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J. S. Sorensen · M. D. Dearing

Elimination of plant toxins by herbivorous woodrats: revisiting an explanation for dietary specialization in mammalian herbivores

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Abstract Constraints on rates of detoxification and elimination of plant toxins are thought to be responsible for limiting dietary specialization in mammalian herbivores. This hypothesis, known as the detoxification limitations hypothesis, suggests that most mammalian herbivores are generalists to avoid overdosing on toxins from a single plant species. The hypothesis also predicts that the few mammalian specialists that exist should have adaptations for rapid detoxification and elimination of plant secondary compounds. We took a pharmacological approach to test whether specialists eliminate toxins from the bloodstream faster than generalists. We compared elimination rate and total exposure of alpha-pinene in closely related dietary specialist and generalist woodrats, *Neotoma stephensi* and *N. albigula*, respectively. Animals were orally gavaged with alpha-pinene, a plant secondary compound present in the natural diets of both woodrat species. We collected venous blood at 3, 6, 10, 15, and 20 min post-ingestion of alpha-pinene. Blood was analyzed for alpha-pinene concentration using gas chromatography. We found that specialist and generalist woodrats did not differ in elimination rates of alpha-pinene. However, specialists had lower exposure levels of alpha-pinene than generalists due to lower initial delivery of alpha-pinene to the general circulation. The levels of alpha-pinene detected in the bloodstream of specialists were 4.7–5.3× lower over all time intervals than generalists. Thus, specialists encounter a functionally lower dose of toxin than generalists. We suggest that the lower exposure level of specialist woodrats may be due to mechanisms in the gut that decrease toxin absorption. Regardless of mechanism, lower exposure to plant toxins may allow specialists to forage on diets with high toxin concentrations thereby facilitating dietary specialization.

Keywords Dietary toxin · Elimination · Generalist · *Neotoma* · Specialist

J.S. Sorensen (✉) · M.D. Dearing
Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
e-mail: sorensen@biology.utah.edu

Introduction

Constraints of the mammalian detoxification system are hypothesized to be a primary factor limiting the occurrence of dietary specialization in mammalian herbivores. This hypothesis proposes that mammalian herbivores cannot rapidly eliminate the toxins consumed in a diet of one plant species due to rate-limitations of detoxification pathways (Freeland and Janzen 1974). According to this hypothesis, the amount of any given plant that can be consumed is defined by the rate at which plant toxins can be detoxified and eliminated from the body. Exclusive consumption of a single plant species would result in ingestion of large quantities of similar toxins that would overload detoxification pathways. In contrast, by utilizing a generalist feeding strategy, mammalian herbivores consume small amounts of a variety of toxins that are processed through a diverse set of detoxification pathways without overloading any one pathway. This hypothesis has become firmly entrenched in the ecological literature to the extent that it is accepted as the predominant factor regulating the foraging ecology of mammalian herbivores (Foley et al. 1999; Freeland 1991; Freeland and Janzen 1974). Yet, there have been relatively few empirical tests of this hypothesis (Dearing and Cork 1999; Freeland and Winter 1975). In addition, alternative hypotheses to explain how specialists overcome detoxification limitations have not been identified. As a result, our understanding of how plant toxins impact diet selection in mammalian herbivores remains rudimentary (Foley et al. 1999).

Although no comparative studies have been conducted on specialist and generalist mammals, independent studies suggest that mammals vary in detoxification capacity. Furthermore, variation in detoxification capacity is generally a result of evolutionary exposure to toxins. For example, the detoxification capacity of geographically isolated populations of mammalian herbivores in Australia is thought to be determined by the availability of plants containing toxins (King et al. 1978; Mead et al. 1985; Oliver et al. 1977). Plants with the secondary

compound fluoroacetate are distributed in Western Australia but not Eastern Australia. As a result, western populations of quokka (*Setonix brachyurus*) in contact with plants containing fluoroacetic toxins have a higher detoxification capacity for monofluoroacetate (a pesticide) than eastern quokka populations (Mead et al. 1985). These results suggest that herbivores can evolve higher detoxification capacities that can overcome the constraints and limitations of typical detoxification systems.

