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CONSEQUENCES OF BIOTRANSFORMATION OF PLANT SECONDARY METABOLITES ON ACID–BASE METABOLISM IN MAMMALS—A FINAL COMMON PATHWAY?

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Abstract—Regulation of acid-base homeostasis is essential for mammals and birds. Biotransformation and metabolism of absorbed plant secondary metabolites (PSMs) results in the production of organic acids that threaten acid–base homeostasis. Consequently these acids must be buffered and excreted from the body. The production of an acid load from detoxified PSMs should occur in herbivorous mammals and birds and with most PSMs and so may provide a unifying theme to explain many effects of PSMs on animal metabolism. Since the organic acids will be largely ionized at physiological pH, disposal of the hydrogen ion and the organic anion may proceed independently. Most hydrogen ions (H^+) from organic acids are eliminated by one or more of three ways: (1) when they react with bicarbonate in the extracellular fluid to form carbon dioxide and the carbon dioxide is exhaled, (2) when they bind to dibasic phosphate and are excreted by the kidney as monobasic phosphate, and (3) when they are buffered and retained in the skeletal system. The secretion of phosphate ions and ammonium excretion are two ways in which the kidney replaces bicarbonate ions that have been eliminated as carbon dioxide. Secretion in the kidney tubule is an important means of excreting excessive organic anions rapidly. This process is saturable and may be subject to competition from a variety of different metabolites. Lagomorphs have limited capacity to form new bicarbonate from ammonium excretion and may therefore be obliged to excrete other cations such as sodium to balance the excretion of organic anions from PSMs. Acidemia has wide-ranging impacts on animals but browsing mammals and birds may have to break down muscle tissues to provide for urinary ammonium in order to generate bicarbonate for

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buffering. Acidemia also can affect the extent of urea recycling. Animals consuming browse diets may have to regulate feeding so that the rate of formation of hydrogen ions does not exceed the rate of disposal. The mechanisms by which this could occur are unknown.

Key Words—Plant secondary metabolites, biotransformation, detoxification, herbivore, marsupial, lagomorph, acidosis, ammonium, phosphorus, sodium, organic acids, kidney.

INTRODUCTION

There is an increasing recognition that plant secondary metabolites (PSMs) are important influences on mammalian foraging and diet selection (Palo and Robbins, 1991). To date, however, our understanding of these interactions is only rudimentary and relies largely on correlations between diet selection and PSM profiles. Some mammals avoid particular plants in their diets because of the occurrence of specific PSMs, but any mammal that feeds on browse cannot avoid ingesting substantial quantities of PSMs such as phenolics and terpenes (Bryant et al., 1992).

Accordingly, the diversity of PSMs ingested by herbivores makes it difficult to measure individual effects. For example, folivorous marsupials eating *Eucalyptus radiata* leaves may ingest up to 60 different terpenes (Foley et al., 1987; Boland et al., 1991) and at least 20–30 broadly different phenolics (Hillis, 1966). With such diverse diets, relating composition and effects is extremely difficult. Clearly some way of characterizing and evaluating the common effects of such a wide range of compounds would be useful.

One of the early dichotomies in the study of plant–animal interactions was between PSMs that exerted a supposed toxic effect and those that acted primarily in the gut to reduce digestibility of foods (Rhoades and Cates, 1976). However, the latter idea has not been supported by detailed studies of digestion and metabolism in a range of animal species. Most investigators now recognize that all classes of PSMs, including broad categories such as phenolics, usually do exert effects beyond the gastrointestinal tract if absorbed (Foley and McArthur, 1994).

Whereas some PSMs are so toxic that they can kill some herbivores quickly (Bryant et al., 1992), most animals are likely to ingest toxic plants that elicit subacute or chronic toxicosis, but the mechanisms are poorly described. Careful studies of the variety of compounds eaten by herbivores may reveal certain adaptive patterns in the metabolism. For example, several studies have now identified apparent thresholds beyond which animals are reluctant to increase their consumption of PSMs (e.g., Jakubas et al., 1993). The best explanation of these effects is that animals would exceed their capacity to biotransform and eliminate toxic PSMs (Freeland and Janzen, 1974).

In this article, we propose that in place of the traditional emphasis on the PSM content of the diet, ecologists should refocus on the biotransformed and excreted metabolites of ingested PSMs (Foley, 1992). Viewed in this way, the effects of absorbed PSMs can be directly explained and evaluated in terms of powerful, existing knowledge about physiological homeostasis and regulation. We emphasize that here we are concerned largely with the PSMs that occur in large concentrations (in particular terpenes and phenolics) that no browser can avoid, rather than alkaloids or cyanogenic glycosides, which typically occur at lower concentrations (Harborne, 1991).

CREATION OF AN ACID LOAD

The effect of PSMs on mammalian herbivores depends on the amount ingested and the degree to which they are neutralized before reaching susceptible tissues. A broader discussion of the effects of PSMs and particular countermeasures employed by mammals can be found in Foley and McArthur (1994).

Most PSMs that are absorbed from the gut must be biotransformed and excreted (Foley et al., 1987). While hydrophilic substances may be readily excreted by the kidney, lipophilic PSMs would tend to accumulate in vivo. Even relatively nontoxic PSMs would eventually produce nonspecific adverse effects at high concentrations. Enzyme systems have evolved to enable animals to transform dietary lipophilic compounds into water-soluble, excretable metabolites (Williams, 1959; Scheline, 1991; Sipes and Gandolfi, 1991).

