THE DIGESTIVE SYSTEM IN MAMMALS: FOOD, FORM AND FUNCTION

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22

The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective

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Mammalian herbivores encounter a diverse range of allelochemicals in their diets (Palo and Robbins, 1991). Clearly, theories of foraging behaviour and diet selection must focus on the effects of these substances as well as on nutrients and energy (Belovsky and Schmitz, 1991). This is particularly true for folivores and other browsers, because it is the leaves of trees and woody shrubs that contain the greatest concentration of allelochemicals (Bryant *et al.*, 1991; Cork and Foley, 1991; Meyer and Karasov, 1991).

Several studies have demonstrated that some allelochemicals are strongly deterrent to some herbivores (Clausen *et al.*, 1990, Bryant *et al.*, 1991, 1992), but many other species ingest significant quantities of, mainly carbon-based, allelochemicals (Cork and Foley, 1991; Meyer and Karasov, 1991; McArthur *et al.*, 1991). In most cases, the effect of these allelochemicals on food intake is unknown. This sometimes reflects problems in the analysis of allelochemicals in plants, but also reflects a lack of knowledge of the effects of ingested compounds on animals. Therefore, the occurrence, effects and costs of ingestion of the allelochemicals contained in different plants available to herbivores must form a central part of any theory of mammal–plant interactions.

The effects of allelochemicals on insect herbivores are much more widely documented (e.g. Bernays *et al.*, 1989); small size makes insects amenable to growth studies and the diets of many insects can be manipulated easily. In contrast, vertebrate herbivores are often difficult to maintain for detailed investigation of the effects of different diets on health and metabolism.

In order to understand the role of allelochemicals in mammal–plant interactions, we need to know the effects that they have on the animal and, in particular, what price the animal pays to ingest and excrete the compounds. Animals process allelochemicals in different ways and the effects and cost of excretion are not the same for all species. Understanding species differences should help us to understand separation of diets, food selection and nutritional ecology.

In this article we do not review the numerous studies that have sought correlations between diet selection and allelochemical content, nor do we discuss the occurrence of allelochemicals in different plants. There are recent reviews of these topics in Palo and Robbins (1991). Rather, we review, from an ecological perspective, what is known of the effects of ingested allelochemicals on mammalian herbivores and suggest some ways that the costs of ingestion and excretion can be measured.

Allelochemicals

Most theories of plant defence have divided allelochemicals into two functional groups. Originally, **toxins** were separated from **digestibility reducers** (Feeny, 1976; Rhoades and Cates, 1976). A more recent division separates **mobile** from **immobile** allelochemicals (Coley *et al.*, 1985), although both divisions produce similar groupings of chemicals. Toxic compounds are small molecules that are rapidly turned over in plants and are, therefore, mobile defences. On ingestion, they are usually absorbed from the gut and exert a specific toxic effect on the consumer. Typical examples are alkaloids, cyanogenic glycosides and non-protein amino acids. In contrast, digestibility reducers are larger molecules that are metabolically inactive in the plant and so are immobile defences. Their site of action in the consumer is supposed to be within the gut and they are thought to interfere with digestion of other nutrients. The best examples are tannins (Bernays *et al.*, 1989).

The effect of these particular allelochemicals on mammals, however, is conditional on the consumer (McArthur *et al.*, 1991). That is, some compounds act as toxins or as digestibility reducers depending on the way in which the consumer deals with them. Therefore, dietary separation may be explained by differences in the effects of allelochemicals in different herbivore species. This is because different effects result in different costs for ingesting the same group of compounds and, consequently, different feedbacks influence diet choice. Two examples illustrate this point.

Tannins have traditionally been regarded as digestibility reducers through their ability to form complexes with dietary and other proteins in the gut. There are many clear examples where ingested dietary tannin reduces protein digestibility (Glick and Joslyn, 1970; Lindroth and Batzli, 1984; Robbins *et al.*, 1991). Nevertheless, there is increasing evidence, both direct and indirect, that indicates that certain tannins sometimes are degraded and absorbed and

thus act as toxins (O'Brien *et al.*, 1986; Mehansho *et al.*, 1987a, b; Clausen *et al.*, 1990; McArthur and Sanson, 1991; Hagerman *et al.*, 1992). How a tannin functions partly depends on its chemistry. For example, the condensed tannin quebracho reduces protein digestion in sheep and deer, whereas the low molecular weight gallotannin tannic acid does not (Hagerman *et al.*, 1992). Tannic acid is more easily degraded than quebracho, and it has been suggested that degradation occurs before strong protein–tannic acid complexes are formed. The resultant low molecular weight phenolics may be absorbed (Hagerman *et al.*, 1992). Consequently, in this example, tannic acid is functionally a toxin whereas quebracho is a digestibility reducer.

