also mentioned the affinity of *B. penicillata* for mallee. Gould (1863) and Krefft (1866) remarked on the abundance of *B. penicillata* along the inland river systems of New South Wales and the Murray River, but offered no explanation for this distribution.

B. penicillata is now confined to the multi-layered open forests of south-western Western Australia, preferring areas with well-drained sandy soils and moderately thick cover.

Bettongia tropica

Little is known of the habits of *B. tropica* although Winter (pers. comm. to Seebeck 1988) described an area of upland grassy open forest and the adjacent rainforest on the western bounds of the north Queensland rainforest zone.

Bettongia gaimardi

Although *B. gaimardi* was once distributed along Australia's south-eastern seaboard, there are no habitat records. In Tasmania, Taylor (1986) has observed the species in grassy, subalpine woodland and snow-gum forest on infertile dolerite soils. *Bettongia gaimardi* were not found in forests supporting dense understoreys, a niche which seemingly belongs to the more quadripedal *Potorous spp.* Instead, the chosen habitat consists of a sparse shrub layer and ground cover of grasses and sedge — the basic materials for nest building.

Caloprymnus

Caloprymnus campestris

Caloprymnus campestris inhabited the most arid environment of any potoroine marsupial, an area described by Gould (1863):

"the stony and sandy plains ... partially clothed with scrub — are its native habitat."

This description was still applicable when Finlayson (1932) rediscovered the animal on the stony flats between sandhills adjoining Lake Eyre. Surprisingly, *Caloprymnus* made little attempt to avoid climatic extremes, choosing instead to construct a rather flimsy nest, often with an open aspect (Finlayson 1932).

Aepyprymnus

Aepyprymnus rufescens

Gould (1863) described *Aepyprymnus* as an inhabitant of:

"the stony, sterile ridges bordering the grassy flats"

In contrast, Finlayson (1931) wrote that they preferred the grassy lands free from lush growth, although he found them also in both open and forested country. Southgate (1980), Schlager (1981) and Dennis (1988 pers. comm.) all pointed to the preference shown by *A. rufescens* for open eucalypt woodland in which the shrub layer is thin and native grasses, particularly *Poa spp* and *Imperata spp.* are abundant. Johnson and Bradshaw (1977) mention its occurrence in hummock grasslands of Queensland.

In a more detailed study of nesting behaviour, Wallis *et al.* (1989; Appendix 5) found that, although *A. rufescens* prefer nesting in open eucalypt forest with some shrub layer, this did not preclude them nesting in open forest with a dense shrub layer, or areas devoid of canopy cover. Feeding occurs in both forested and cleared country (Wallis *et al.* 1989).

3.3 Ecology of potoroine marsupials

Potoroine marsupials, because of their small body size and selective feeding habits, usually lead a nocturnal, solitary existence, leaving their shelter at dusk or after dark and returning before dawn. Nevertheless, there are exceptions. The behaviour of *P. tridactylus*, for example, is debatable. Guiler (1958) claimed strict nocturnality for this species, whereas Kitchener (1967) reported activity commencing at dusk. In contrast, Seebeck (1981) cited his own research in which 36% of captures occurred during daylight. Other potoroines — for example, *B. penicillata* (Christensen and Leftwich 1980) and *B. gaimardi* (Taylor 1990 pers. comm.) — vacate their nests at dusk; *A. rufescens* (Schlager 1981; Wallis *et al.* 1989), *P. longipes* (Scotts and Seebeck 1989), *B. leseur* (Stodart 1966) and *C. campestris* (Finlayson 1932) are strongly nocturnal. Unfortunately, how potoroines divide their time between different activities — eating, sleeping, nest-building, and so on, is not known.

Apart from courtship and maternal behaviour, potoroines are usually solitary. The exceptions break the rule in interesting and diverse ways. Up to 50 *B. leseur*, for example, may live collectively in a warren but they do not group outside this shelter (Dahl 1926). *A. rufescens* shelter as individuals but sometimes feed in pairs or loose aggregations. Schlager (1981) reported that adult-adult pairs and subadult-adult pairs comprise 12% and 3% respectively of all observations. However, because it is difficult to determine the age and sex of wild potoroines, the significance of this pairing is obscure. This aside, the fact that male and female *A. rufescens* sometimes feed together, and nest within a short distance of each other, implies that some pairing may occur (Dennis pers. comm.; Wallis *et al.* 1989)

Little significance can be attached to estimates of animal density and home range because there are few published values, and those vary markedly with habitat quality and the means of determination. This is well illustrated by comparison between Bennett's (1987) estimates of 2.55 *P. tridactylus* per hectare, with a home-range of 2 hectares, with Kitchener's (1967, 1973) values of 0.2, 19.4 and 5.2 for population density and home ranges of males and females respectively. Because animals may travel long distances to their feeding areas and concentrate loosely within them, radio-telemetry is almost essential for estimating home ranges and population densities. This was realized by Scotts and Seebeck (1989), who, by confining their research to trapping, underestimated the home range of *P.longipes*. Similarly, Southgate's (1980) high population density estimates of 0.4 *A. rufescens* per hectare were almost certainly due to his failure to track animal movements. This also introduces doubt to his claim that population numbers are highest in January and September. It is known, at least for *A. rufescens* (Wallis *et al.* 1989), *B. penicillata* (Christensen 1980) and *B. gaimardi*

(Taylor pers. comm.), that some species may travel more than a kilometre to core feeding areas. Thus, the distance between nesting and feeding areas may be a prime determinant of home range.

Sexual dimorphism in body mass has been reported for *A. rufescens* (Schlager 1981; Johnson and Bradshaw 1977), *P.longipes* (Scotts and Seebeck 1989) and *B. leseur* (Prince, cited by Seebeck *et al.* 1989) but the differences are usually negligible or the evidence conflicting. However, it seems that male *P.longipes* are 20% heavier than females (Seebeck *et al.* 1989).

Body mass of potoroines tends to remain stable, suggesting that food is usually in adequate supply. For example, radio-collared *A. rufescens* weighed the same in winter as they did in summer (Wallis *et al.* 1989). Bennett (1987), however, reported that male *P. tridactylus* were heavier in autumn, a feature he associated with nutrient availability. Kitchener (1967) observed a similar, although non-significant trend in Tasmanian *P. tridactylus*, and Christenen (1980) noted that male *B. penicillata* were heaviest in December.

Potoroines breed continuously, which again implies that at least within their present ranges, food supply is reliable. Alternatively, continuous breeding may reflect also the group's ability to practise embryonic diapause. This would terminate breeding during nutritionally harsh times. Tyndale-Biscoe (1968), however, provided evidence that both wild and captive *B. penicillata* show breeding peaks, a characteristic not shown by other potoroines.

Gestation is 20-22 days in the genera *Aepyprymnus* and *Bettongia*, but lasts 38 days in *P. tridactylus* (Tyndale-Biscoe and Renfree 1987). No data are available for *P.longipes*, so it is unknown whether the long gestation is a generic trait. Although *B. penicillata* and other potoroine species may occasionally bear two young, only one survives pouch life. This lasts 95 days in *B. penicillata* and up to 150 days in *P.longipes*. Hence, annual potoroine fecundity is 2-3 young per female. Animals attain sexual maturity between 8 and 12 months, usually before mature body mass is reached (Tyndale-Biscoe and Renfree 1987).