The principal enzyme systems for metabolizing PSMs are located in the liver and gut wall (Smith, 1992), and they are characterized by an extremely low substrate specificity, in accordance with their function of metabolizing a virtually unlimited variety of substances. Thus most types of PSMs can be biotransformed, including terpenes, phenolics, steroids, and alkaloids.

Biotransformation has two phases and is similar for most vertebrates, although there can be important differences between species in specific reactions. Phase I reactions involve oxidation, reduction, or hydrolysis and introduce or uncover a functional group in the PSM. Phase II reactions are conjugations of the PSM or its phase I metabolite (via a functional group) and an endogenous molecule such as glycine, glutathione, or sulfate, or glucuronic acid. Phase I reactions usually make the PSM more polar, while phase II conjugation almost always produces a very water-soluble and highly ionized product. This greatly increases excretion by the renal or biliary–fecal routes, often involving active transport systems. The chemical change from PSM to metabolite(s) can also alter biological activity, but this is incidental to the increase in water solubility. Often toxicity is lost through metabolism, especially after conjugation, but in some cases the metabolite is more toxic than the parent PSM.

The best understood phase I enzymes are the P-450-dependent mixed-function oxidases (MFOs), which are located in the endoplasmic reticulum (Nebert and Gonzalez, 1987). Four P-450 gene families are considered to have evolved and diverged in animals during the past one billion years in response to their exposure to PSMs. About two dozen enzymes are involved, with overlapping substrate specificities. The types and amounts of P-450 present vary between and within species, and polymorphisms also occur. Glucuronide formation is a major phase II reaction, catalyzed by UDP-dependent glucuronosyltransferase, which has several forms leading to species differences in this reaction (Sipes and Gandolfi, 1991).

Glucuronosyltransferase is also located in the endoplasmic reticulum of the liver, kidney, gut wall, and other tissues, where it may be integrated with P-450 MFOs, enabling an efficient sequence of oxidation and conjugation. Most other conjugating enzymes are found in the cell cytosol. Large-molecular-weight (>350) glucuronides are secreted in the bile, while smaller ones are excreted in urine. Glutathione conjugates are also secreted in bile, while conjugates with sulfate, amino acids, mercapturic acid, and acetate are usually excreted renally.

Conjugation is a key process in our argument because, as well as making the metabolites water soluble, conjugation with glucuronide, sulfate, or amino acids can convert compounds that are neutral or weakly acidic into strong organic acids (Robinson et al., 1953; Scheline, 1991; Sipes and Gandolfi, 1991).

Some examples emphasize the importance of the process. For example, the pK_a of the terpene menthol is about 18 and of menthol glucuronide 3.7; the pK_a of benzoic acid is 4.2 and that of benzoyl glycine (hippuric acid) is 3.7. Significant species differences occur in these phase II reactions, largely related to the type of compound used for conjugation. For example, common brushtail possums (*Trichosurus vulpecula*) conjugate phenol with glucuronic acid, while carnivorous marsupials employ sulfate conjugation (Baudinette et al., 1980). However, the excreted products are strong organic acids.

Not all PSMs are prepared for excretion by being conjugated, and substantial quantities can be excreted in a nonconjugated form. For example, in common ringtail possums (*Pseudocheirus peregrinus*) fed *Eucalyptus radiata* foliage, only a minor proportion of leaf metabolites were excreted as conjugates. The bulk was excreted as polyoxygenated molecules (mainly dicarboxylic acids), which are highly polar and acidic (McLean et al., 1993). Presumably, these dicarboxylic acids are not conjugated because they are not sufficiently lipophilic to reach the site of glucuronosyltransferase in the endoplasmic reticulum membrane.

Other PSMs that may be ingested as acids are often sufficiently polar to be excreted without further metabolism. One of the best examples is salicylic acid, which in many species is largely excreted unmodified (Scheline, 1991). Whereas most alkaloids are bases, some have ester groups that can be metabolized to

acids (Harborne, 1991). Alkaloids generally occur in substantially lower concentrations compared with terpenes and phenols, and accordingly we expect their influence (whether basic or acidic) on acid–base metabolism to be minor.

In summary, the excretion of PSMs is aided by their metabolism to polar acidic compounds usually through phase II conjugation reactions but often through phase I reactions alone. Our thesis, therefore is that the processes of biotransformation and excretion of the vast majority of absorbed PSMs in mammals and birds result in the need to excrete a load of organic acids.

This means that once a compound has been absorbed and becomes a substrate for the MFO system, we can simply regard it as part of an organic acid load. Instead of being concerned with the effects of the native terpene or phenol, the effects may be viewed as those of a metabolically produced organic acid. We believe this is a useful rationalization because the effects of acid loads on animals is well studied and there are clear links between dietary acid loads and whole body protein and energy balance. This may allow nutritional ecologists to integrate better the effects of dietary PSMs with the traditional measures of dietary nutrients.

BUFFERING AND DISPOSAL OF AN ACID LOAD

All acid metabolites will be almost completely ionized at physiological pH (Robinson et al., 1953). Thus, the hydrogen ion and accompanying anion need not necessarily be disposed of together. The hydrogen ion can be either neutralized in the body by reaction with bicarbonate, buffered and retained in the body, or excreted in the urine as titratable acid. The accompanying anion is most often excreted into the urine by the organic anion transport system of the kidney and passed as ammonium or sodium salts. In the ensuing discussion of these processes, we focus on possible rate-limiting steps and, where appropriate, emphasize differences in mechanisms between species. We conclude the section with a discussion of the factors limiting the excretion of PSMs. A schematic illustration of the disposal of H^+ and organic anions is given in Figure 2 below.