The effect of tannins also depends on the physiology of the consumer. Quebracho reduced the digestibility of protein in ruminants (Robbins *et al.*, 1987) and in some macropodoid marsupials (C. McArthur and G. D. Sanson, unpublished data), but it had no such effect in two marsupial possums (C. McArthur and G. D. Sanson, unpublished data). We believe this is because the possums deal with the tannin differently. Tannin–protein complexes appear to be broken down in the gut of common ringtail (*Pseudocheirus peregrinus*) and brushtail possums (*Trichosurus vulpecula*) because not all of the quebracho is recovered in the faeces. This suggests that the animal has absorbed a part of the quebracho phenolics. Again, quebracho tannin functions as either a digestibility reducer or a toxin, depending on the physiology of the consumer.

The second example concerns volatile monoterpenes which are a common component of many woody plants such as *Eucalyptus* ((Foley *et al.*, 1987) and *Artemesia* (sagebrush; Welch *et al.*, 1982). Monoterpenes are widely known for their anti-microbial effects, and early studies (Nagy and Tengerdy, 1968; Oh *et al.*, 1968) showed that some monoterpenes from sagebrush and Douglas fir (*Pseudotsuga menziesii*) could inhibit the cellulolytic activity of rumen microbes *in vitro*. Monoterpenes were consequently regarded as digestibility reducers (Nagy and Tengerdy, 1968, Connolly *et al.*, 1980). However, later studies *in vivo* found that monoterpenes were rapidly absorbed from the gut before exerting significant effects on microbes (Welch *et al.*, 1982; Foley *et al.*, 1987). Although absorption avoids possible digestibility-reducing effects, the absorbed compounds can now exert a toxic action: potentially disrupting cell membranes and causing liver damage (McLean *et al.*, 1993). The animal must rapidly excrete the compound from the body and this is presumed to involve some cost. These results suggest that the principal mode of action of monoterpenes is not an effect on digestibility but more likely a toxic action.

These examples illustrate the difficulty of trying to predict the effect of an allelochemical based solely on its chemistry. An understanding of both the chemistry of the allelochemical and the physiology of the consumer is essential for determining the cost of allelochemical ingestion.

Methods of dealing with allelochemicals

The effect of a particular allelochemical depends on the amount ingested and the rate and degree to which it can be neutralized or eliminated from the body. How quickly and to what extent this occurs depends on the method used to deal with it.

Salivary modification

Mammalian saliva plays a variety of roles including digestive, protective and buffering (Mandel, 1987). In human saliva, there is a group of 'proline-rich' proteins and peptides that influence mineral homeostasis (Bennick, 1982; Mandel, 1989; Madapallimattan and Bennick, 1990). Similar salivary proteins have been observed to interact strongly with dietary tannins and they are now more generally referred to as tannin-binding salivary proteins (TBSP) (Austin *et al.*, 1989).

Austin *et al.* (1989) postulated that TBSP form stable complexes with tannin in the gut and that these complexes are resistant to degradation in the gut. TBSP have a higher affinity for tannins than do many other proteins (including the major plant protein ribulose bisphosphate carboxylase/oxygenase), because of their open conformation and enhanced hydrogen bond acceptor capacity (Hagerman and Butler, 1981). Consequently, tannin binds preferentially with TBSP even where there is an excess of other proteins. It has been assumed, but not explicitly demonstrated, that TBSP also bind more tannin per unit protein. This idea is supported by recent studies which showed that, in the presence of tannins, more protein is digested in those species that possess TBSP than in species which lack them (Robbins *et al.*, 1991).

The occurrence of TBSP has been examined more extensively among eutherians than marsupials. In eutherians, the production and effectiveness of TBSP varies and is loosely related to an animal's feeding niche. TBSP are constitutive and effective in browsers, e.g. deer, moose, beavers (Hagerman and Robbins, 1992), and some omnivores, e.g. humans (Mehansho *et al.*, 1987b) and bear (Hagerman and Robbins, 1992); they are inducible in other omnivores, e.g. rats (Mehansho *et al.*, 1983) and mice (Mehansho *et al.*, 1985); or uninducible by tannins and, therefore, ineffective in grazers, e.g. cattle (Austin *et al.*, 1989) and others, e.g. hamsters

(Mehansho *et al.*, 1987a). Some TBSP may have a high affinity for a variety of structural types of tannin, while others bind with a fairly restricted group of tannins which are apparently chemically similar (Hagerman and Robbins, 1993). There is some evidence that specialist browsers produce specific TBSP while generalist browsers produce non-specific TBSP (Hagerman and Robbins, 1992).