The populations studied seem to exhibit sexual parity, but the paucity of comprehensive life-history studies encourages caution when assessing this parameter. In one of the more thorough population studies, Bennett (1987) found more females than males in his population of *P. tridactylus*. The fact that there is sexual parity of pouch young indicates possible differences in maternal investment at a later stage.

In conclusion, the disappearance of potoroines from many areas limits our potential understanding of their ecology. The degradation of present habitats undoubtedly affects

population parameters such as density and home range. Furthermore, the paucity of ecological studies on most potoroines prevents inter-species comparisons, and further restricts our knowledge of potoroine ecology.

3.4 The diets of potoroine marsupials

Surgeon John White (1790) wrote in his diary:

"As to mere conjectures (and such too often are imposed upon the public for incontestable facts), it cannot be improper to suppress them."

The failure to suppress such conjectures has led to confusion about dietary habits of potoroines, as exemplified by the dietary classifications assigned to them. Hume (1982) labelled them herbivores; Lee and Cockburn (1985) preferred the term fungivore/omnivore; Seebeck and his co-workers (1989) state that all modern potoroids are omnivores.

This disagreement raises the question: indeed, do we know about the diets of potoroine marsupials? There appears to be a wealth of information on potoroine feeding habits. More than 30 reports (Table 3.1) refer in varying degrees to the diets of this marsupial sub-family. Unfortunately, no-one has previously subjected this literature to critical appraisal. When this is done, we find that most reports are anecdotal or mere conjectures based on few observations. Only five reports involve systematic diet studies: Guiler (1971) and Bennett and Baxter (1989) with *P. tridactylus*; Scotts and Seebeck (1989) with *P. longipes*; Christensen (1980) with *B. penicillata*; and Schlager (1981) who studied *A. rufescens*. Recently, dietary studies have been made of *B. gaimardi* (Taylor pers. comm. 1990).

By comparison, in studies with primates, Chivers and Hladik (1980) emphasized the need for long-term diet studies comprising at least one annual cycle and animals of each age and sex class. In another study, Tevis (1953) examined the diets of rodents by analysing the gut contents of 509 chipmunks of four species, and 273 mantled squirrels. Gut contents were sampled in each season. Clearly, our knowledge of potoroine diets is negligible relative to these other studies, and must be treated with caution. Furthermore, the distribution of potoroines, their habitats, and no doubt their diets, are remarkably different from those prior to European influence. Rather than classify the feeding habits of potoroines on limited data from remnant populations, it seems wiser to integrate this information with relevant anatomical, physiological and ecological data, and then draw conclusions. This approach is adopted in the following sections which review the current knowledge of digestion and metabolism in potoroines. Because little

Table 3.1 An inventory of observations of potoroine feeding habits.

Species	Food items	Methods and comments	Reference
H. moschatus	insects, worms, tubers, berries	observation; detail unknown	Ramsay (1876)
H. moschatus	insects, worms, tubers, berries	probably citing Ramsay (1876)	Troughton (1943)
H. moschatus	four spp fruit, insects	one stomach	Harrison (1962)
H. moschatus	fruits,insects, plant matter	observation; probably few	Johnson and Strahan (1982)
A. rufescens	tubers	observation; probably few	Finlayson (1931)
A. rufescens	roots, grasses	probably citing other work	Troughton (1944)
A. rufescens	grass	one stomach	Harrison (1962)
A. rufescens			Calaby (1966)
A. rufescens			Johnson and Bradshaw (1977)
A. rufescens	tubers, fungi	observation; very few	Southgate (1980)
A. rufescens	tubers, hypogeal and epigeal fungi, insects, seeds, gum	observation;	Schlager (1981)
A. rufescens	tubers, insects, grass	observation; semi-detailed	Wallis (unpub)
C. campestris	fibrous plant tissue	communication with aboriginals	Finlayson (1932)
C. campestris			Calaby (1966) Schlager
C. campestris	coleoptera	gut analysis; one preserved gut	Dixon (1988)
P. tridactylus	hypogeous fungi,	scat analysis	Seebeck (in Seebeck *et al.* 1989)
P. tridactylus	fungi, plants, seeds, fruit	scat analysis?	Kitchener (1967)
P. tridactylus	fungi, insects, berries, sedges	scat analysis; detailed, seasonal	Guiler (1971)
P. tridactylus	hypogeous fungi, arthropods, fruits, seeds, plants	scat analysis; detailed, seasonal	Bennett (1987)
P. longipes	hypogeous fungi, seeds, plants	scat analysis; detailed	Scotts and Seebeck (1988)

Table 3.1 An inventory of observations of potoroine feeding habits (continued).

Species	Food items	Methods and comments	Reference
B. gaimardi	tubers	observation; probably few	Gould in Troughton (1944)
B. gaimardi	hypogeous fungi		Rose (1982, 1985)
B. gaimardi			Johnson and Rose (1983)
B. gaimardi	hypogeous fungi, seeds, roots		Taylor (1986a,b; in prep)
B. leseur	crops especially peas and beans		Gould (1863)
B. leseur	marine refuse, dead matter	?	Shortridge (1909)
B. leseur	ground nut — *nalgoa*	observation	Dahl (1926)
B. leseur	green plant parts, bulbs, tubers, quondong fruits/nuts	observation	Finlayson (1958)
B. leseur	roots	speculation	Ride and Tyndale-Biscoe (1962)
B. leseur	tubers and roots	observation	Main and Yadav (1971)
B. leseur	quandong and sandalwood nuts	probably citing Finlayson (1931)	Lyne (1974)
B. penicillata	garden plants? check	observation	Jones (1924)
B. penicillata	fungi, plant tissue, roots, tubers, arthropods	observation	Sampson (1971)
B. penicillata	gum	observation; very few	Kinnear (1979)
B. penicillata	fungi, seeds,		Christensen (1980)

is known about potoroines their closest relatives — the Macropodidae — are often discussed.

3.5 Dentition in the Potoroinae

Owen (1859) and Waterhouse (1846) both recognized that different kangaroo species occupy different habitats and presumably have different diets. However, it was Krefft (1875) who first reported morphological differences. Among the large kangaroos he recognized a broad upper third incisor, smaller deciduous premolars and molar progression. In contrast, a group of smaller marsupials, that included potoroine marsupials, had narrower third incisors, permanent premolars and no molar progression.

Garrod (1875) too, identified the divergence in dental morphology. On the basis of premolar size, molar pattern and gut morphology, he revised the taxonomy of the Macropodidae. This classification was revised further by Bensley (1903) and Raven and Gregory (1946).

In a series of papers, Ride (1957, 1959, 1962, 1964, 1971, 1978) recognized the inverse relationship between premolar size and specialisation for grazing. Like previous studies, Ride's focussed on taxonomy. It was Sanson (1976, 1978) who confirmed an often stated trend that, in terms of functional morphology and diet, there is, within the Macropodoidea, a basal herbivorous grade — the browser. The dental anatomy of the browser is functionally different from those of the concentrate feeders (potoroines) at one extreme and of the grazers at another. Later, Sanson (1989) acknowledged that several intermediate forms occur.

It is difficult to generalise about the diet of a species or family from dentition alone. However, much can be gained by comparing species from diverging nutritional environments. This section examines the functional morphology of potoroine mastication by drawing comparisons with the other extreme — the grazing macropodids. By necessity, the discussion relies heavily on the writings of Morton (1981) and Sanson (1978, 1980, 1989).

Section 3.4 concluded that a unifying feature among potoroines is their preference for a concentrated diet, that is presumably lower in plant-cell walls (fibre). The diet is probably more heterogeneous than the grass diet eaten by the grazing macropodids.