Intracellular and Extracelluar Buffers

Ingestion or endogenous production of an acid results in a decline in plasma bicarbonate, which is the major extracellular buffer in mammals. The degree to which plasma bicarbonate concentrations are diminished depends on additional intracellular buffers, especially hemoglobin. In humans and laboratory animals, about half of an acute acid load can be titrated by intracellular buffers (Schwartz et al., 1957). Generally these processes are very rapid, occurring within 4–6 hr of an acute dietary acid load.

Following this initial buffering, respiratory adaptations become important.

Depletion of blood bicarbonate will eventually lead to changes in blood pH and therefore a fall in arterial P_{CO_2}. This in turn will tend to return blood pH to normal levels. Interestingly, the response in terms of plasma bicarbonate is in fact maladaptive (Madias et al., 1977) because hypocapnia induced by the respiratory response will decrease plasma bicarbonate beyond the level induced by the initial acidosis.

Buffering of Acid Loads by the Skeletal System

The skeletal system plays a role in the buffering of acid loads, but there is disagreement as to its importance under different conditions. Enormous reserves of alkaline salts are held in bone, and acidemia dissolves bone, releasing its alkaline, calcium salts. Bicarbonate is also leached from the active surface of bone and drives hydrogen ions into the bone matrix (Barzel and Jowsey, 1969).

Balance experiments over several weeks (Lemann et al., 1965, 1966) have shown that not all acid is excreted or buffered by intracellular or extracellular fluids. The remainder is believed to be buffered by the skeletal system. In humans made acidotic for six days, there was no progressive depletion of plasma bicarbonate, but a substantial excretion of calcium, magnesium, and phosphorus. These and other lines of evidence (Green and Kleeman, 1991) suggest that bone is a sink for hydrogen ions (protecting plasma bicarbonate stores) and the source of the extra urinary calcium. Although the skeletal system is accepted as a buffer of acid in the short term (days to months), there is less agreement about its role in long-term (three to five years) acidosis. Green and Kleeman (1991) suggest that skeletal sodium and potassium are more important in the defense against acute acidosis whereas efflux of calcium carbonate is augmented in the chronic acidotic state. Oh (1991) has argued that bone reserves of alkali salts are too limited to explain long-term responses, and this remains a controversial topic.

At least in the short-term chronic state, however, it is clear that metabolic acidosis leads to losses of bone calcium via an increase in urinary calcium loss. The mechanisms by which this occurs are not fully elucidated, but appear to involve an integrated response of the kidneys with an accompanying increase in the rate of bone resorption (Green and Kleeman, 1991). In lambs made acidotic with dietary ammonium chloride, no changes have been detected in the uptake of calcium from the gut (Abu Damir et al., 1991) even though urinary excretion was elevated.

Differences in the retention of calcium and phosphorus have been observed in lambs eating acid-promoting diets or which have been made acidotic by the addition of ammonium chloride to their food (Abu Damir et al., 1990). Mineral wastage in response to acidosis is important in domestic animal production, but we are aware only of anecdotal evidence to suggest that these effects are realized in browsing species. Robbins (personal communication) observed frequent bro-

ken bones in several species of deer that were maintained on browse diets in captivity but we can not be certain as to the cause. Although Pehrson (1983) and Reichardt et al. (1984) found that hares (*Lepus timidus* and *L. americanus*) were very often in negative calcium balance when fed browse, this was largely because of fecal calcium loss rather than urinary losses. Unfortunately, there are no simple markers of bone metabolism that reflect changes in acid–base status, but attempts have been made to use hydroxyproline as an index of bone mobilization during acidosis in domestic ruminants (Takagi and Block, 1991).

Given the central role played by the skeletal system in buffering acid loads, future studies of mineral balance in response to acid-loading, browse diets could be worthwhile. In such studies it will be important to distinguish between the excretion of endogenous and diet-related sources of calcium.

Excretion of Acid–Urinary Titratable Acid

Only a small percentage of the daily H^+ production can be excreted directly in the urine. In humans, dogs, and rats, the minimum urine pH is about 4.5. The limit to urine acidity is set by the gradient between blood and urine, and evidently the difference between blood hydrogen ion concentration and urine cannot be greater than 1000-fold. There is no reason to suspect that minimum urine pH is any different in wild species.

This being so, all animals need buffers so that the necessary acid load can be excreted in the urine. As with any buffer system, the capacity (in this case acid excretion) depends on the pK_a of the buffer and the total amount of buffer present. Buffers in the urine include organic anions such as citrate, acetate, hydroxybutyrate, and even creatinine (Hamm and Simon, 1987). However, all these have a $pK_a < 6$ and so can contribute little to hydrogen ion excretion over the physiological range of urine pH. Buffers are only effective one unit either side of the pK_a. In contrast, phosphate has a pK_a of about 6.8 (the exact value depends on a range of factors) and so is an ideal buffer at the typical pH values found in mammalian urine (Figure 1). At pH 7.4, 80% of phosphate is in the dibasic form (HPO_4^{2-}) and is capable of buffering hydrogen ions. At pH of 4.8, 99% will be in the monobasic ($H_2PO_4^-$) form. The bulk of the titration occurs between 6.8 and 5.8, so the phosphate can contribute to H^+ excretion at typical urine pH (Hamm and Simon, 1987).