Preliminary evidence in marsupials (C. McArthur and G. D. Sanson, unpublished data; C. McArthur, A. M. Beal and G. D. Sanson, unpublished data) suggests that browsing macropodoids (e.g. Tasmanian pademelon *Thylogale billardieri*) produce TBSP but grazing macropodoids (e.g. red kangaroo *Macropus rufus*) do not. These marsupials, thus, show a similar pattern to ruminants. However, in folivorous hindgut-fermenting marsupials, (koala *Phascolarctos cinereus*, greater glider *Petauroides volans*, common ringtail possum, common brushtail possum) TBSP, although sometimes present, appear functionally unimportant. Proline-rich proteins from the parotid salivary glands of koalas and ringtail possums have an extremely low affinity for tannins compared with TBSP from other animals (Mole *et al.*, 1990). Furthermore, there appears to be little or no protein cost when these folivores consume a tannin-rich diet (Foley and Hume, 1986; McArthur and Sanson, 1991 and unpublished data). Interestingly, brushtail possums do produce non-specific TBSP (C. McArthur, A. M. Beal and G. D. Sanson, unpublished data) but the parotid gland responsible for their production and secretion is very small and flow rates are low (A. M. Beal personal communication). Consequently, we suggest that TBSP are functionally useless in brushtail possums. This observation emphasizes that TBSP not only have to be produced but also they have to be secreted in sufficient quantities to bind the tannin.

There are three advantages to producing TBSP. First, smaller amounts of protein are lost in the faeces of animals with TBSP than in animals which lack them. Secondly, there may be a saving of dietary essential amino acids when salivary protein–tannin complexes are excreted because proline is a 'cheap' non-essential amino acid. This may be particularly important for hindgut fermenters that have a greater reliance on dietary amino acids. Thirdly, by forming stable salivary protein–tannin complexes, tannin is no longer susceptible to degradation and absorption (Robbins *et al.*, 1991), so the risk of converting a digestibility reducer to a toxin is reduced. While the relative cost of producing specific or non-specific TBSP has yet to be investigated, there is presumably some advantage to specialist browsers in producing proteins which can bind specific tannins. An animal feeding on a diet with few tannin types may realize a greater protein saving if salivary

proteins with high specificity bind a greater proportion of the tannin per unit protein than proteins that are non-specific. This argument, however, relies heavily on what might be a very small protein pay-off. It remains to be determined what, if any, are the benefits of specific TBSP. Competitive binding assays comparing specific versus non-specific salivary proteins may resolve this point, by indicating the relative capacity of each type for particular tannins.

In conclusion, salivary modification plays a role in some species in dealing with dietary tannins. In animals where TBSP are produced, toxic costs of tannins may be avoided and protein costs are reduced but still measurable. The effectiveness of TBSP may depend on having sufficiently high salivary flow rates and these may have evolved for other needs (for example buffering of forestomach contents in ruminants and macropodoids). The apparent lack of functional TBSP in folivorous, marsupial, hindgut fermenters may be due to a greater selective pressure in these animals to conserve protein. *Eucalyptus* foliage has a low nitrogen content and those species that feed on *Eucalyptus* may have offset the cost of excreting TBSP by evolving other mechanisms for dealing with tannin (McArthur and Sanson, 1991).

Microbial modification

Most mammalian herbivores house large microbial populations in expanded regions of their guts: the forestomach and/or the caecum–proximal colon. The microbial degradation of cellulose and associated carbohydrates is generally viewed as the primary role of these symbionts. However, it has also been suggested that microbial detoxification of ingested allelochemicals was a major evolutionary force in the development of such symbiotic relationships (Janzen, 1979).

Gut microorganisms have been shown to detoxify a range of allelochemicals including oxalates, alkaloids, cyanogenic glycosides and non-protein amino acids (O'Halloran, 1962; Galtier and Alvinerie, 1976; Smith, 1986). The potential importance of microbial detoxification is demonstrated by studies in Australian goats fed the shrub legume *Leucaena leucocephala*. The use of *Leucaena* as a stock food in Australia was limited by the presence of the toxic amino acid mimosine. In contrast, goats from Hawaii fed on *Leucaena* without problem. Experiments showed that the infusion of rumen fluid from Hawaiian goats into Australian goats negated the toxic effect and all treated animals started to eat the plant within hours (Jones and Megarrity, 1986). In this case, a specific microorganism existed that could detoxify

mimosine. This spectacular result has led to a number of projects to engineer rumen microbes able to detoxify other allelochemicals (Smith, 1992).