We evaluated whether a specialist mammalian herbivore is capable of overcoming detoxification limitations using two species of woodrats (*Neotoma* spp.). We hypothesized that specialist woodrats will have lower systemic exposure to plant toxins than generalist woodrats through faster rates of toxin elimination. We tested this hypothesis by comparing the elimination rate of alpha-pinene in specialist, *Neotoma stephensi*, and generalist, *N. albigula*, woodrats.

N. stephensi and *N. albigula* represent a model study system to initially test detoxification limitations in specialist and generalist mammalian herbivores. These species occur sympatrically, are similar in body size, and have equal access to juniper (*Juniperus monosperma*), which is high in plant secondary compounds (Adams 1994; Adams et al. 1981). Despite these similarities, the two species have disparate foraging strategies in both the field and the laboratory (Dearing et al. 2000; Dial 1988). In the wild, *N. stephensi* feeds almost exclusively on juniper (80–95% juniper) across its range, whereas *N. albigula* is a dietary generalist consuming 24–33% juniper along with other plant species (Dial 1988; Vaughan 1982). In the laboratory, specialists voluntarily consume twice as much juniper as generalists (Dearing et al. 2000).

The differences in foraging behavior between these woodrat species are not due to food availability (Dial 1988) or nutritional constraints (Dearing et al. 2000). Rather differences are attributed to the presence of plant secondary metabolites found in juniper, particularly alpha-pinene. Alpha-pinene is the predominant monoterpene in juniper (2% by dry weight; Dearing et al. 2000). Following ingestion or inhalation, alpha-pinene is readily absorbed due to its high solubility in blood and tissues (Falk et al. 1990a; Filipsson 1996). In mammals, alpha-pinene can accumulate in peripheral fat, kidneys, brain and other organs resulting in central nervous system depression, allergic contact dermatitis, lung function impairment, liver and kidney cysts, and death (Falk et al. 1990b; Savolainen and Pfaffli 1978; Sperling et al. 1967).

The physiological and behavioral consequences of alpha-pinene ingestion in specialist and generalist woodrats are similar to those associated with the intake of whole juniper (Dearing et al. 2000). As with juniper consumption, alpha-pinene has negative physiological and behavioral consequences on generalist woodrats but not specialists. Generalist woodrats (*N. albigula*) decrease food intake when fed a diet containing high concentrations of alpha-pinene. In contrast, specialists (*N. stephensi*) maintain food intake on the same diet. In addition, urine pH is lower in generalists than specialists fed diets containing alpha-pinene, suggesting that the acid-base homeostasis of generalists is challenged to a greater extent (Dearing et al. 2000).

We took a pharmacokinetic approach to test whether specialists are more efficient than generalists at detoxifying and eliminating dietary toxins. We compared the concentration of orally ingested alpha-pinene in the blood over time in specialist and generalist woodrats (*Neotoma* sp.). Evaluating the time course of a toxin provides information about the rate of detoxification and elimination as well as its potential toxicity (Neubig 1990). The rate of elimination is determined from the slope of the concentration-time curve, whereas toxin exposure level is determined from the area under the concentration-time curve. Higher exposure levels to toxins are indicative of toxic effects. We predicted that specialist woodrats would have lower total exposure to toxins from their preferred plant species due to faster rates of toxin elimination than generalist woodrats.

Materials and methods

Animals

Specialist (*N. stephensi*) and generalist (*N. albigula*) woodrats were trapped outside the south border of Wupatki National Park, 45 km NE of Flagstaff, Ariz. (35°30'N, 111°27'W) and transported to the University of Utah Animal Facility. Animals were housed in shoebox cages (48×27×20 cm) with bedding and cotton batting on a 10-h light and 14-h dark cycle at 20°C. All animals were fed Harland Teklad ground rabbit chow (formula 2120) and water ad libitum prior to the study.

Anesthesia and dosing

Nine specialist and seven generalist woodrats were fasted 15 h prior to dosing. Animals had not been exposed to any dietary toxin within 3 months prior to experimentation. Water was provided ad libitum throughout the experiment. Animals were anesthetized with Metafane (methoxyflurane) for approximately 2–3 min until they exhibited uncoordinated movement but no loss of consciousness. This degree of mild anesthesia was necessary for animals to be properly held for dosing. Woodrats were gavaged with a single oral dose of alpha-pinene (128.7 μg/g body mass; Aldrich cat. no. 14,752–4). Because body mass did not differ between specialists and generalists (ANOVA: $F_{(1, 15)}$=2.06, P=0.17), both species received the same oral dose of alpha-pinene.