Urinary excretion of phosphate is the major contribution to titratable H^+ excretion and changes in phosphate excretion alter urinary titratable acid (Scheiss et al., 1948). Acute dietary phosphate loading leads to a greater urinary titratable acid in direct proportion to the increase in phosphate excretion. However, this occurs because inorganic phosphate (P_i) stimulates hydrogen ion excretion, not because buffer excretion is a rate-limiting step for H^+ disposal (Gennari and Maddox, 1992). Similarly, animals that are P_i depleted retain part of an H^+

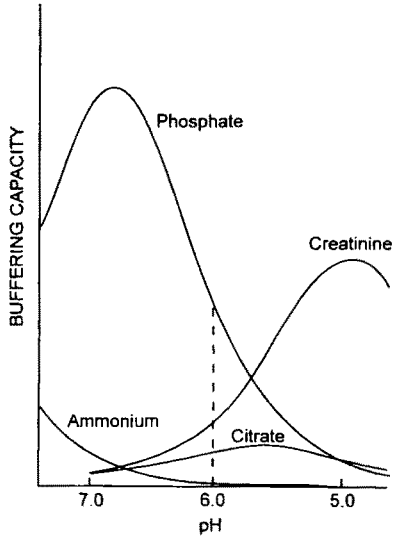

FIG. 1. Schematic representation of the relative importance of urinary buffers of H^+ in common ringtail possums. Concept and data on pK_a from Hamm and Simon (1987). Data for common ringtail possums from Foley (1992), Foley (unpublished), Johnson and Foley (unpublished). Note that ammonium is a poor buffer at typical urine pH and that phosphate is the predominant urinary buffer.

load, but again this is not because buffer excretion is limiting but because P_i depletion inhibits the secretion of H^+ ions in the distal tubules of the kidney. However, during metabolic acidosis in humans, phosphate excretion does increase and so does titratable acid excretion. The mechanisms involved are complex and interact with other factors affecting phosphate excretion, such as dietary phosphorus intake and parathyroid hormone (Hamm and Simon, 1987).

In summary, the excretion of titratable acid is an important route to dispose of excessive plasma H^+, but there is no evidence that acid excretion is regulated by changes in renal phosphate handling. An animal eating a diet that is rich in dibasic phosphate has the potential to excrete more titratable acid depending on how well that phosphate is absorbed. At present it is difficult to say how actively this is regulated.

Several studies of folivorous marsupials (Cork, 1992; Hume and Esson, 1993) and other herbivores (e.g., prairie voles, *Microtus ochragaster*) (Lindroth and Batzli, 1984) and ruffed grouse (*Bonasa umbellus*) (Jakubas and Guillon, 1991) have shown a correlation between diet choice and ratios of phenolics to nitrogen or phenolics to phosphorus in the foliage. Such correlations could be

explained by a need to maintain intakes of nitrogen and/or phosphorus sufficient to provide glutamine for production of new bicarbonate (see below) or for excretion of titratable acid in the urine. Cork and Foley (1991) and Cork (1992) suggested that ratios of nutrients to PSMs are important in relation to toxicity; if ratios are below thresholds, long-term utilization of a diet is not possible. Above the threshold, other factors might determine the selection and utilization of the diet.

Urinary Ammonium—The Measure of "New" Bicarbonate

The augmentation of urinary ammonium excretion is one of the most widespread responses to loading an animal with either exogenous or endogenous acids. For mammals, the major nitrogen-containing compound in the urine is typically urea; ammonium, although present, is a minor constituent. During acid loading, however, ammonium production is increased 10 to 20-fold and forms the dominant part of urinary nitrogen.

Until recently, the idea that ammonium was a urinary buffer of acid was firmly entrenched. Renal physiologists believed that since NH_3 diffuses into the lumen of the kidney tubules and then combines with a secreted H^+ ion, ammonium was in effect a buffer to convey acid out of the body (Pitts, 1964). There are two problems with this scheme. First, metabolism of amino acids gives rise to NH_4^+, not NH_3, so no matter how the resulting ammonium was changed in the body, urinary NH_4^+ could not represent any net transfer H^+ from the body (Atkinson and Bourke, 1987). This is shown below for alanine (equation 1).

$$CH_3-\underset{\displaystyle NH_3^+}{\underset{|}{CH}}-COO^- + 3O_2 \rightarrow 2CO_2 + HCO_3^- + NH_4^+ + H_2O \qquad (1)$$

Secondly, the pK_a of the NH_3/NH_4^+ buffer system is 9.2, so at physiological pH almost all the ammonia is in the acid form (NH_4^+) and no buffering could occur. It follows that ammonium does not in any way carry acid out of the body as many textbooks still claim. Nonetheless, ammonium does play a very special role in acid–base regulation. To understand how this is so, we need to consider the biochemistry of ammoniagenesis (Figure 2).

Urinary ammonium arises from the metabolism of glutamine (and to a lesser extent alanine and asparagine) in the cells of the proximal kidney tubules. Glutamine extracted from circulating plasma is metabolized to NH_4^+ and α-ketoglutarate. The α-ketoglutarate is then metabolized to generate HCO_3^-. The ammonium, after a somewhat elaborate transport process, ends up in the kidney's collecting duct. Therefore, each molecule of ammonium ion that appears in the urine represents a process that also generates one molecule of bicarbonate. If the ammonium from glutamine had been transported back to the liver, it

