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DIGESTION AND ABSORPTION OF *Eucalyptus* ESSENTIAL OILS IN GREATER GLIDER (*Petauroides volans*) AND BRUSHTAIL POSSUM (*Trichosurus vulpecula*)

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Abstract—Measurements were made of the quantity and composition of the steam-volatile essential oils in gastrointestinal tract contents of greater gliders fed *Eucalyptus radiata* foliage and brushtail possums fed *E. melliodora* foliage. In both species, there was less oil in the stomach contents than in an equivalent mass of foliage. Only minor losses of leaf oils occurred during mastication by greater gliders, and absorption from the stomach appeared to be the major reason for the difference in the oil content of ingested leaves and of stomach contents. The apparent digestibility of oils over the whole gut was 96–97%, although oils from the cecum and feces of both species contained compounds not present in the original leaf oils. Absorption of oils before they reach the hindgut should reduce the severity of antimicrobial effects but may involve a metabolic cost to the animal in detoxification and excretion.

Key Words—Folivores, marsupials, allelochemicals, transformation, detoxification.

INTRODUCTION

The greater glider (*Petauroides volans*) and the brushtail possum (*Trichosurus vulpecula*) are folivorous marsupials. Greater gliders feed almost exclusively

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on eucalypt leaf (Marples, 1973). Although the leaves of eucalypts form an important part of the diet of the brushtail possum in southeastern Australia, they are usually supplemented with foliage from other species of trees and shrubs, fruits, flowers, and herbage (Kerle, 1984). The importance of noneucalypt foods in the diet of the brushtail possum has led several authors (e.g., Freeland and Winter, 1975) to speculate that the consumption of eucalypt foliage is limited by the presence of "toxic" allelochemicals such as essential oils.

Although there has long been speculation about the effects that essential oils of *Eucalyptus* spp. might have on phytophagous animals (Pratt, 1937; Fleay, 1937; Betts, 1978), recent studies have failed to find relationships between the level and/or composition of leaf oils and the feeding perferences of some mammals (e.g., koala: Southwell, 1978; and insects (*Paropsis*): Morrow and Fox, 1980).

Irrespective of the effects of oils on gross food preference, their ingestion results in a metabolic cost for detoxification (Cleland, 1946; Hinks and Bollinger, 1957a, b), and their biological actions have the potential to affect populations of microbes in the digestive tracts of animals (Freeland and Janzen, 1974). Several studies have demonstrated a deleterious effect of mono- and sesquiterpenes on ruminal fermentation (Nagy et al., 1964; Nagy and Tengerdy, 1968; Oh et al., 1967, 1968). However, all these studies were performed in vitro, consequently no allowance was made for absorption of the oils, and in some cases the concentrations of oils used were unrealistically high. In contrast to ruminants, essential oils in hindgut fermenters may be absorbed and detoxified before they reach the site of microbial activity.

This paper describes the pattern of absorption of essential oils from the gut of the greater glider and brushtail possum. Volatile material extracted from digesta at five points along the gut was separated into its component compounds by gas–liquid chromatography. Also, the hypothesis that leaf oils may be lost during mastication was tested in greater gliders.

METHODS AND MATERIALS

Animals. Greater gliders were caught by hand during logging operations in a forest dominated by New England blackbutt (*E. andrewsii* ssp. *campanulata*) (Forest Type 161: Forestry Commission of New South Wales, 1965) in northern New South Wales. Brushtail possums were caught in wire cage traps in eucalypt woodland dominated by *E. melliodora*, *E. blakelyi*, *E. viminalis*, and *E. caliginosa* near Armidale, NSW. Both species were housed in metabolism cages in an air-conditioned room (20 ± 3°C) on a 12 : 12 light–dark regime for at least three weeks prior to each experiment. The greater gliders were fed *E. radiata* foliage and the brushtail possums fed *E. melliodora* foliage which was collected fresh each week and stored in plastic bags with the stems in water

at 8°C. Further details of these procedures and those used in the sampling of foliage and the collection of feces and urine are given by Foley and Hume (1987).

Experiment 1: Digestion of Essential Oils. Six greater gliders (one male, five females) were fed foliage from one *E. radiata* tree and three male brushtail possums foliage from one *E. melliodora* tree for 14 days. Samples of the leaves offered and feces collected for the last five days were stored in plastic bags at −15°C. These samples were steam distilled and the distillates analyzed by gas–liquid chromatography (GLC) and mass spectrometry (GLC-MS) as described below.

Experiment 2: Sites of Oil Absorption. Three female greater gliders were fed foliage from one *E. radiata* tree, and three male brushtail possums were fed foliage from one *E. melliodora* tree for 10 days. Samples of the diet and feces were collected for the last five days. The animals were then killed by an overdose of sodium pentabarbitone at 1200 hr, five hours after foliage was last available. The digestive tract was quickly excised, and the contents of the stomach, small intestine, cecum, proximal colon, distal colon, and rectum were removed, bulked within each species, frozen in liquid nitrogen, and stored at −15°C. These samples were steam distilled, and the distillate was analyzed by GLC as described below.

No marker substance was used in this experiment since in preliminary experiments only traces of oil could be recovered from gut contents of greater gliders that had received the marker Cr-EDTA in the drinking water (0.28 mg/ml). The oil appeared to be polymerized and oxidized and would not pass through the GLC column. The chelated chromium may have catalyzed the autoxidation of essential oil components (Garnier and Gaiffe, 1967).