Alpha-pinene was suspended in peanut oil (34.32 μg alpha-pinene /μl peanut oil) to provide dose volumes between 0.3 and 1.0 ml. The doses were administered by oral gavage using a blunt feeding needle (18 gauge ×5 cm) with a rounded end. Doses represented approximately twice the alpha-pinene dose generalists consume per meal and half the dose specialists consume per meal, based on meal size, percent juniper in diet, and alpha-pinene concentration in juniper (Dearing et al. 2000; Dial 1988). Previous studies showed that both species maintain body weight on a daily dose of alpha-pinene used in this study (Dearing et al. 2000). After dosing, woodrats were immediately placed in ventilated tubes that limited both the movement of woodrats and provided access to the tail for blood collection.

Blood sampling and analysis

Blood samples were collected 3, 6, 10, 15, and 20 min post-gavage from the distal end of the tail. Approximately 80 µl of whole blood was collected in heparinized capillary tubes and transferred into an Eppendorf tube. Blood was immediately placed on dry ice and stored at –20°C prior to analysis.

We conducted preliminary studies to examine the effects of blood source, Metafane, and peanut oil on detection and quantification of alpha-pinene. Blood was collected from specialists, generalists and laboratory rats (*Rattus norvegicus*) and spiked with alpha-pinene. The detection of alpha-pinene in the blood of each of these species did not differ. Because standard curves require large quantities of blood for serial dilutions that could not be collected from live woodrats, we used blood from laboratory rats to prepare alpha-pinene standard curves. Laboratory rats were on a diet free of toxins within 3 months prior to blood collection and had never been dosed with alpha-pinene. Serial dilutions for the standard curve were prepared by spiking blood with alpha-pinene and making concentrations ranging from 0.008 µg/ml to 42 µg/ml. To determine the effects of Metafane and peanut oil, we analyzed 80 µl of blood for alpha-pinene from one specialist and generalist without anesthesia or peanut oil dosing, after Metafane alone, and after Metafane and peanut oil dosing. Alpha-pinene was not detected in the blood of any of these control animals. In addition, the chemical components of Metafane and peanut oil were easily identified as separate compounds from alpha-pinene and did not influence the chromatography of alpha-pinene in experimental animals.

Alpha-pinene in the blood was quantified using headspace-solid phase microextraction (HS-SPME) and gas chromatography (GC) (Hewlett Packard 5890) (Namera et al. 1997). Headspace analysis is preferred to standard extraction procedures due to greater sensitivity of the method for volatile compounds. In addition, standard extraction procedures for terpenes require blood volumes greater than those that could be serially collected from woodrats. Twenty microliters of blood and 20 µl of distilled water were added to CG vials and homogenized. Samples were then placed in a dry bath incubator at 50°C for 3 min. After heating, samples were immediately exposed to a replaceable extraction fiber, coated with 100 µm of polydimethylsiloxane (Supelco cat. no. 57300-U), for 5 min under gentle shaking. The fiber was then exposed to the GC injector for 3 min. Operating conditions were: helium flow 2 ml/min; splitless injection; oven temperature 60°C for 2 min, increasing by 15°C/min to a final temperature of 260°C; injector 250°C; detector 260°C. A blank run was done on the fiber between each sample to insure that the fiber was free of contaminants. Samples were run in triplicate and averaged to determine concentration values.