Likewise, several dental features separate the potoroines from the grazing macropodids. Of most importance, functionally, are the prominent premolars, the absence of molar progression, the twisted molar row, the medial rotation of the potoroine mandible and the deep insertion of jaw muscles in the extensive masseteric canal.

The large premolars are developed as specialized shearing blades in the three extant potoroine genera. They are used in preference to incisors for initial food preparation. Morton (1981) showed that the efficient use of the premolars is hampered by their size and configuration which suggests simultaneous occlusion of molars and incisors. This effect is diminished by the medial rotation of the mandible which causes the tubercular molars to occlude sequentially from posterior to anterior. Moreover, the same medial rotation, made necessary by the twisted molar row and probably assisted by the deep insertion of the jaw muscles, allows engagement of even worn premolars (Morton 1981). This is clearly advantageous in potoroines because much of their diet is hypogeous and presumably abrasive. The ability to occlude worn premolars is even more important when we consider that potoroines lack the "classic" adaptations to an abrasive diet — continuous open-rooted teeth (Sanson 1978) and hypsodonty (Simpson 1953).

The large potoroine premolars inhibit lateral mandibular movement. This is not so in large, grazing macropodids in which the premolar is so small, or even absent, that lateral movement of links across lophs is important in the second occlusal phase (Sanson 1980).

The large potoroine premolar seems to form a buttress preventing molar progression. Furthermore, rows of teeth in potoroines are straight, rather than curved dorso-ventrally as in the large macropodids. Sanson (1989) sees the vestigial premolar, curved tooth row and affiliated molar progression in the grazing macropodids as adaptations to their poor quality, abrasive, grass diets.

In contrast to the molariform teeth of specialized grazers, the potoroine molars are low rather than high-crowned and erupt simultaneously rather than sequentially (Sanson 1989). The potoroine molars show also far greater size variation (Morton 1981). Sanson believes that these differences reflect distinct diets. If we assume that the outer covering of potoroine food items is the major barrier that the teeth must rupture, and if we assume that the specialized premolar is adept at processing this covering, then the molars need only triturate the liberated contents. The low structural-carbohydrate content and high water content of the heterogeneous diet gives it a low impact strength. Also, the premolars may contribute to molar function. Thus, in potoroines there is no need for the specialized high-crowned molars with elaborate enamel ridges seen in the grazing macropodids.

The teeth of potoroines have other features which are presumably important to the animal, but have not been studied. Canines, although rare in macropodids, are well developed in potoroines and give them a short diastema or gap. The longer diastema in

macropodids is thought to orientate ingested food particles (Sanson 1989). Is it possible that this function in potoroines is taken by the manus?

The incisors of potoroines and grazing macropodids also differ. In potoroines, the first upper incisor is more recurved and extends below the level of adjacent incisors. This anatomy prevents the occlusion of all incisors simultaneously as occurs in grazing macropodids (Sanson 1989). It is unclear how this geometry affects ingestion by potoroines. In macropodids the incisors are finely-tuned cutting-implements.

Archer (1984) drew attention to several features that differ among the potoroine genera. For example *Potorous spp* have elongated premaxillae; *Bettongia* show an increase in length and serration number of P3; *Caloprymnus* have a vestigial C\1 and *Aepyprymnus* has a reversed molar size gradient so they increase in size from front to back.

Character states tell us little about the advantages they evoke on the organism. Morton (1981) recognized this and conducted a form-function study of the masticatory apparatus using a species from each extant potoroine genus. The premolars are functionally similar. This, she attributes to the similar physical properties of the diets processed by each species. The molars, however, show both morphological and functional differences between species. Morton proposes that this is related to the different amounts of structural carbohydrates in the diet of each species. *A. rufescens*, which is thought to consume the most fibrous diet, has higher molar cusps, longer shearing blades and a relatively larger masticatory apparatus than does *Potorous*. *Bettongia* has intermediate dental characteristics.

Morton's conclusions may be criticised in two ways:

1. The physical properties of the diets to which each species responded in an evolutionary sense are assumed similar. We have a limited knowledge of potoroine diets but if we adopt the food categories of Lucas and Luke (1984) the rhizophagous diet of *Aepyprymnus* falls in "hard, brittle foods" and the mycophagous diet of *Potorous* in "tough and/or soft foods".

2. Premolar similarities across species are explained by assuming the diets are physically similar. However the molars are assumed to have undergone adaptive changes in response to physical differences between diets. Hence an adaptive argument is used to explain molar disparity but is ignored when explaining premolar resemblance.

Despite these criticisms, there is no doubt that differences in cheek-teeth morphologies occur. While the degree of shearing edge and incisor development in *A. rufescens* fails to match that in the grazing macropodids, it is still present. Kay and

Highlander (1978) reported a similar dental morphocline across primate species as the level of dietary plant-cell walls increased.

The earliest potoroines recorded by Stirton *et al.* (1961) were later placed in the genus *Bettongia* by Stirton *et al.* (1968). The permanent premolar is very similar to that of living bettongs, but the molars differ. This suggests a slow evolutionary change compared with the diprotodonts described by Stirton *et al.* (1967). It further suggests that the potoroines were sufficiently generalized to allow them to exist with limited modification under changing ecological conditions. Bartholomai (1972) reports that the genera *Potorous, Bettongia* and *Aepyprymnus* are all represented in Pleistocene deposits and the material is usually similar or identical to recent forms.

3.6 Comparative morphology of the potoroine digestive tract

Two distinct stomach morphologies occur in the Potoroidae. One of these, a "simple", glandular, sacciform-type stomach belongs to *Hypsiprymnodon moschatus*, and has been described by Carlsson (1915) and Heighway (1939). Both authors recognized the simple stomach. Carlsson (1915) made comparisons with the stomach of *Trichosurus vulpecula*, and Heighway (1939) concluded that *Hypsiprymnodon's* stomach is morphologically midway between the simple phalangerid type and the complex macropodid form. This simple-stomach morphology is one reason why this species was placed in its own sub-family Hypsiprymnodontinae within the family Potoroidae. Other distinguishing features are its notable plagiaulacoid premolars, and unique hind-foot anatomy (Johnson and Strahan 1982). The species usually produces two young, something rarely, if ever, recorded in other potoroids.

The stomachs of all three extant potoroine genera (*Aepyprymnus*, *Potorous* and *Bettongia*) are more specialized for fermentation than that of *Hypsiprymnodon*. They bear such resemblance to the macropodid stomach that similar terminology can be used to describe all.

A good description of macropodid foregut structure and function is provided in the studies of Langer (1979, 1988); Dellow (1982); Gemmell and Engelhardt (1977); Hume and Dellow (1980); Langer *et al.* (1980) and in the review by Hume (1982). In contrast, information on potoroine stomach morphology comes from a much more limited information base: the research of Owen (1868), Schultz (1976) and Langer (1980). Even less is known about stomach function in potoroine marsupials, although the research by Hume and Carlisle (1985), Frappell and Rose (1986) and Kinnear *et al.* (1979) offers some preliminary insight.

Because little is known about the form-function complex of the potoroine forestomach, the following discussion compares what is known with the better understood processes in the Macropodidae.