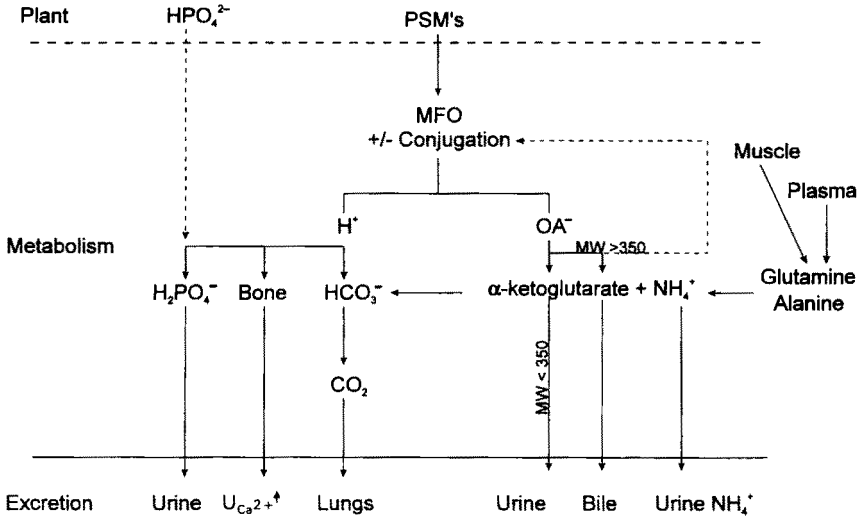

FIG. 2. Pathways of disposal of organic acids produced during the biotransformation of phenols and terpenes in mammals. Note the relationship between the urinary excretion of ammonium and the formation of "new" bicarbonate.

would have been converted into urea (equation 2):

$$2NH_4^+ + 2HCO_3^- \rightarrow H_2N{-}CO{-}NH_2 + CO_2 + 3H_2O \qquad (2)$$

This equation shows that urea formation consumes bicarbonate, and the overall process would have made no change to acid–base status. If, instead, the ammonium is excreted in the urine, then the new bicarbonate from the metabolism of α-ketoglutarate is available to titrate part of the acid load. Therefore, urinary ammonium excretion does not represent H^+ excretion per se, but it is an index of the amount of "new" bicarbonate produced and hence the amount of the acid load that is neutralized in the body.

There are several limitations to the excretion of ammonium as a metabolic response to acidemia that are worth noting. First, it takes some days for ammoniagenesis to reach full capacity. In humans and laboratory animals, this lag time between an acid load and maximum ammonium excretion is on the order of three to six days (Halperin et al., 1992). This means that if an H^+ load is to be produced every day during this adaptation period, then it must either be buffered within the body until there is sufficient ammoniagenesis or else excreted as titratable acid.

The other limitation of renal ammoniagenesis as a counter to metabolic acidosis concerns damage to the kidney that occurs during prolonged periods of

elevated ammonium production (Halperin et al., 1989). These disorders include nephrocalcinosis. Finally, circulating glutamine may be insufficient to meet the demands of ammoniagenesis and additional glutamine may have to be derived from muscle protein catabolism. We will discuss this final point in more detail later.

Renal Organic Acid Secretion—An Active and Saturable Process

Thus far we have mainly discussed the excretion of hydrogen ions arising from an endogenous acid load. Recall that, at physiological pH, organic acids will be almost completely ionized, and so disposal of the hydrogen ion is only part of the solution. It remains to dispose of the organic anion as well.

The kidney plays a major role in eliminating metabolites of biotransformation processes from the body. Many organic metabolites can be filtered from the blood and so become part of the excreted urine. However, of particular importance is the secretion of organic anions by the organic acid transport system of the kidney. There has been a large amount of research to elucidate the nature and controls on these processes (reviewed in Moller and Sheikh, 1983).

The renal clearance rate should be highest for secreted substances, and it can equal renal plasma flow when there is no reabsorption from the kidney tubules, which should be the case for most conjugates. In contrast, clearance by glomerular filtration alone will be much lower even when there is no reabsorption because only 20% of plasma is filtered.

One important feature of the renal transport system is the wide variety of substrates that are accepted—the number of compounds is unlimited and includes many (but not all) glucuronides, hippurates, mercapturic acids, dicarboxylic acids, and hydroxy acids of both endogenous chemicals and metabolites of xenobiotics. As a general rule, conjugated metabolites are far more likely to be actively secreted than their parent compounds (Caldwell, 1982). A good example is the amino acid conjugates of aromatic acids; hippuric acid is actively secreted while benzoic acid is not. Conjugates are also far less readily reabsorbed from the kidney tubule than are the free acids because of their greater polarity and lower lipophilicity (Caldwell, 1982).

Early studies show that secreted anions were capable of competitively inhibiting the secretion of other anions, and so organic acid transport was believed to be a single system (Pritchard and Miller, 1992). This concept is important to our arguments because it suggests that the system can be easily saturated and, in these circumstances, the capacity of the organic anion transporters may set some limit on the speed with which the body can rid itself of organic anions formed by biotransformation of PSMs.

Whether or not there is a single renal organic acid transport system has bearing on our argument that biotransformed PSMs can be seen to have a com-

mon effect. If saturation of the organic anion transport system does constitute a rate-limiting step to the disposal of acid loads, then it is of little importance whether the anions are derived from the PSMs of one plant or another. In other words, animals choosing a mixed diet should not be advantaged necessarily by different pathways of biotransformation unless the cosubstrates of elimination (e.g., glutathione, glycine) are in short supply.

Ullrich and Rumrich (1988) questioned the notion of a single organic anion transport system and have revealed at least three separate organic acid transporters. One system handles oxalate (and sulfates), a second handles dicarboxylates, while the third was elucidated with paraamino hippurate and so corresponds to the classical model of organic anion transport.

Puzzlingly, some mammals (e.g., common ringtail possums) excrete ingested terpenes as dicarboxylic acids rather than as conjugates of glucuronic acids, as might be expected (but see above). However, it is unlikely that this is to avoid saturating the main transport system. The dicarboxylate anion produced by common ringtail possums are all 10-carbon compounds derived from monoterpenes, and the dicarboxylate acid transporter elucidated by Ullrich and Rumrich (1988) is restricted to compounds of five to seven carbons. We would not anticipate many PSM metabolites to be less than 10 carbons, and so it seems unlikely that much capacity is spared by the use of these two alternative organic anion transports. Sulfate conjugates, however, may be able to be excreted via an alternate transporter.