Pharmacokinetics

Rate of elimination was calculated by determining the slope of the concentration time curve for each species. Slope was calculated using the point-slope method of exponentially decreasing concentrations:

$$\text{Slope} = \ln(C_1/C_2)/(t_1 - t_2) \quad (1)$$

where C is the concentration of alpha-pinene in the blood (µg/ml) and t is time (min). Percentage of the oral dose woodrats encountered 3 and 20 min after oral administration of alpha-pinene was calculated from the dose of mass specific alpha-pinene (µg) administered orally to woodrats and the quantity of alpha-pinene detected in the body (µg) during each time interval (t=3 and 20 min). The quantity of alpha-pinene detected in the body at each time interval was calculated as the product of alpha-pinene concentration (µg/ml blood) at each time interval (from GC analysis) and estimated mass specific blood volume (ml) (Davies and Morris 1993). Total alpha-pinene exposure was determined by calculating the area (µg min^{-1} ml^{-1}) under the concentration-time curve (AUC) with the linear trapezoidal method:

$$\text{AUC} \cong \sum [(Ci + Ci - 1)/2 \times (t_i - t_{i-1})] + C_n/\lambda \quad (2)$$

where C is the concentration of alpha-pinene in the blood (µg/ml), t is time (min), and λ is the negative slope. The equations used in calculating elimination rate and AUC are standard equations employed in pharmacological studies (Biosse and Okamotom 1978; Iwamoto and Klaassen 1977; Klippert and Noordehoek 1983; Weiss 1990).

Statistics

A repeated measures analysis of variance on log-transformed data was used to detect differences in the elimination rates of alpha-pinene between species across all sampling periods. Due to failure to meet assumption of sphericity, a Geisser and Greenhouse adjusted univariate correction was used to test time and interaction effects of the model (Muller and Barton 1989). Independent one-way ANOVAs were used to analyze for differences between species in rate of elimination, maximum alpha-pinene concentration, percentage of oral dose detected at 3 min and 20 min, and total alpha-pinene exposure. All data, other than total alpha-pinene exposure, were log transformed to normalize distributions prior to analysis.

Results

Rates of elimination

Blood levels of alpha-pinene were maximal in generalist and specialist woodrats and declined in a log-linear fashion. Blood concentrations of alpha-pinene decreased by 76.71% for generalists and by 74.43% for specialists between 3 and 20 min (Table 1, Fig. 1). We did not find a significant difference between species in rates (i.e. slope of line) of alpha-pinene elimination (Fig. 1, $F_{(1,\ 15)}$=0.58, P=0.46).

Alpha-pinene exposure

Generalists had higher blood levels of alpha-pinene than specialists across all collection periods as indicated by a significant species effect without a significant interaction effect (Table 1, Fig. 1). The maximum alpha-pinene concentration (t=3 min post-ingestion) detected in the blood of generalists was 5.2 fold higher than maximum concentrations in specialists (Table 2, $F_{(1,\ 15)}$=6.82, P=0.02). The maximum quantity of alpha-pinene (t=3 min) detected in the body was 1.6% of the orally

Table 1 Repeated measures ANOVA results for the experiment comparing the concentration of alpha-pinene in the blood over time for generalist (*Neotoma albigula*) and specialist (*N. stephensi*) woodrats

	F	*df*	*P*
Species	42.1	1, 14	0.016
Time	34.2	1.4, 20	<0.0001
Interaction	2.87	1.4, 20	0.09

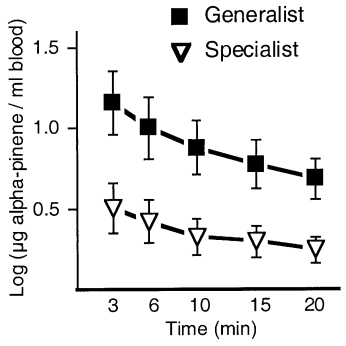

Fig. 1 Concentration (log values) of alpha-pinene in blood of generalist (*Neotoma albigula*) and specialist (*N. stephensi*) woodrats after oral administration of 128.7 µg alpha-pinene / gram body mass. Generalists had higher blood concentrations of alpha-pinene than specialists over all time periods (Tukey HSD, $P<0.05$). *Bars* represent ±SE

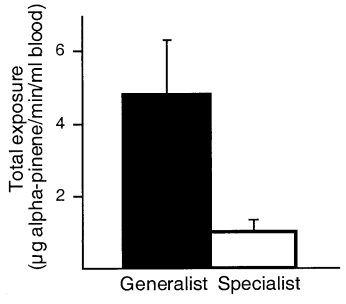

Fig. 2 Total exposure level (AUC in µg min^{-1} ml^{-1}) of alpha-pinene in generalist (*N. albigula*) and specialist (*N. stephensi*) woodrats after oral administration of alpha-pinene. Alpha-pinene exposure was determined by calculating the area under the concentration-time curve from Fig. 1. *Bars* represent ±SE