Excepting that of *Hypsiprymnodon*, the macropodoid stomach can be divided into a sacciform forestomach (SFS), a tubiform forestomach (TFS) and an acid-secreting hindstomach. Despite these general similarities, there are, nevertheless, important internal and external differences between potoroine and macropodid stomachs. The most obvious is the dominance of the potoroine SFS, which may compose 80% of total foregut capacity. In macropodids, by comparison, the TFS usually dominates. Even so, in macropodids there is a general trend — the smaller the species, the larger the SFS (Fig 3.2).

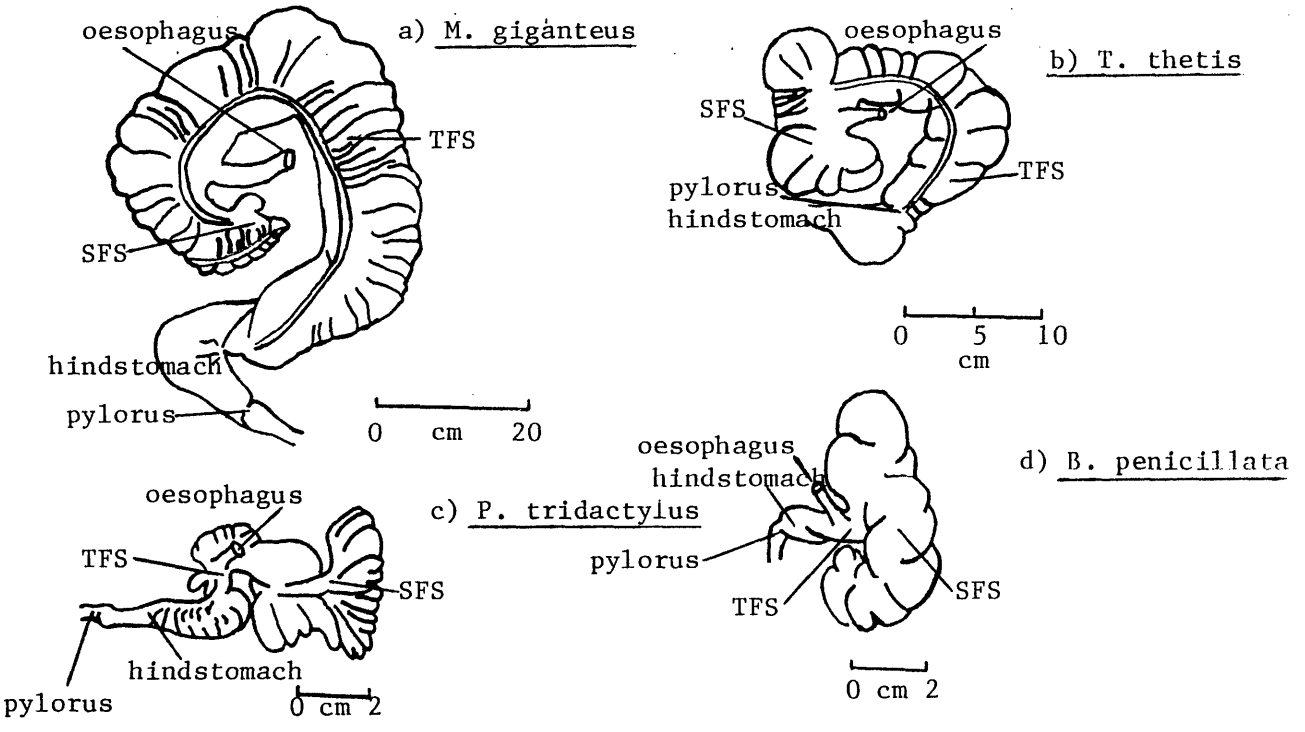

Fig 3.2 The stomachs of *Macropus giganteus*, *Thylogale thetis*, *Potorous tridactylus* and *Bettongia penicillata* (After Hume 1982).

In studying the gastrointestinal tracts of potoroines and macropodids, Langer (1980) described the topographical differences that are caused by variations in capacity of foregut regions. Whether topographical differences result in functional differences remains unknown.

Perhaps the major difference between potoroines and macropodids is that, in the former group, the point of entry of the oesophagus into the foregut (ie the cardia) is very close to the fold separating the TFS from the hindstomach. In macropodids (Langer 1980), the cardia is found near the sacciform-tubiform junction. As a consequence, the distance between the cardia and hindstomach in potoroines is short, and offers — at

least theoretically — an opportunity for digesta to bypass the fermentation region. This may account for the absence of a gastric sulcus in some potoroines, for example *B. penicillata* (Kinnear *et al.* 1979) and only weak development in others, for example *P. tridactylus*. Most macropodid species, by comparison, have a prominent gastric sulcus (Hume 1982). Langer (1980) raises the separate possibility that the offset forestomach region in potoroines prolongs the retention of digesta. Both theories remain untested.

The potoroine forestomach lacks stratified squamous epithelium; instead the forestomach is lined entirely by cardiac glandular mucosa. This contrasts with the macropodid stomach in which squamous epithelium lines at least part of the SFS. In larger species the trend for more stratified squamous epithelium may reflect the mechanical stimulation of a more abrasive diet (Moir 1968; Chapter 2).

Gemmell and Engelhardt (1977) reported that cardiac glands in *Macropus eugenii* are mucigenous, and Cummings *et al.* (1972) reported similar glands in the llama. Furthermore, Gemmell and Engelhardt recognized three types of granules in the cardiac glands of *M. eugenii*, suggesting three different secretions, but did not elaborate on possible functions. Rübsamen (1976), cited by Langer (1980), revealed that llama cardiac glands are closely associated with absorption of short-chain fatty-acids. We can speculate, at least, that this epithelial type has a similar function in macropodoids. Thus, the more extensive cardiac glands in potoroines, relative to the larger macropodids, reflects the higher fermentation rates expected for potoroines due to their more concentrated diet (Hungate *et al.* 1959; Hofmann 1989).

Differences exist also between potoroines and macropodids in forestomach musculature. In the latter group, the musculature of the forestomach wall is differentiated into three longitudinal bands or taeniae (Langer *et al.* 1980). Non-permanent semi-lunar folds formed by stomach wall contractions extend between the taeniae, creating haustrations which give the foregut its colon-like appearance (Owen 1868). The hindstomach contains no such musculature. In potoroines, Langer (1980) found an opposite situation, noting weak haustrations in the SFS and hindstomach but not in the TFS. Hume and Carlisle (1985), also, examined the gastrointestinal tracts of *A. rufescens* and *P. tridactylus*. Their description accords generally with Langer's, except that they identified weak haustrations in the TFS.

Dellow (1982) considered that the extensive haustrations, in macropodids, are responsible for the marked separation of digesta phases and trajection of digesta in a caudal direction. Hume and Carlisle (1985) reported extended retention of some radio-opaque particles within the SFS, and concluded that phase separation might occur also in the potoroine forestomach. To test this suggestion requires rate-of-passage studies using specific digesta phase markers.

The parotid salivary glands of *B. penicillata* are large (3.12 g per kg; Forbes and Tribe 1969). Kinnear *et al.* (1979) suggest that the large potoroine glands indicate a well-developed buffering system, presumably designed to cope with high fermentation rates. These might result from ingestion of concentated foods. Kinnear's hypothesis, however, must be viewed with caution. We have no information about other salivary glands, saliva composition and production rates or other modes of foregut buffering — for instance, glandular secretions and short-chain fatty-acid absorption (Chapter 2).

The lower parts of the digestive tract in potoroines, like that of most other PGF, remains a mystery. In their studies of potoroine alimentation, Hume and Carlisle (1985) commented that "the gross anatomy of the small intestine is unremarkable". Richardson (1989) and Richardson and Wyburn (1980) noted similar features in *B. penicillata* and *M. eugenii* respectively.