Renal organic anion transport is highly complex, and there are few data of relevance to whole animal metabolism of PSMs. However, in spite of the recent research on alternative transport pathways, it seems reasonable to continue with the classical view of regarding organic anion excretion as a saturable system whose capacity may impose a rate-limiting step on the excretion of anion loads from the body.

Differences between Species—Sodium Wasting in Rabbits

Often, animals are divided into those that excrete net acid in the urine and those that excrete net base. Digestive physiology and the particular metabolism of a species affects whether it will be an acid excreter or a base excreter. The focus of our argument is that some diets may often have an overriding effect on this, but metabolic differences between species can mediate dietary effects.

In this context, the acid–base metabolism of lagomorphs is of particular interest. Many lagomorphs eat diets that expose them to significant levels of absorbable PSMs, and studies of domestic rabbits indicate that most of these are metabolized to acid end products (Williams, 1959; Scheline, 1991). Thus we might expect lagomorphs to display the responses seen in species such as common ringtail possums to endogenously produced acid loads. However,

domestic rabbits cannot normally augment urinary ammonium excretion in response to acid loads (Richardson et al., 1978) and so, even during severe metabolic acidosis, urinary ammonium remains at low levels. In contrast to laboratory rats at least, rabbits convert a greater proportion of dietary components into bicarbonate in the gastrointestinal tract, and this bicarbonate is then absorbed. Consequently, it is possible that rabbits are rarely, if ever, faced with the problem of generating new bicarbonate in the kidneys and so have no need to excrete substantial quantities of ammonium in the urine. Furthermore, there is some evidence (Cruz-Soto et al., 1982) that early exposure to acid-loading diets may alleviate this metabolic defect in domestic rabbits. Thus, browsing lagomorphs may be better able to augment urinary ammonium than their grazing kindred.

To our knowledge, there are no data on net acid excretion (or even urine pH) of any lagomorph fed browse diets. It is difficult, therefore, to evaluate whether rabbits are essentially immune to acidemia resulting from biotransformation processes. Nonetheless, the metabolism of absorbed PSMs will still result in the creation of organic anions and, even if the H^+ formed is neutralized by bicarbonate absorbed from the gastrointestinal tract, the animal must still excrete the organic anions in the urine.

These organic anions must be accompanied by some cation and, in the absence of ammonium, a highly probable alternative cation is sodium or potassium. Consistent with this suggestion is the observation that several species of lagomorphs, when fed browse or browse extracts, excrete substantial quantities of sodium in the urine (Pehrson, 1983; Reichardt et al., 1984; Iason and Palo, 1991) sufficient to ensure that they are in severe negative sodium balance for the duration of the browse diet. In addition, Iason and Palo (1991) found that mountain hares (*Lepus timidus*) lost significantly less sodium in the urine than did grazing European hares (*Lepus europaeus*) when both were fed browse.

Normally urinary losses of sodium are closely regulated by the renal system. The kidney would be expected to reabsorb sufficient filtered sodium to prevent a negative sodium balance unless that loss was obligatory. Pehrson (1983) suggested that negative sodium balance in mountain hares 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 wasting.

We suggest that in lagomorphs, sodium is excreted in the urine in amounts equivalent to urinary excretion of organic anions and that the loss is therefore obligatory. In other words, organic anions arising from biotransformational reactions are excreted as sodium salts in lagomorphs, whereas in most other species they are excreted as ammonium salts.

However, there are alternative explanations that need to be explored. Most of the experiments in which sodium wastage has been observed were of short duration, and it may be that the sodium loss is not sustained in the longer term. In other species, urinary sodium is often elevated in the early days of a metabolic acidosis because it takes several days for renal ammonium excretion to be elevated (Halperin et al., 1992). It should also be noted that changes may have occurred in the animals' extracellular fluid volumes, and this alone may have been sufficient to account for the extra urinary sodium.

EFFECT OF ACIDEMIA ON ANIMAL METABOLISM

Disturbances of acid–base status have wide-ranging effects on biochemical and physiological processes of animals (Hills, 1973; Gennari and Maddox 1992). Very often the effects of acute and chronic acidosis differ, and so it is important to consider which state prevails in each instance. However, metabolic acidosis has wide-ranging effects on organ systems and whole body homeostasis. In particular, effects on the respiratory system, the cardiovascular system, and the skeletal system have been described. There are pronounced effects on carbohydrate metabolism (e.g., an impaired insulin response) (De Fronzo and Beckles, 1979) and on protein metabolism. All these may involve some compensatory response from the animal. However, from an ecological perspective, the effects of most interest are those on protein and nitrogen metabolism.

Protein Metabolism

We have described above the mechanisms that allow the rapid augmentation of urinary ammonium during acidosis. Urinary ammonium is derived largely from plasma glutamine and, to a lesser extent, alanine. The key question involves the source of these ammoniagenic amino acids. Previously, it was thought that there was a quantitative switch between urea excretion and ammonium excretion during acidosis so that total urinary nitrogen excretion remained constant (Hills, 1973). However, there is now clear evidence that in many species and under different conditions, this simple switch from urea to ammonium does not occur and that during acidosis urinary ammonium excretion is increased without changing net urea production. For example, in calves (Scott et al., 1971), red deer (*Cervus elaphus*) (Scott, 1971a), and pigs (Scott, 1971b) ammonium excretion was elevated during infusions of hydrochloric acid, but there was little change in urinary urea excretion.