Table 2 Alpha-pinene as a concentration in the blood and as a percentage of the mass specific dose of alpha-pinene orally administered to woodrats. Values represent maximum concentrations and percentages (t=3 min post-ingestion) of alpha-pinene in generalist and specialist woodrats. SEs are given in parentheses

	Concentration (µg/ml)	Percent of oral dose
Generalist	21.6 (7.9)	1.6 (0.52)
Specialist	4.2 (1.6)	0.26 (0.10)

administered dose in generalists and 0.3% in specialists (Table 2). Moreover, the proportion of orally administered alpha-pinene detected in the body of generalists 3 min after oral administration of alpha-pinene was 5.3 fold greater than in specialists ($F_{(1, 14)}$=9.1, P=0.01). Generalists had a higher overall exposure level to alpha-pinene than specialists (Fig. 2, $F_{(1, 15)}$=7.89, P=0.02) due to initially higher maximum alpha-pinene concentration in generalists coupled with a similar rate of elimination.

Discussion

We tested the hypothesis that specialist woodrats would be more efficient than generalist woodrats at detoxifying and eliminating alpha-pinene, a plant secondary compound common in the diet of both species. We found that specialists and generalists did not differ in elimination rate of alpha-pinene. However, specialists had lower systemic exposure to alpha-pinene than generalists. Lower exposure levels in specialists were due to lower initial concentrations of alpha-pinene in the bloodstream. In the subsequent paragraphs, we discuss the potential mechanisms that could yield similar elimination rates yet different exposure levels and their implications.

Rates of elimination

Similar rates of alpha-pinene elimination in specialist and generalist woodrats did not support the hypothesis that specialists have faster rates of elimination than generalists and suggest that these species have similar detoxification enzymes and activity. The close phylogenetic relationship between specialist and generalist woodrats (Planz et al. 1996) and exposure to alpha-pinene in the diet over evolutionary time may explain these results. Several studies demonstrate that detoxification enzymes and activities may be conserved even among distantly related taxa (Gregus et al. 1983; Ritschel et al. 1991; Riviere et al. 1997; Walker 1978). For example, the elimination rate of tetracycline is similar in humans and sheep (Riviere et al. 1997), and coumarin is eliminated at a similar rate in sheep and monkeys (Ritschel et al. 1991). In addition, preliminary studies comparing liver enzymes of woodrats (*N. stephensi* and *N. lepida*) and laboratory rats (*R. norvegicus*) indicate that these species utilize the same major detoxification pathways (Lamb et al. 2001; Dearing, unpublished data).

Shared evolutionary history is not a requisite for herbivores to have similar elimination rates as evolutionary experience alone can generate similar elimination rates among unrelated herbivores. Several studies demonstrate that elimination kinetics among wild mammalian herbivores is determined by evolutionary exposure to toxins (King et al. 1978; Mead et al. 1985; Oliver et al. 1977). For example, the grey kangaroo (*Macropus fulginosus*) shares a high detoxification capacity for the toxin fluoroacetate with distantly related taxa (brush-tailed possum: *Trichosurus vulpecula*, and bush rat: *Rattus fuscipes*) and has a much higher capacity than a more closely related species (red kangaroo; *Macropus rufus*) (Oliver et al. 1977). Plants with fluoroacetate are present in the habitat of the grey kangaroo, brush-tailed possum, and bush rat, but absent in the habitat of the red kangaroo. These results suggest that phylogeny, evolutionary exposure to alpha-pinene or a combination of both may explain similar detoxification capacities in specialist and generalist woodrats. Further studies are necessary to discern among these possibilities.