It is possible that wild species harbour other microbes that may be equally effective in expanding an animal's feeding niche. Osawa (1992) has recently identified a biotype of *Streptococcus bovis* in the caecum of koalas which degrades protein–tannic acid complexes but it is not known whether the bacterium uses the protein or the tannic acid (or both) as substrate. Differences in microbial detoxification capacity are unlikely to be found between individuals within a population but it may be worthwhile comparing different populations within a species if significant differences in food choice are observed.

The role and importance of microbial detoxification depends in part on the nature of the ingested allelochemical and the digestive physiology of the animal. For example, some allelochemicals are actually made more toxic following microbial modification in the foregut (O'Hara and Fraser, 1975; Carrlson and Dickinson, 1978). Other compounds with antimicrobial actions, such as terpenes, may in fact be best dealt with by rapid absorption from a simple stomach rather than interaction with the microbial population. Furthermore, many ecologists have argued that herbivores with an extensive foregut fermentation may be better able to cope with diets rich in allelochemicals than hindgut fermenters because the foregut microbes act as a first-line of defence (Janzen, 1979; Langer, 1986). In contrast, hindgut fermenters are thought to be at a disadvantage for microbial detoxification. This notion has been very influential in the study of mammalian folivory (McKey *et al.*, 1981; Waterman *et al.*, 1988; DaSilva, 1992).

The most widely cited example of the potential usefulness of microbial detoxification in the foregut is the degradation of pyrrolizidine alkaloids in the rumen of sheep (Russell and Smith, 1968; Lanigan and Smith, 1970). However, studies by Cheeke (1984) showed that resistance to intoxication from pyrolizidine alkaloids was not simply a function of digestive anatomy. He found that while some foregut fermenters were resistant to the toxic effects, others were susceptible and a similar difference was observed in hindgut fermenters. For pyrrolizidine alkaloids at least, the potential toxic effect does not depend on the site of microbial action.

While the detoxifying ability of microbial symbionts is undoubtedly important in expanding the feeding niches of some species, it seems unwise to regard one system or the other as superior. Certainly, the evidence for a consistent advantage of foregut fermentation for detoxifying allelochemicals such as alkaloids is equivocal and cannot be used to explain the evolution of foregut fermentation in different groups.

Biotransformation of absorbed compounds

Allelochemicals that are absorbed from the gut usually undergo transformation prior to excretion. Compounds that are absorbed across the gut are usually lipid-soluble, non-polar compounds while those excreted in urine or bile are water soluble. To this extent, the general nature of the biotransformational processes are similar in all mammals (Caldwell, 1982). However, the particular enzymatic pathway and the level of enzymatic activity vary widely amongst species.

Biotransformation processes usually occur in two steps. The first step (often called a Phase I reaction) serves to introduce, or expose within the structure of the allelochemical, a functional group and this mostly (but not always) results in a less toxic product. The second step (Phase II) conjugates this modified product with a small molecule such as glucuronic acid, sulphate or glycine (Caldwell, 1982). The general nature of these processes is well known and was described in Freeland and Janzen's (1974) seminal work. Toxicologists have continued to study the mechanisms involved in great detail. However, given the potential importance of biotransformational processes in the interaction of mammals and woody plants, it is surprising that so few studies have been performed in wild species. In contrast, study of the role of biotransformational enzymes in insect–plant interactions is considerably more advanced. There is compelling evidence that differential toxicity of allelochemicals to insects is linked to differences in the pattern of activity of biotransformational enzymes (Lindroth, 1991; Brattsten, 1992).

The biotransformational systems of mammals, like those of insects, are highly complex. However, studying the mammalian systems from an ecological viewpoint is much more difficult. Some recent data on domestic animal species gathered and reviewed by Smith (1992) are relevant to the issues involved here.

1. The level of activity of biotransformational enzymes varies greatly amongst species and cannot be explained by phylogenetic or dietary groupings (e.g. carnivore/herbivore).
2. The inducibility of biotransformational enzymes makes it difficult to determine, from studies under standard conditions, what the effects of prior exposure to particular toxins will be (Smith, 1992). Almost all studies to date have been of domestic species and have used standard or model substrates rather than a relevant plant allelochemical. Little consideration has been given to the effects of prior exposure to the compound.
3. The site of biotransformational enzymes may be significant: the gut mucosa may be far more important in the biotransformation of ingested

allelochemicals than has been previously appreciated. For example, the activity of one biotransformational enzyme (UDP glucuronosyl transferase) was three times greater in the rumen wall than in the liver of sheep and cattle and many other enzymes in ileal tissue showed high activity. Given that the gut is the first organ exposed to ingested allelochemicals, these results may be very significant.