Like the macropodid caecum, the potoroine organ lacks also taeniae and haustra. Although the colons of both groups are similar, having ascending and descending parts connected with a region of colonic coils, this latter region or transverse colon is more distinct in the potoroines (Hume and Carlisle 1985). It is interesting that the potoroine hindgut is relatively larger than that in the Macropodidae. Hume and Carlisle (1985) reported that up to 35% of total digesta is held in the hindgut, and Frappell and Rose (1986) stated that "the proximal colon and caecum have a volume approximately one third of the sacciform forestomach". They did not, however, report values for the distal colon. In contrast, the hindgut of macropodids is diminutive. In three species from diverging habitats it comprised only 11-17% of total gut capacity (Dellow 1979).

3.7 The research

Given that studies of macropodoid nutrition, digestive physiology and metabolism have focused on the larger kangaroos and wallabies, and neglected the smaller potoroines, it seemed justified to study the latter group. However, the paucity of published information on potoroines made it inappropriate to study just one aspect of their metabolism. Instead, in the hope that it might stimulate further research, a general comparative study was planned using one species from each extant genus. Three aspects of digestive physiology and metabolism were of particular interest. First, was the role of the potoroine foregut; second, physiological explanations for the recent distributions of the modern potoroine species; and thirdly, the similarities and differences between potoroines and macropodids.

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CHAPTER FOUR

Materials and methods

4.1 Introduction

THE nature of the experiments and the methods used were constrained by a shortage of suitable animals and the need to complete daily balance measurements within a reasonable time. Females, were used mainly for breeding purposes and they were therefore unsuitable for most experiments; similarly, juveniles, because of their changing metabolism, were not used. Other factors limited the work: some animals housed in metabolism cages for several weeks experienced stress; others spilt their food making quantitative measurements of intake and excretion difficult; legal constraints prevented surgical intervention — for example, fistulation of the gastrointestinal tract; because few balance studies had ever been conducted with potoroines, cage architecture and measuring techniques were subject to continuing change. With these limitations, the experiments described and the methods used are a compromise between the ideal and the achievable.

The animal husbandry, sampling procedures and analytical techniques common to most experiments are described in this chapter; more specific methods accompany the experiments to which they relate.

Several parameters — for example, water intake, cell-wall digestibility and nitrogen balance — were measured in most experiments. Where feasible, the data generated have been pooled in separate chapters devoted to each of these parameters to facilitate interpretation.

4.2 Animals

A captive colony of *A. rufescens* was first established at the University of New England in 1980 (Schlager 1981). This colony, supplemented with animals captured at Drake during the present study, provided the experimental animals. The *P. tridactylus* and *B. penicillata* colonies were established from eight pairs of each species obtained from the Sir Colin MacKenzie Zoological Park, Healesville, Victoria, in 1984 and 1985.

4.3 Animal husbandry

All animals were housed at the University of New England, Armidale, N.S.W. (30.31° S, 151.39° E, altitude 1000 m). Typical ambient temperatures in summer and

winter are given in Chapter 10. When not being used in experiments, animals lived in outdoor enclosures measuring 9 m x 3 m. The 2 m walls were constructed of wire mesh (30 mm mesh) which extended 30 cm below ground level to prevent animals escaping. A wire roof of similar mesh kept potoroines in and predators out. Rodents, in particular *Rattus rattus*, caused a major problem in the enclosures by eating food intended for potoroines; wire of a smaller mesh might have avoided this problem.

P. tridactylus thrived when several animals of each sex were kept together in an enclosure. In both *A. rufescens* and *B. penicillata*, however, aggression occurs between adult males housed together in small enclosures. For this reason, enclosures housed only one male with one or more females.

All species displayed activity patterns resembling those of wild animals. The *A. rufescens* and *B. penicillata* remained strictly nocturnal while *P. tridactylus* were active also in the early morning and evening. Hollow logs placed in each enclosure were intended to provide shelter during periods of inactivity, but only the *P. tridactylus* made any use of them. Instead, the *A. rufescens* and *B. penicillata* preferred to build their typical grass nests, while the *P. tridactylus* usually squatted together in depressions dug beneath the numerous tussocks of *Phalaris aquatica*. When the grass cover became sparse, during the colder months, straw was provided for nesting material.

The basal ration (Table 4.1) and water were provided *ad libitum*. A detailed description of the development of this diet is given in Appendix 1. Judging from the green colouration of their faecal pellets, the *P. tridactylus* occasionally supplemented their diet by eating the succulent growing tips of *P. aquatica*. This was not observed in the other species although both gathered *P. aquatica* for use as nesting material.

All species bred freely in captivity, and births outnumbered deaths in *A. rufescens* and *P. tridactylus*. However *B. penicillata* seemed more prone to both handling and climatic stress, and maintaining their numbers was a constant struggle.

In winter, particularly when conditions were cold and wet, mortalities peaked among all species. Stress was thought to be a major factor. Dead animals were submitted for necropsy to the NSW Department of Agriculture's Regional Veterinary Laboratory in Armidale, which at first identified pneumonia as the most common cause of death. However, in 1987, further inquiry established lungworm, *Capillaria sp* as an additional contributing factor. Where this was a confounding factor the animals were usually stressed, with clear lung lesions. Lungworm has been isolated also from the populations of *A. rufescens* and *P. tridactylus* at the University of New South Wales. A search through animal records shows that in 1982 an *A. rufescens* was borrowed from that institution (I.D Hume pers. comm.). This animal is suspected of being the vector of the parasite. As soon as lungworm was diagnosed, faecal egg counts were monitored regularly by the Regional Veterinary Laboratory, which advised all animals to be

captured and dosed orally with a broad-spectrum anthelminthic (Panacur) at the rate of 0.3 ml per kg body mass for five consecutive days. These measures reduced faecal egg counts to zero, but in the absence of adequate quarantine facilities the lungworm infection persisted.

Table 4.1 Composition (g.kg^{-1} ADM) and typical chemical composition (g.kg^{-1} ODM) of the basal diet

Ingredient	Level of inclusion
maize	555
wheat	200
soybean meal	140
oaten straw	75
mineral mix (Table A1.6)	29
mineral/vitamin premix (Table A1.6)	1
Analysis	
dry matter	910
organic matter	947
ash	53
nitrogen	22
acid detergent fibre	110
neutral detergent fibre	223
cellulose	95
hemicellulose	113
lignin	15

4.4 Experimentation

All experiments with animals housed in metabolism cages were of the balance type, in which ingestion of food and excretion of faeces and urine were monitored. These were conducted between 1984 and 1989. Numerous animals of each species were used in these studies and some in several experiments. However, there was no evidence to suggest that individuals responded differently to experimental treatments and individual animals, therefore, are not identified in the chapters describing the experiments.

4.4.1 Metabolism cages

Animals selected for balance studies were moved from the outdoor enclosures to metabolism cages (Fig. 4.1) in a semi-controlled environment room. The cages were designed to allow quantitative measurement of food and water intake and urinary and faecal excretion. They were arranged in racks, each containing two tiers of three cages.