Elimination rates of specialists and generalists in this study represent the constitutive capacity of woodrats to detoxify and eliminate an acute dose of alpha-pinene. Elimination rates of alpha-pinene in the wild could be enhanced compared to constitutive capacity due to chronic exposure to dietary toxins. Several studies demonstrate that chronic exposure to toxins increases detoxification capacity (Biosse and Okamotom 1978; Igimi and Nishimura 1974; Khanna and Israel 1980) and intake levels of toxins in mammals (Atsatt and Ingram 1983; Foley 1992). In our study, neither species had exposure to plant toxins within 3 months prior to the start of the experiment. In nature, woodrats are constantly acclimated to dietary toxins and may have induced levels of enzyme activity that differ considerably from constitutive levels (Sipes and Gandolfi 1986). Thus, it is possible that under field conditions, specialists may have much higher elimination rates of alpha-pinene than generalists.

Although the oral dose and resultant concentration of alpha-pinene in the blood may appear extremely low, the doses used are biologically and pharmacologically relevant. The dose used is comparable to amounts of alpha-pinene woodrats naturally ingest in 1–2 meals (Dearing, unpublished data). Assuming alpha-pinene is readily absorbed (Falk et al. 1990a; Filipsson 1996), the low percentage of orally ingested alpha-pinene detected in the blood of woodrats (0.3–1.6%) suggests that the majority of alpha-pinene is detoxified during the first circulatory pass through the liver and excreted as metabolites (Boyes et al. 1970; Falk et al. 1990b; Igimi and Nishimura 1974; Iwamoto and Klaassen 1977; Neubig 1990). Rapid first-pass kinetics and resultant low concentrations of toxin in the blood are consistent with previous studies on alpha-pinene and similar monoterpenes (Falk et al. 1990b; Filipsson 1996; Igimi and Nishimura 1974; Koppel et al. 1981; Sperling et al. 1967). For example, oral administration of *d*-limonene (a monoterpene similar to alpha-pinene) in laboratory rats resulted in maximal blood concentrations that were only 1.8% of the oral *d*-limonene dose, while 60% of the oral *d*-limonene dose was excreted as urinary metabolites (Igimi and Nishimura 1974). In addition, studies demonstrate that even low blood concentrations of alpha-pinene have severe pharmacological consequences (Koppel et al. 1981; Sperling et al. 1967). For example, the dose used in this study resulted in alpha-pinene concentrations in the blood of woodrats that were twice as high as the LC_{50} for laboratory rats (LC_{50}= blood concentration at which mortality occurs in 50% of animals) (Sperling et al. 1967).

Alpha-pinene exposure

Despite similar elimination rates, generalists had a 4.8-fold higher systemic exposure to alpha-pinene than specialists. The major factor contributing to lower exposure levels in specialists compared to generalists was the lower delivery of alpha-pinene to systemic circulation. Delivery of alpha-pinene into circulation can be regulated by absorption, first-pass elimination and distribution into tissues and organs (Minchin and Ilett 1982). Absorption is minimized when a toxin is chemically degraded or physically inactivated in the gut or if faster passage rates of digesta minimize contact time of the toxin with gut epithelia. High activity of detoxification enzymes along the gut wall and in the liver may also minimize delivery of a toxin into circulation. Finally, the distribution of toxins to various tissues can reduce systemic concentrations of toxins.

We argue that specialists do not minimize systemic exposure of alpha-pinene through more efficient liver detoxification or increased distribution of alpha-pinene in non-blood tissues. Higher enzymatic activity in the liver of specialists compared to generalists would have resulted in differential elimination rates, with the slope of specialists being steeper than generalists. This outcome was not demonstrated in this study. Although it is possible that enzymatic activity is higher in specialists than generalists during the time prior to the first or after the last blood sampling periods, this result requires non-linear elimination in specialists. Non-linear elimination is unlikely, as alpha-pinene and similar lipophilic monoterpenes follow linear, first-order elimination kinetics (Falk et al. 1990b; Filipsson 1996; Igimi and Nishimura 1974; Koppel et al. 1981; Sperling et al. 1967). Our results might also be explained by differences in the distribution of alpha-pinene to various tissues. Alpha-pinene is highly soluble in blood and fat and accumulates in adipose, kidneys and brain of mammals (Falk et al. 1990a; Savolainen and Pfaffli 1978; Sperling et al. 1967). If specialists had greater adipose stores or larger kidney and brain mass than generalists, alpha-pinene may rapidly reach equilibrium in these tissues prior to the first blood collection. However, permanent storage of toxin in such tissues is not a viable strategy for minimizing toxin load under chronic ingestion of toxin. If animals stored alpha-pinene in adipose, they would have to continually deposit fat with little catabolism to prevent large concentrations of alpha-pinene from being released into the circulation. Storage of alpha-pinene in organs such as the kidney and brain (Savolainen and Pfaffli 1978) is even more detrimental than adipose storage and would ultimately cause organ toxicity.