4. The action of biotransformational enzyme systems may sometimes result in toxication rather than detoxification, although this is relatively rare.

The possibility that biotransformational enzymes are induced by dietary constituents together with the effects of gender (e.g. Bergeron and Jodoin, 1991), developmental stage and nutritional status (Parke and Ioannides, 1981; Boyd and Campbell, 1983) suggests that it will be difficult to make specific predictions about the role of biotransformational enzymes in ecological interactions between plants and mammals. Nonetheless, given the probable significant interaction between nutritional state and biotransformational capacity, we urge that this aspect of the system be given attention. Factors such as body fat content, level of protein intake and mineral status can all affect the capacity of biotransformational enzymes. We know of no ecologically relevant example where these interactions have been studied.

Such a study will require us, initially, to accept the detailed mechanisms as a 'black-box' and to concentrate on the end-products of these processes. In effect, we need some index of the capacity of the biotransformational system in intact animals that can be measured under different nutritional states. Lindroth and Batzli (1983) have suggested a measure of urinary glucuronic acid excretion as a general index of detoxifying ability, but recent studies in arboreal marsupials (McLean *et al.*, 1993) have shown that the majority of terpenes and phenolics ingested as part of a diet of eucalypt leaves are excreted unconjugated. The measurement of total urinary organic acids may be a more appropriate parameter. This is much more likely to account for variable pathways (e.g. carboxylic acids, glycine conjugates) and the methods involved are relatively straightforward and accessible to nutritional ecologists. The development of indices of detoxification is an important research need in the study of interactions between mammals and woody plants.

Our coverage of the methods used by mammals to neutralize allelochemicals has, of necessity, been selective. However, there is little evidence that mammals use some of the other strategies adopted by insects for dealing with similar compounds. For example, many insects sequester allelochemicals, and, in others, high gut pH (Berenbaum, 1980) and the presence of surfactants (Martin and Martin, 1984) are important counters to allelochemicals. Genetic

resistance through other, unknown mechanisms is certainly important in some cases (King *et al.*, 1978; Smith *et al.*, 1991), but presumably the conservative nature of mammalian physiology limits the adaptive options for dealing with allelochemicals.

Assessing the effects and costs of allelochemicals: what currency to use?

A single allelochemical can have different effects in different herbivores. Therefore, if we are to measure differences in the cost of ingestion and excretion of these compounds, we need to decide how to express the costs. Ingested allelochemicals affect animals on various levels, reflecting different types of costs with different units. We identify four major ways in which these costs can be assessed. First, there are effects related to site: both digestive (pre-absorptive) effects and metabolic (post-absorptive) effects. Secondly, there may be measurable changes in whole-animal nitrogen or energy needs that are attributable to allelochemicals. Thirdly, and more specifically, there may be costs associated with metabolic pathways and routes of excretion of allelochemicals. Finally, on a whole-animal scale, allelochemicals may affect fitness parameters such as growth and reproduction.

Effects of allelochemicals on digestion

The putative effect of tannins on digestion has probably been the most studied consequence of ingesting allelochemicals. Effects on digestibility are easily translated into a measurable cost (reduced protein digestion, reduced fibre digestion, reduced dry matter digestion) that, in theory, should allow simple, quantifiable comparisons between animals.

However, several recent reviews of tannins have failed to identify any clear effects on digestibility (Mole and Waterman, 1987; Bernays *et al.*, 1989). In those cases where effects on digestibility of dietary components have been identified, the mechanism of the effects is not well understood. Inhibitory effects of tannins on digestive enzymes have often been suggested as an important mechanism, but several studies have questioned this because of the apparent lack of free tannin available to bind digestive enzymes in the small intestine or caecum-colon (Foley and Hume, 1986; Mole and Waterman, 1987; Blytt *et al.*, 1988) or because of the apparent insensitivity of membrane-bound enzymes to condensed tannins (Blytt *et al.*, 1988). The limited effects of tannins on membrane-bound enzymes observed by Blytt *et al.* (1988) suggest that other membrane-associated processes such as nutrient

absorption could also be little affected by tannins. There are few data to evaluate this possibility, but Karasov *et al.* (1992) showed that acute (but not sub-chronic) exposure to the hydrolysable tannin tannic acid inhibited carrier-mediated intestinal uptake of glucose and amino acids in mice. Although Karasov *et al.* (1992) stressed the complex nature of these processes, it may be worthwhile to use this approach in a range of species fed a range of different tannins.