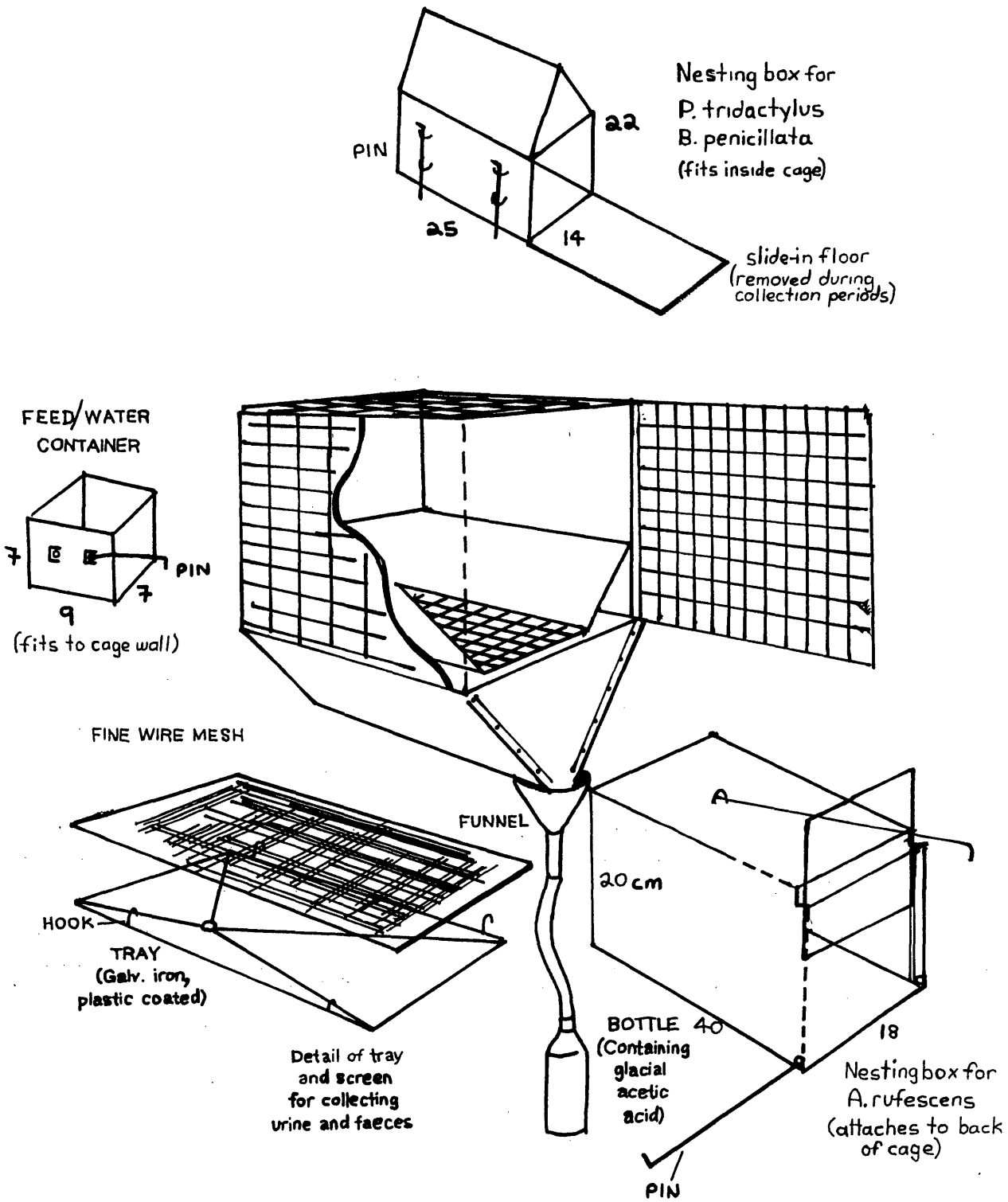

Figure 4.1 Schematic diagram of the metabolism cage and collection apparatus

4.4.2 Environment

Whenever animals were moved inside, the experimental room was set to the prevailing natural daylength. It was not altered again during the span of the experiment. At set times, two 60 watt globes were automatically turned on and off; a heavily-shaded 25 watt lamp operating continuously, gave sufficient background lighting for observations and sampling when the main lights were off. While animals were indoors, room temperatures were recorded daily. The ambient temperature was usually maintained at 22 $\pm$ 2°C, although on very cold nights this sometimes dropped as low as 15°C. Humidity was not controlled.

4.5 Balance measurements

When animals were brought inside, they were placed separately in cages, at random, and introduced to the experimental diets. Also, in each balance experiment, animals were randomised among experimental treatments. Animals were given a seven-day general adaptation period. During the next seven days — the pre-collection period — intake and output were monitored daily to ensure that animals were in a steady state for the start of the seven day collection period. The animals were weighed when first brought inside and then at weekly intervals.

At the same time each day of the collection period and in the same sequence, cages were cleaned and measurements made of food and water intake and excretion of faeces and urine. Maximum and minimum temperatures and evaporation for the previous 24 h were recorded at this time and a food sample (ca 75 g) taken for later analysis.

The peak feeding period lasted for four or five hours after dark. During this time, to minimise contamination of spilt food by excreta, faeces were removed at regular intervals and placed in plastic bags; spilt food was placed back into feeders. This procedure also made the quantitative measurements of intake and excretion more reliable. Because most faeces are voided during the peak feeding time, this practice made it possible to estimate faecal-water loss. When spilt food was obviously contaminated by urine, the food was oven dried at 60°C for 24 h and 90% of the weight loss assumed to be urine; the remaining weight loss was assumed to be moisture already present in the food. In the preliminary investigation of nitrogen requirements (Chapter 5) this latter procedure was compared with the alternative of analysing contaminated food; the difference ($\pm$ 2%) did not justify the additional laboratory work required to analyse food refusals.

Urine was collected into plastic bottles containing 5 ml glacial acetic acid, which was sufficient to reduce the pH to 2 and hence prevent microbial catabolism of urinary compounds. The collection trays below each cage were washed daily with 50 ml of water to retrieve any adhering urine.

4.6 Sample preparation

Urine samples and tray washings were bulked daily and stored frozen until analysed.

Each animal's total daily output of faeces was transferred to aluminium trays and dried in a forced-draught oven at 60°C for 24 h. It was then stored in an open plastic bag to allow equilibration with atmospheric moisture. This procedure was repeated on each day of the collection period. The bags of faeces were allowed a further 3 days equilibration at the completion of the collection period, and the faeces were then weighed and ground to pass through a 1 mm sieve. Representative samples (ca 100 g) of the ground material were stored in plastic jars prior to analysis.

The food sample resulting from samples taken on each day of the collection period was ground to pass through a 1 mm sieve and 100 g was retained for analysis.

4.7 Blood sampling

In all three potoroine species, blood was withdrawn from a lateral vein located at the base of the tail, using a heparinised winged infusion kit (Terumo Surflo 0.80 x 19 mm). The animal was placed in a hessian sack and its tail was threaded through a hole in the sack. After fur was removed from the target area the needle was inserted into the vein, which was occluded with a thumb. The syringe was not attached until blood flow accelerated. Even then, minimal suction only was used to draw blood. Samples could be obtained without assistance if the animal was restrained by holding it between one's legs while seated. However bleeding animals housed indoors, particularly the smaller species, always proved difficult due to the reduced blood flow, caused by the animal's limited activity.

4.8 Pouch examinations and milking

Pouches were examined with the animals immobilized as described previously for bleeding. However, for pouch examination, the animal was held in a seated position facing away from the handler, with its head covered by the lip of the hessian sack. In this position, the animal's pouch was accessible and could be examined, pouch young could be removed and the female milked by hand.