Absorption model

We suggest that differences in absorption may explain differences in exposure levels of alpha-pinene in specialist and generalist woodrats. Specialists may have greater capacities of mechanisms in the gut that reduce the amount of toxin absorbed and transported to the liver and general circulation. P-glycoproteins and the cytochrome p450 enzyme, "3A1" in rats (CYP3A1=CYP3A4 in humans) are two candidate mechanisms hypothesized to operate in concert to reduce absorption of toxins and limit bioavailability (Silverman 1999; Sparreboom et al. 1997;

Watkins 1997). These mechanisms have received almost no attention from scientists studying plant-mammal interactions but are well known to pharmacologists (Bellamy 1996; Wang et al. 2001; Washington et al. 2001). *P*-glycoproteins and CYP3A enzymes are associated with the brush border cells of gut epithelium as well as in other tissues such as the liver. *P*-glycoproteins are energy-dependent pumps that efflux a wide range of lipophilic toxins from cells (Washington et al. 2001), while CYP3A is an enzyme subfamily that detoxifies a wide range of toxins. The following model has been proposed for their action in the gut epithelium: *P*-glycoproteins efflux toxins to reduce the concentration inside the cell such that CYP3A can detoxify compounds to less reactive metabolites prior to absorption into the circulation. For example, the presence of *P*-glycoproteins in mice results in a 6-fold decrease in drug exposure levels and an increase in drug recovery in the feces compared to mice lacking *P*-glycoproteins (Sparreboom et al. 1997). Thus, *P*-glycoproteins may reduce toxin exposure levels and increase fecal excretion directly by effluxing toxins out of the cell back to the gut lumen and indirectly by facilitating pre-absorptive detoxification in cells of the brush border (Washington et al. 2001).

Preliminary data suggest that a large percentage of alpha-pinene is excreted unchanged in the feces of woodrats and that specialist woodrats excrete more alpha-pinene than generalists. The concentration of alpha-pinene excreted in the feces of woodrats was 59% and 24% of the oral dose for specialist (n=3) and generalist (n=3) woodrats, respectively. Furthermore, specialists excreted 2.8× more alpha-pinene per gram of dry feces than generalists ($F_{(1, 5)}$=3.8, P=0.12). These preliminary results suggest that absorption, in contrast to rates of detoxification, may be dictating toxin exposure in woodrats. We hypothesize that specialist woodrats minimize overall exposure to alpha-pinene through decreased absorption of alpha-pinene in the gut, not through more efficient liver detoxification or higher tissue distribution. We are currently testing the absorption model and the detoxification limitations hypothesis more rigorously in additional specialist and generalist mammalian herbivores.

Freeland and Janzen (1974) proposed that foraging behaviors of mammalian herbivores are governed by the detoxification system and elimination rates of toxins. This work is widely cited, but few empirical tests of this hypothesis have been conducted on natural herbivore-plant systems. Although our results are limited in the extent to which they apply to the specialist-generalist paradigm in general, our study is the first to utilize pharmacological methods to comparatively test the predictions of the detoxification limitations hypothesis. Our findings demonstrate that specialists have lower total exposure levels of alpha-pinene than generalists. However, lower exposure levels were not due to differences in elimination rates as proposed by Freeland and Janzen (1974). We provide the absorption model as a testable alternative mechanism that may lower toxin exposure and explain the differences in foraging behavior of mammalian herbivores. Specifically, we predict that *P*-glycoproteins may play a role in preventing the absorption of toxins along the gut wall and that specialists have greater activity of *P*-glycoproteins than generalists. Our results suggest that ecologists investigating the role of plant toxins on herbivore ecology should reevaluate the elimination of toxins and consider both detoxification and absorption as mechanisms mediating foraging behavior.

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