However, digestive costs are often only part of the overall cost of ingesting tannins. There are two other aspects which must be considered. One relates to the fate of the tannin; the other relates to the overall effect of tannin on nutritional components of the diet. Unless it can be demonstrated that no part of the tannin fraction in a diet is degraded and absorbed, the cost is incomplete without considering post-absorptive or total metabolic effects. In some cases, it does appear that the tannin is fully recoverable in faeces, but this is by no means universal. Hydrolysable tannins in particular are often degraded. When tannins become functional toxins, part of the cost of ingesting them will be related to the processes leading to their excretion. As Robbins *et al.* (1991) pointed out, animals with tannin-binding salivary proteins may reduce the toxic cost of tannins. The measurement of toxic effects and costs is discussed in the next section.

Metabolic effects

The physiological effects of allelochemicals on mammalian browsers are poorly defined. Some allelochemicals are so toxic that they kill some herbivores very rapidly. However, this is probably relatively rare. Of greater ecological importance is the concept of toxicity at the sub-acute or chronic level; it has been widely argued that allelochemicals can restrict the amount of food consumed by a herbivore because animals have a limited capacity to detoxify allelochemicals (Freeland and Janzen, 1974; Freeland and Saladin, 1989).

Recently, two major physiological effects linked to the ingestion of allelochemicals have been identified. The first of these effects is a disturbance to the acid–base balance of marsupials fed *Eucalyptus* foliage and the second is a disturbance of sodium balance in lagomorphs fed woody shrubs.

Acidosis in folivorous marsupials

Studies of arboreal marsupials fed *Eucalyptus* foliage have shown that animals eating the leaves of some trees (e.g. *E. radiata, E. citriodora, E. dives*) excrete an acid urine whereas an alkaline urine is excreted following the

ingestion of other species (e.g. *E. ovata*). Acid urines are characterized by a high level of ammonium and a low concentration of urea, a pattern characteristic of a metabolic acidosis. Foley (1992) argued that the acid load arises from the detoxification of terpenes and phenolics that have been absorbed from the diet. Subsequent chemical studies have confirmed this; the major urinary acids are derivatives of terpenes and phenolics found in the diet (McLean *et al.*, 1993; L. Johnson and W. J. Foley, unpublished data). The acid load is formed within hours of a change of diet and thus is clearly diet-related. This pattern has now been established in common ringtail possums, greater gliders and koalas (W. J. Foley, unpublished data) and suggests that diet-induced acidosis is a physiologically normal state in these animals.

Acid–base disturbances have wide-ranging effects on many organs and metabolic processes. For example, the conservation of nitrogen by urea cycling is diminished, muscles may be catabolized when nitrogen intakes are low and the animal is acidotic, and an animal's ability to concentrate urine may be lowered.

While there are certainly some differences between species in the metabolism of allelochemicals, the end-products are always strong organic acids. Therefore, Foley (1992) argued that effects similar to those seen in marsupials should be observed in other species consuming diets rich in allelochemicals.

Sodium wastage in rabbits

When lagomorphs are fed diets of browse or browse extracts, there is a marked increase in urinary sodium losses. For example, in mountain hares (*Lepus timidus*) fed a range of browse diets, urinary sodium losses resulted in a negative sodium balance (Pehrson, 1983). Similar results have been reported in mountain hares fed heather (G. Iason, unpublished data), mountain hares and European hares (*Lepus europaeus*) fed extracts of birch (Iason and Palo, 1991) and in snowshoe hares fed several browse species (Reichardt *et al.*, 1984).

What is surprising about all these results is that most of the sodium loss occurs via the urine. Normally, urinary losses of sodium are closely regulated by the renal system and the kidney would be expected to reabsorb sufficient filtered sodium to prevent negative sodium balance – unless that loss was obligatory. Pehrson (1983) suggested that negative sodium balance could occur through one of two mechanisms. Sodium imbalance could be due to the effects of a high dietary potassium intake. However, there is no evidence that the levels of potassium in any of these diets was notably high. Pehrson's

second hypothesis was that many browse plants contain (unspecified) compounds that lead to sodium leakage. A third possibility is that sodium is lost as a result of an acidosis similar to that described in marsupials.