4.9 Analytical methods

All chemical analyses of dietary constituents were conducted in quadruplicate; analyses of urinary, faecal, blood and milk components were undertaken in duplicate.

4.9.1 Dry matter and ash

All results are expressed on an oven-dry-matter (DM) basis determined by drying samples (ca 1.00 g) of feed, faeces and digesta in porcelain crucibles for 24 h at 80°C. The resulting oven-dry samples were ashed immediately, in a muffle furnace at 600°C for 12 h, to determine organic matter and ash. These determinations were made simultaneously with other chemical analyses to negate any effects of moisture absorption during storage.

4.9.2 Gross energy

Gross energy determinations were made on samples of faeces (ca 1.5 g), feed (ca 1.0 g) and freeze-dried urine (ca 2.5 g) using an automatic adiabatic bomb calorimeter (Gallenkamp) which was routinely standardized using benzoic acid. Feed and faeces were compressed into pellets before combustion; the dried urine, which resembled a thick paste, combusted completely without any additional treatment.

4.9.3 Total nitrogen

Total nitrogen was determined on 0.25 g samples of feed and faeces and 1 ml of urine using a semi-micro Kjeldahl digestion with a Cu-Se catalyst (A.O.A.C. 1975). The ammonia produced upon alkalisation was collected into a boric acid solution using the distillation apparatus described by Ivan *et al.* (1974). The recovery of ammonia-nitrogen was estimated using a standard ammonium-sulphate solution and blank (double-distilled water). All experimental samples were corrected to 100% recovery.

4.9.4 Urea

Urea-nitrogen, in the urine and the plasma of captive animals, was determined colorimetrically with the diacetyl monoxine method of Marsh *et al.* (1965), adapted for use with a Technicon autoanalyser. Samples were initially diluted 100-fold, but often this dilution had to be adjusted according to urinary urea concentration.

4.9.5 Acid-Insoluble Ash

A modification of the method of Van Keulen and Young (1977), as used by Mollah (1982), was followed to determine acid-insoluble ash.

An appropriate weight of sample (2-5 g) containing at least 100 mg of acid-insoluble ash was placed in a tared, oven-dried sintered-glass crucible (Pyrex, porosity 4, pore size index 5-15 μm) and combusted at 480°C for 14 h. When cool, the crucibles were placed in an evaporating dish containing 4N hydrochloric acid and simmered for 15 minutes. The hot crucibles were then placed under reduced pressure and thoroughly washed, first with hot 4N hydrochloric acid and then with boiling distilled water. After redrying, the combustion, boiling and washing stages were repeated. Finally, the crucible containing acid-insoluble ash was dried and reweighed.

The ash from the initial combustion and washing appeared black, indicating a residue of carbon. The repeat treatment resulted in a variable mass loss (2-8%) and

removal of the remaining carbon.

The use of sintered-glass crucibles has advantages over other methods — for example, those of McCarthy *et al.* (1974, 1977) — in that the quantitative transfer of samples is unnecessary. Nevertheless, the method restricts combustion to <500°C, invoking the need for two combustions to oxidize carbon.

4.9.6 Cell-wall constituents

The cell-wall constituents: neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined in feed and faeces by the procedures of Goering and Van Soest (1970). The NDF and ADF were determined on separate samples weighing ca 1.00 and 2.00 g respectively, while the ADL was determined by extracting the ADF residue for four hours with 72% sulphuric acid. Cellulose was taken to be the difference between ADF and ADL, and hemicellulose that between NDF and ADF.

The detergent system of fibre analysis has undergone several modifications since 1970, including the exclusion of sodium sulphite from the NDF solution. However, owing to the comparative nature of the present work, in which the interest was directed towards the reaction of the animal to "fibre", rather than the absolute levels of fibre present, the original method was followed.

4.9.7 Particle size analysis

The particles in pelleted feed and wet faecal pellets were graded by wet-sieve analysis according to their size. The samples (0.75-1.2 g DM) disintegrated readily when gently shaken in water. The resulting particles were separated on five stainless-steel sieves of mesh size 1118, 600, 300, 150 and 75 μm. These also were shaken for five minutes as water flowed through at constant pressure (20 kPa). The residues were transferred to preweighed Whatman No. 41 filter papers and oven-dried at 80°C for 24 h.

4.9.8 Recovery of barium carbonate

Carbon dioxide was removed from the respiration chambers by circulating the air through a potassium hydroxide trap containing ca 1200 ml of solution (100-400 g $KOH.l^{-1}$). After the measurement period the solution was transferred to a 2000 ml volumetric flask and diluted to volume. The production of carbon dioxide was determined on duplicate 20 ml aliquots in dry, tared centrifuge tubes. The excess hydroxide was removed with ammonium ions, and then the carbonate was precipitated with barium ions. The tubes were spun (3000 rpm for 20 minutes), the supernatant was poured off, the precipitate was washed and then respun. Finally, the tubes were dried at 105°C for 24 h and reweighed.

4.9.9 Short-chain fatty-acids

The molar concentrations and proportions of acetic, propionic, butyric, iso-butyric, iso-valeric and valeric acids were estimated in the supernatant of centrifuged digesta samples by following the method of Erwin *et al.* (1961), using a dual-column gas-liquid

chromatograph (model 427, Packard Instrument Co., Illinois, U.S.A.) connected to a model 604 Packard data processor. Results were calculated with reference to the internal standard, iso-caproic acid.

4.9.10 Digesta pH

The pH of digesta samples collected in the experiments described in Chapter 9 was measured within 1-2 minutes of collection, using a pH meter calibrated with standard buffers (pH 4.0 and pH 7.0).

4.9.11 Blood parameters

Several blood parameters were measured in plasma collected from free-living *A. rufescens*. In each case the standard method (A.O.A.C 1975) was automated with a Roche Cobas-Bio analyser.

Glucose

Plasma glucose was determined by an automated version of the enzymatic procedure described by Bondar and Mead (1974 cited by A.O.A.C 1975).

Lactate

L-lactate in the plasma was determined by measuring its enzymatic conversion to pyruvate.

Creatinine

Plasma creatinine concentrations were determined by an automated version of the method of Folin and Wu (1919).

Plasma urea-nitrogen

Plasma urea-nitrogen was determined kinetically using the urease method described by MacKay and MacKay (1927).

Plasma protein

Plasma protein was determined by the biuret method (A.O.A.C. 1975).

4.9.12 Milk constituents

Milk solids

The milk-solid fraction was determined gravimetrically to 0.1 mg by freeze-drying a known mass of milk, between 25 and 100 mg, in preweighed plastic vials.

Milk carbohydrates

Carbohydrate concentrations were determined as total hexose using the modified phenol-sulphuric acid reagent method of Messer and Green (1979). To 200 µl aliquots of diluted milk (five µl milk in 2.000 ml double-distilled water) were added 1 ml phenol (3.55 % w/v) and 3 ml concentrated sulphuric acid. Upon cooling the absorption was read at 490 nm.

Milk protein

The total protein in milk was determined using the Coomassie Brilliant Blue G250 spectrophotometric method described by Bradford (1976) and Spector (1978). 100 µl of

diluted milk (five µl milk in 2.000 ml double-distilled water) were mixed with 5 ml of protein reagent and then read in a spectophotometer at 595 nm.

Milk fat

Milk fat was determined as the difference between total solids and the combined protein and carbohydrate, assuming an ash content of 5% of the total solids fraction.