In common ringtail possums, Foley (1992) found that animals excreting an acid urine lost more nitrogen at similar nitrogen intakes than animals excreting an alkaline urine. These possum data are confounded by the different species of leaf offered to produce the different excretory patterns, and so it is not certain

that the effects observed in other species during acid infusions occur during diet-induced acid loading. An increased loss of urinary N would be a clearly measurable cost of biotransformation and could be linked directly to measures of nitrogen requirements and the suitability of different diets.

When dietary protein levels are adequate, glutamine required for urinary ammonium can be met from circulating plasma glutamine. However, the diets consumed by browsing mammals often contain only maintenance levels of protein. When humans and laboratory animals are acidotic and are consuming insufficient dietary protein, their is insufficient plasma glutamine to meet requirements for urinary ammonium (Hannaford et al., 1975).

The maintenance of acid–base homeostasis is of such importance that animals may break down skeletal muscle to provide the necessary glutamine for ammoniagenesis. Skeletal muscle can supply the necessary glutamine in the short term, but clearly extensive muscle wasting can take place if the acidemia is prolonged and if energy balance is negative. This is why obese humans lose valuable body protein as well as body fat during starvation. The ketoacidosis that develops requires them to consume their muscle to provide for urinary ammonium (Halperin et al., 1992).

Chronic acidosis in children (Cooke et al., 1960) and rats (Williams et al., 1991) can lead to retarded growth. That the growth retardation can be corrected by giving sodium bicarbonate confirms that the effects are linked to the effect of acidosis on whole body protein metabolism. Ruminant herbivores fed silage or grain diets are particularly susceptible to acidosis, and a review of a number of studies suggests that addition of sodium bicarbonate spares body protein and improves nitrogen balance (Phillip, 1986).

In conclusion, there is growing evidence in a variety of species that metabolic acidosis changes the balance between body protein synthesis and breakdown. Studies with rats suggest that these effects are mediated by glucocorticoids (May et al., 1986), but the exact mechanisms have yet to be determined (Reaich et al., 1992).

Recycling of Endogenous Urea

In most herbivores, urea is not a waste product but a valuable source of nitrogen that can be recycled back to the gut and used by microorganisms for growth. It is likely that all mammals recycle a substantial proportion of endogenously produced urea and incorporate the nitrogen into microbial protein, but only foregut fermenting herbivores and coprophagic species can use the microbial protein as a source of essential amino acids. Therefore, the diminution of urea production and recycling could be deleterious to the protein metabolism of these species. The extent of urea recycling is most closely related to rates of fermentation in the gut (Kennedy and Milligan, 1980), but during acidemia,

urea production is substantially reduced and the quantity recycled may be independent of fermentative activity.

Foley (1992) showed that, in common ringtail possums, animals fed on foliage diets that led to net acid excretion (*Eucalyptus radiata*) synthesized and degraded in the gut far less urea than animals fed foliage that resulted in net alkali excretion (*E. ovata*)—even though the nitrogen intake of each group was the same (Figure 3).

If urea recycling is as important to the nitrogen economy of marsupial folivores, as Chilcott and Hume (1984) have argued, then clearly the consumption of acid-producing plants can have a significant impact on the process.

Ability to Concentrate Urine

Many animals fed high protein diets are able to concentrate their urine more than other animals (Jamison, 1981). This has been attributed to an effect of urea in the urinary concentrating process. This accumulation of urea in the interstitium of the kidney medulla allows a greater concentration gradient can be reached than possible when urea is absent. Most folivores eat low protein diets but, in the absence of acidosis, urinary urea concentrations can be substantial (Cork, 1981; Foley and Hume, 1987; Foley, 1992). In cases where acidosis leads to a rapid cessation of urea production in favor of ammonium production, acidotic animals may be unable to concentrate their urine as well as can those in the nonacidotic state. Controlled experiments are needed to evaluate whether the magnitude of the effects could mean a significantly greater water demand for animals during acidemia.

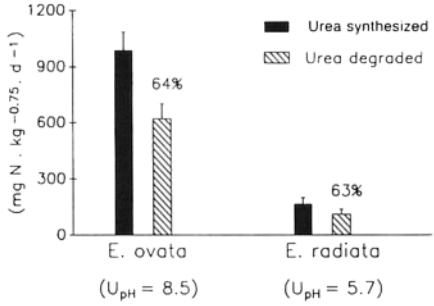

FIG. 3. Effect of diet on urea synthesis and urea degradation in common ringtail possums fed two foliage diets, *Eucalyptus radiata* and *Eucalyptus ovata*. Note the interrelationships between diet, urine pH and urea synthesis and degradation. The percentage value is the proportion of urea synthesized which was degraded in the gut (Foley, 1992).

CAN ACID-BASE HOMEOSTASIS PROVIDE FEEDBACK TO REGULATE FOOD INTAKE?

We have shown that in arboreal marsupials, at least, feeding on diets rich in absorbable PSMs leads to a large organic acid load that must be excreted. The H^+ ions are clearly diet-related and are a consequence of the metabolism and biotransformation of foliage PSMs (Foley, 1992). Given that the maintenance of acid–base homeostasis is among the most important regulatory necessities of any animal, we argue that factors that promote acid production must be controlled so that the capacity to buffer and excrete acids is not exceeded.