In contrast to most other species, lagomorphs appear to have a reduced capacity for ammoniagenesis during acidosis (Richardson *et al.*, 1978). We predict that all the lagomorph species in which sodium imbalance has been observed were excreting a load of organic acids from detoxified phenolics. Given the limited ability of lagomorphs to augment urinary ammonium, it may be that sodium is exchanged for hydrogen and excreted in the urine and that this is responsible for the negative sodium balance.

If sodium wastage in lagomorphs has a similar cause to the acidosis seen in folivorous marsupials, we will be better able to understand the effects of absorbed allelochemicals in mammals. Instead of having to understand the effects of the many different allelochemicals in the diet, we may be able to focus on the end-products of their metabolism. Since acid–base homeostasis is the most important regulatory necessity of any animal, processes that threaten it should be closely controlled. In particular, the speed at which acid metabolites are excreted is important. Acidic metabolites must not be allowed to accumulate and drive down systemic pH. In the next section, we briefly review the importance of kinetic studies of allelochemical excretion.

Rate of elimination of allelochemicals

The toxicity of an absorbed allelochemical depends on its concentration at the sites at which it causes damage. The rate at which it can be transformed or eliminated from the body is therefore an important factor in determining the degree of toxicity.

The speed of excretion of allelochemicals depends on a wide range of factors such as whether the allelochemical is metabolized before absorption, its lipid solubility and whether the metabolite is excreted in bile or urine or both. Details of these processes can be found in most recent textbooks of pharmacology (e.g. Klaasen and Rozman, 1991).

However, from an ecological perspective, the effect of body size on detoxification processes is particularly important. Walker (1978) has shown a relationship between body mass of mammals and the rate of metabolism of xenobiotics. Smaller species are capable of more rapid detoxification of xenobiotics than larger species. Freeland (1991) has used these data to argue that small species are more likely to evolve specialized food habits because they can maintain lower concentrations of allelochemicals in the plasma.

A slow rate of excretion of an allelochemical from the body increases the potential toxicity of the compound (Klaasen and Rozman, 1991), and therefore increases the cost. We suggest that examining the elimination characteristics of allelochemicals will be useful for estimating costs of consumption of simple or mixed diets. For example, explaining the limited intake of a single plant species may depend on establishing the intake of allelochemicals in that diet at which the detoxification or excretion pathways are saturated. Similarly, a common argument to explain the mixing of certain plant species in the diet of a herbivore is that it avoids overloading any one particular pathway for detoxification and elimination (Freeland and Janzen, 1974). Comparing elimination characteristics of allelochemicals from single plant species may reveal excretory differences related to pathway saturation. Intuitively, saturation of pathways at lower intakes implies greater costs for the consumer.

These ideas have been canvassed before (e.g. Freeland and Janzen, 1974; Freeland, 1991) but we are not aware of any studies that have attempted to explain the differential utilization of plants by mammals in these terms. We believe that this approach could be of great utility in studying the effects of allelochemicals on food selection and intake, and we urge its adoption.

Energy budgets and energy metabolism

Energy has been used as the currency for assessing the costs of allelochemicals in a number of studies (Cook *et al.*, 1952; Cork *et al.*, 1983; Foley, 1987). The advantages of doing so are that the energy intake and expenditure of the whole animal can be measured readily. Decreases in the energy available to the animal then represents an integrated cost of the ingestion and excretion of allelochemicals. The nutritional value of foods has traditionally been measured in terms of the metabolizable or net energy yield and so, in theory at least, it should be possible to express the costs of ingestion and excretion of allelochemicals in an ecologically meaningful way.

For example, Cook *et al.* (1952) and Foley (1987) showed that animals fed tree leaves rich in terpenes lost 40–50% of the digestible energy in the urine whereas on other diets the loss was only 10–15%. The difference was attributed to the excretion of metabolites of the ingested terpenes. On the surface, the cost of excreting terpenes appears to be enormous but it should be remembered that the gross energy of terpene-rich diets is significantly greater than other diets. Therefore, the net cost of excreting terpenes may not be nearly so great. Without detailed chemical examination of the urine, it is difficult to apportion the urinary energy loss to determine the 'energy

increment' of the excretion of absorbed allelochemicals from these sort of data.

One disadvantage of measuring costs in terms of overall energy retention is that the results tell us little about the energy-demanding processes involved. Of more value are concurrent measures of heat production, but even then it is hard to attribute changes in whole animal heat production to the effects of a particular allelochemical.