4.9.13 Isotope analyses

Preparation and administration of isotopes

The isotopes ^{14}C-urea, ^{3}HOH and ^{103}Ru-chloride were obtained from Amersham, U.K.; ^{51}Cr-ethylene diamine tetraacetic acid from the Australian Atomic Energy Agency, Lucas Heights, N.S.W.; and H^{18}OH from Yeda Stable Isotopes, Israel.

The ^{14}C-urea and ^{3}HOH were diluted where necessary with physiological saline and injected intramuscularly into the hind leg. The precise dose was determined by weighing the syringe before and after injection.

Counting of isotopes

All samples were corrected for background levels of isotopes using faeces, urine or plasma from unlabelled animals.

a) Beta emitters

All samples were counted in a Packard Tri-Carb automatic liquid scintillation spectrometer model 3255, using the automatic external standard (A.E.S.) to correct for quenching. The counting efficiencies were corrected using a series of known standards.

^{14}C-urea

Urine samples for the determination of ^{14}C-urea activity were prepared in two ways. First, 0.10 g of urine was mixed with 0.90 ml distilled water and 10 ml scintillation fluid. Alternatively, scintillation vials containing ca 0.20 g urine were dried under reduced pressure in a dessicator containing sulphuric acid and then mixed with 1.0 ml distilled water. The reconstituted urine, blanks (1.0 ml distilled water) and diluted dose solutions were mixed with 10 ml scintillation fluid prior to counting.

^{3}HOH

Water (ca 0.100 g) isolated by vacuum sublimation (Vaughn and Boling 1961) from whole blood, blood cells, plasma and urine was counted with 0.9 ml distilled water in 10 ml scintillation cocktail.

b) Gamma-emitters

^{51}Cr-EDTA and ^{103}Ru-Phenanthroline

The radioactivity emitted by ^{51}Cr-EDTA and ^{103}Ru-P in faeces, urine and diluted injection solutions was counted in channels "A", (with an energy spectrum ranging from 270 to 400 keV), and "B" (400 to 800 keV) respectively, of the Packard spectrometer. Separate ^{103}Ru-P and ^{51}Cr-EDTA standards were used to calculate the proportion of the

^{51}Cr-EDTA appearing in channel "B" (0.80%) and the ^{103}Ru-P in channel "A" (12.7%). The correction described by Tan, Weston and Hogan (1971) was applied.

Differences are known to occur in counting efficiency with variation in the height of the sample in the gamma tube (Dixon 1978). This problem is usually circumvented by packing the tubes to a constant height, which is straightforward when large volumes of faeces are excreted. However, as described in the rate-of-passage studies in Chapter 7, experiments with potoroines had to deal sometimes with extremely small samples. Because individual defaecations were of considerable interest one was left with three options:

1) *Pack all tubes to the same height, dictated by the smallest defaecation.* This would require extensive mixing and subsampling of most samples.

2) *Pack all tubes to a common height* — for example, that of the average defaecation, — and distribute the small defaecations through a filler to expand them to this level; or

3) *Count at different heights all the faeces deposited, and correct for the height of counting.*

The third approach was investigated in the "height study" described below.

Height-of-counting study

Dry faeces were ground through a 1 mm sieve and 86.5 g were taken to which were added 430 g of water containing 0.5 ml of a ^{103}Ru-P and ^{51}Cr-EDTA dose solution emitting 3.6 x 10^6 cpm. The slurry was mixed and then dried at 80°C for 24 h prior to being reconstituted with water to 35% dry matter.

The labelled faeces were packed into gamma counting tubes at increments of 0.5 cm from 0.5 to 8.0 cm. These were counted at machine settings between 0 and 3.5 cm at intervals of 0.5 cm.

From this study it was decided to set the scintillation counter at 2.0 cm because *B. penicillata* and *P. tridactylus* usually void sufficient faecal pellets to fill the tubes to between 1.0 an 4.0 cm. Over much of this range a machine setting of 2.0 cm gives maximal counting efficiency (Fig. 4.2). Furthermore, although *A. rufescens* often void greater quantities of faeces than the other species, additional tubes can be used for single defaecations. When more than one tube was needed, these were always packed to <4.0 cm.

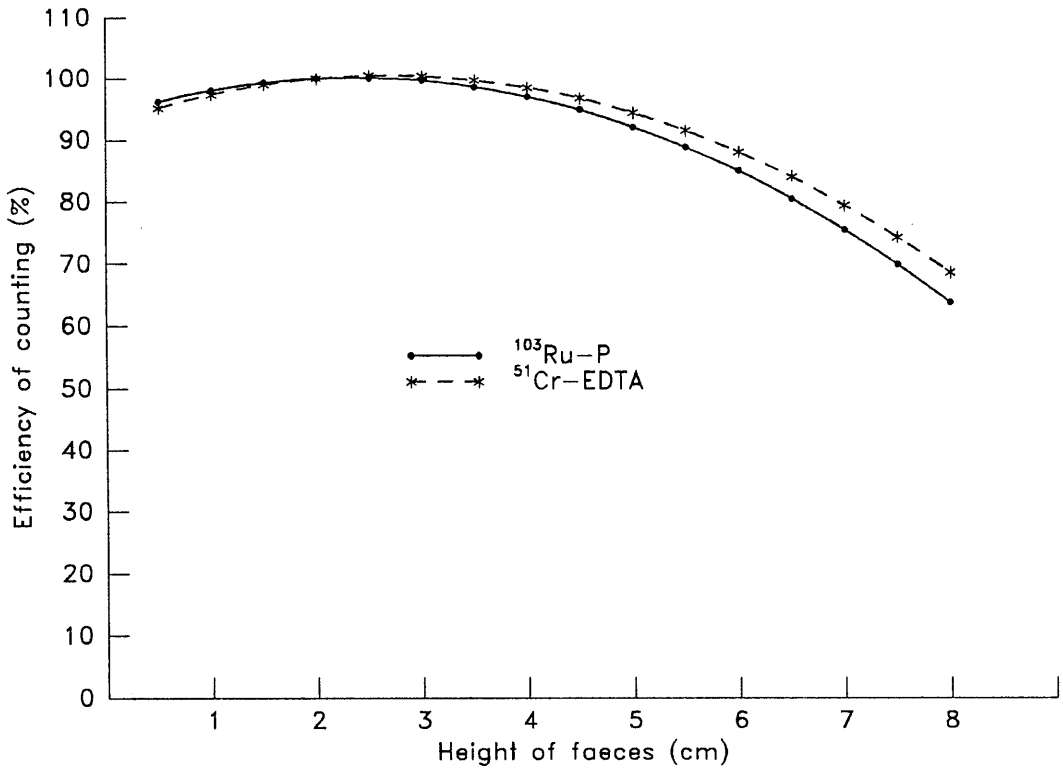

Fig. 4.3 The efficiency of counting, at a gamma-counter setting of 2 cm, the radiation emitted from tubes packed to different heights with labelled faeces. Efficiencies are relative to those measured in tubes containing 2 cm of faeces.

4.10 Statistical methods

The results of the present studies are presented, where possible, in the form of treatment means together with the associated standard errors of differences between means (sed). In those experiments with more than two treatment groups with uneven replication (for example, Section 8.2), it was not possible to give single sed. Instead error mean squares (ems) are provided. Analysis of variance was carried out using BMDP statistical packages. When significant variance ratios were detected, differences between treatment means were tested using the least significant difference procedure.

Further details on statistical methods are provided, where necessary, in the individual experimental sections.

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