We have already discussed the mechanisms that allow animals to buffer and excrete acid loads from the body very rapidly. Although these processes are not a universal panacea (see discussion above on ammonium excretion), the immediate control problem is to prevent the capacity of these buffering and excretory systems from being exceeded. What then are the possible mechanisms that might provide an appropriate signal for the animal to increase hydrogen ion excretion?

Changes in Systemic pH

It is tacitly assumed that a change in systemic pH is the signal needed by the kidney to increase urinary acid excretion (Tannen, 1980). This is a controversial area because important experiments by De Sousa et al. (1974) showed that administration of some acid loads did not lower the blood pH or plasma bicarbonate content of dogs. Doses of hydrochloric acid led to a prolonged decrease in blood pH and plasma bicarbonate, whereas nitric acid had no detectable effects. These results were important in formulating a theory of H^+ excretion that is based on the nature of the accompanying anion. They suggested that since the nitrate ion is poorly reabsorbed, it will be delivered rapidly with endogenous sodium to the distal nephron, whereas anions such as chloride will be largely reabsorbed and reenter the circulation. For example, Bank and Schwartz (1960) showed that sodium-depleted subjects excreted acid urine when infused with sodium sulfate, but that sodium chloride had little effect on urine acidity.

Although these ideas appear to be largely correct, a repeat of the experiments by Madias and Zelman (1986) showed that the failure of De Sousa et al. (1974) to observe declines in blood pH and plasma bicarbonate was because they collected only a single blood sample 24 hr after acid loading. In fact, more frequent sampling showed that animals fed nitric acid did show a decrease in blood pH and plasma bicarbonate but that the effects were only transitory. It would seem, therefore, that although the anion that accompanies the hydrogen ion is an important mediator of the length of acidosis, changes in blood pH cannot be excluded in urine acidification.

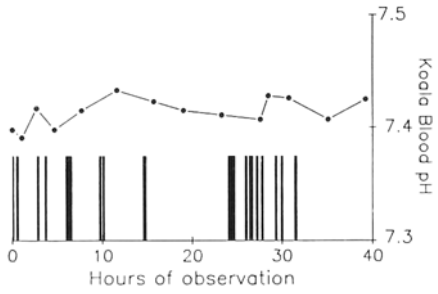

FIG. 4. Arterial blood pH in relation to feeding on *Eucalyptus robusta* by a koala. The vertical bars show duration of feeding bouts. Blood was drawn from an in-dwelling catheter in the femoral artery and analysed within four minutes (Foley and Handasyde, unpublished).

Nonetheless, this does not necessarily mean that systemic pH exerts control over renal acid excretion (Tannen, 1980). The types of anions derived from the biotransformation of absorbed PSMs are, like nitrate, likely to be poorly reabsorbed. In fact, as we have described above, many are secreted by the kidney. Thus any acidemia that results from the accumulation of organic acids, such as after a feeding bout, may itself be only transitory. There are few data to support or refute this suggestion. Foley and Handasyde (unpublished) measured the arterial acid–base status in relation to feeding bouts in a pilot study with koalas (*Phascolarctos cinereus*). Data for a single animal are shown in Figure 4. During these experiments, the animals were excreting urine of pH 5.3–5.5, which contained substantial concentrations of ammonium but the variation in blood pH and plasma bicarbonate were only minor. In addition, there was no relationship between blood pH or bicarbonate and the cessation or intensity of feeding bouts. Although these data are very limited, they suggest that changes in blood pH during the ingestion of browse diets are only small and not involved with the regulation of renal acid excretion or feeding.

Changes in Arterial P_{CO_2}

Changes in the arterial carbon dioxide tension (P_aCO_2) have a direct effect on renal hydrogen ion secretion. High P_aCO_2 depresses renal hydrogen ion secretion whereas the reverse is true under conditions of low P_aCO_2. These effects occur even within the normal day-to-day variation in P_aCO_2, but it is generally believed that they do not mediate routine adjustments in renal hydrogen ion excretion (Gennari and Maddox, 1992).

Aldosterone

The mineralocorticoid aldosterone has been implicated as a controller of renal hydrogen ion excretion because it stimulates hydrogen ion secretion in the distal nephron. An aldosterone deficiency directly depressed hydrogen ion secretion and results in a sustained metabolic acidosis. Whether or not the effects are sufficient to account for day-to-day regulation in acid excretion is uncertain because animals without adrenal glands and given constant doses of mineralocorticoids can respond to acid loads normally (Gennari and Maddox, 1992).

Potassium Status

Changes in potassium supply affect an animal's ability to produce ammonium in the kidney. In laboratory animals and in humans, ammoniagenesis is stimulated by deficiencies of potassium and depressed by potassium loading. However, the differences in ammonium excretion are countered by changes in titratable acid excretion, and there is no change in net acid excretion. Therefore it seems that the potassium status of the body is unlikely to play a major role in controlling renal acid excretion (Tannen, 1980; Gennari and Maddox, 1992).

CONCLUSION

In spite of widely varying daily acid loads that animals can encounter, plasma bicarbonate and systemic pH are kept within very narrow ranges in all homeotherms. A major question is whether these effects are sufficiently great that animals need to regulate their diets in order to control acid production. Humans eating typical diets and with normal renal function probably do not need to control their diets. However, the acid loads of mammals eating diets rich in PSMs are substantially greater. Any theory of food intake must invoke some sort of feedback signal so that intake can be modified, but at present, simple factors such as systemic pH cannot be invoked as part of the feedback process. A similar conclusion has been reached in the study of acid–base effects on silage intake in ruminants (Philip and Simpson, 1991). Nonetheless, even if the regulatory process is not known, it is clear that acidemia is a major consequence of the ingestion of diets rich in PSMs.

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