This approach has been little used but one example illustrates both the utility and problems of such measures. It has been known for some time that the previous nutritional history of an animal can affect its basal metabolic rate (e.g. Marston, 1948). However, Thomas *et al.* (1988) observed that when voles (*Microtus pensylvannicus*) were maintained on diets containing allelochemicals, fasting or basal metabolic rate was significantly higher than in animals fed diets lacking allelochemicals. These data suggest that there is some carry-over effect of the allelochemicals that affects basal energy expenditure. It is possible that part of the reason is an increased protein synthesis for the production of biotransformational enzymes.

In future it will be necessary to partition the effects of allelochemicals far more carefully when measuring energy retention and heat production. For example, some allelochemicals can be excreted without metabolism because of their low pK_a and high water solubility (Scheline, 1978). Is there a measurable increment in heat production as a result? Manipulation of the routes of administration of allelochemicals (such as direct infusion into the bloodstream) may allow measurement of the costs of microbial detoxification. Careful choice of model allelochemicals may allow the costs of Phase I transformations to be measured separately from Phase II conjugations.

Specific nutrients and excretion of allelochemicals

The excretion of allelochemicals from the body may involve a cost in terms of specific nutrients. In mammals, allelochemicals are most often excreted conjugated to small molecules. The conjugating moiety is either a sulphate group (derived from sulphur-containing amino acids), glycine or glucuronic acid. The type of conjugate excreted depends on the nature of the allelochemical, the availability of the specific nutrient and the diet/digestive physiology of the animal.

It is possible to express the cost of excretion of allelochemicals in terms of the amount of conjugate present in the urine (or faeces). For example, Cork (1981) measured urinary glucuronic acid excretion in koalas fed an allelochemical-rich diet (*E. punctata* foliage) and calculated that it represented about 20% of the animals' fasting glucose production. Although it seems relatively simple to measure the quantity of conjugated product in this way and then determine how much nitrogen or carbohydrate it represents, in practice the approach is complicated by a number of factors. These include different routes of excretion and interconversion of the conjugating moieties within the body. For example, many glucuronide conjugates of terpenes are excreted partially in the urine and partially via the bile. Secondly, when loads of allelochemicals are high, the pathways of excretion may be different than at lower doses (Møller and Sheikh, 1983; Klaasen and Rozman, 1991).

Nonetheless, when excretory pathways are well known and when the sources of allelochemicals in the diet are relatively simple, this approach may have considerable utility. For example, Lowry and Sumpter (1987) fed sheep a range of tropical grasses containing varying amounts of phenolic acids (mainly *p*-coumaric and ferulic acids). Following absorption, these acids were excreted as the glycine-conjugated metabolite, hippuric acid. Only minor amounts of other metabolites were observed (e.g. benzoyl glucuronide). Lowry and Sumpter (1987) estimated that urinary excretion of hippurate represented up to 16% of the nitrogen ingested and a significantly greater proportion of the digested nitrogen. In cases where nitrogen availability is limited, this represents a significant cost.

Effects on growth

The ingestion of allelochemicals has been shown to have significant effects on the growth and survival of herbivores. For example, Jung and Batzli (1981) found that voles grew poorly and suffered significant mortality when fed extracts of a range of unpalatable arctic plants. Similarly, Lindroth and Batzli (1984) and Lindroth *et al.* (1986) observed reduced growth rates in voles fed a range of natural phenolics. Although these effects appear to be easily quantifiable, it is difficult to attribute them to any particular action of allelochemicals. This is illustrated by a recent study which showed that warfarin-resistant rats grew much more slowly than other rats but that the growth reduction was connected to differences in metabolic pathways rather than to the 'cost of detoxification' (Smith *et al.*, 1991).

Conclusions

Our understanding of the effects of allelochemicals on mammalian herbivores is still in its infancy. We have learnt that simple classification of plant allelo-

chemicals as either toxins or digestibility reducers is no longer tenable and future theories of plant defence and foraging must accommodate a more dynamic view of the effects of allelochemicals. The effects of any allelochemical on a mammalian consumer will depend on the methods it uses to counteract and excrete the particular compound. Ultimately we need to understand both the chemistry of the allelochemicals and the physiology of the consumer.

Incorporating these ideas into foraging and plant defence theory may be difficult as we cannot yet make broad generalizations about the effects or costs of allelochemicals. Nonetheless, several of the areas identified in this review, such as the occurrence and utility of tannin-binding salivary proteins, the effects of absorbed allelochemicals on acid–base status and the possibility of differences in the kinetics of metabolism and excretion of allelochemicals between species, have the potential to provide this integrating framework. We urge that future studies of the interaction between mammalian herbivores and their food plants try to develop new ways to evaluate the costs of ingestion and excretion of allelochemicals.

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