Experiment 3: Oil Losses during Mastication. The amount of terpene lost during mastication of leaf by greater gliders was measured after conversion of the respirometers described by Foley (1984) to an open-flow system. Expired air was bubbled through two flasks containing cyclohexane which had been shown in a preliminary experiment to trap expired terpenes. At 0600 hr on day 1, leaves from one *E. radiata* tree were placed in the chamber and the pump started. At 1800 hr, a greater glider was placed in the chamber and allowed to feed normally. Fresh cyclohexane was placed in the traps. At 0600 hr on day 2, uneaten leaves, feces, and urine were removed from the chamber, fresh cyclohexane was placed in the traps, and the animal was left until 1800 hr when the experiment was terminated. This procedure was replicated three times with different animals. Samples for the two controls (leaf only, animal only) and the experimental treatment were bulked over the three replicates. Two runs in which a known volume of *E. radiata* essential oil was evaporated in the chamber were conducted to estimate recoveries. The cyclohexane was removed from each sample by fractional distillation on a series of Vigreaux and packed columns.

The remaining material was analyzed by GLC-MS after addition of *n*-dodecane as an internal standard.

Analytical. Essential oils were extracted from wet leaves, feces, and gut contents by steam distillation with cohobation in an all-glass apparatus (Hughes, 1970). Eucalypt leaves were distilled for 8–12 hr; gut samples were distilled for 12–24 hr. All oil samples were stored in air-tight glass bottles over sodium sulfate at −20°C.

Analytical GLC was carried out on a Perkin-Elmer 900 instrument using a quartz-silica SCOT column (50 m × 0.5 mm ID) coated with FFAP (free fatty acid phase polyethylene glycol reacted with nitroterephthalic acid) and with helium as the carrier gas. A Hewlett Packard 3370A Integrator was used to determine peak areas.

Combined GLC–mass spectrometry (GLC-MS) was performed on a Shimadzu GC6-AMP instrument with a SCOT column (70 m × 0.5 mm) coated with FFAP and programmed from 80°C to 225°C at 3°C/min. This system was connected to an AEI MS12 mass spectrometer via an all-glass straight split. Mass spectra were recorded at 70 eV ionization voltage with an ion source temperature of 150°C. Spectra were recorded every 6 sec on a VG Digispec Display data system which produced standard bar graphs for direct comparison with published spectra. Chemical ionization mass spectrometry was performed on an AEI MS902 mass spectrometer fitted with a Chemspect source. Ammonia was used as reagent gas at a pressure of 0.5 torr. High-resolution mass spectrometry was performed on this instrument under the same conditions using perfluorokerosene as reference and a peak timing method (Brophy et al., 1979). Identification of compounds was based on comparison of mass spectra with those of known compounds and coinjection with authentic compounds.

The oil distilled from the feces of greater gliders (20 mg in 1 ml methanol) was added to 4 ml of 1 M aqueous sodium hydroxide, and the mixture was heated at reflux for 4 hr. The basic solution was extracted with pentane (3 × 2 ml), the aqueous layer acidified with conc. hydrochloric acid and then reextracted with methylene chloride (3 × 2 ml). Both solutions were dried over sodium sulfate and the solvent removed under a stream of nitrogen. The residue resulting from the methylene chloride solution was taken up in 1 ml ether and treated with diazomethane.

RESULTS

The yield of steam-volatile oils from foliage, gut contents, and feces in all experiments is given in Table 1. The percentage composition of the major components of the oils from *E. radiata* leaves and the corresponding greater glider feces and from *E. melliodora* leaves and brushtail possum feces is given in Tables 2 and 3. GLC traces of the oil from leaf and feces of each species are shown in Figure 1.

TABLE 1. YIELD OF STEAM-VOLATILE ESSENTIAL OILS FROM EUCALYPT FOLIAGE AND FROM DIFFERENT PARTS OF THE GUT OF THE GREATER GLIDER AND BRUSHTAIL POSSUM

Experiment	Species		Yield (ml/100 g dry matter)					
			Leaf	Stomach	Small intestine	Cecum/Proximal colon	Distal colon	Feces
1	Greater glider	*E. radiata*	7.45	—	—	—	—	0.10[a]
	Brushtail possum	*E. melliodora*	0.82	—	—	—	—	0.02[b]
2	Greater glider[b]	*E. radiata*	11.05	6.59	Trace	0.32	Trace	0.09
	Brushtail possum[b]	*E. melliodora*	1.35	0.66	Trace	0.28	0.04	0.03

[a] $N = 6$.
[b] $N = 3$.

TABLE 2. MAJOR COMPONENTS (>1.0%) OF STEAM-VOLATILE OIL FROM *E. radiata* AND CONCENTRATION IN DIGESTA FROM DIFFERENT PARTS OF THE GUT OF THE GREATER GLIDER

Peak number (Figure 1A, B)	Identification	Experiment 1		Experiment 2			
		Leaf (% composition)	Feces (% leaf)	Leaf (% composition)	Percentage leaf		
					Stomach	Cecum	Feces
1	α-pinene	4.9	23	6.6	81	34	5
5	α-phellandrene	9.3	40	16.5	103	8	40
6	α-terpinene	4.4	42	6.8	101	?[a]	74
8	1,8-cineole + β-phellandrene	4.3	43	5.1	100	36	192
9	γ-terpinene	7.5	30	14.6	105	?[a]	33
10	*p*-cymene	10.5	23	3.4	55	147	89
11	terpinolene	2.2	41	4.3	105	37	49
15	*trans*-*p*-menth-2-en-1-ol	4.5	107	2.6	124	157	190
16	terpinen-4-ol + caryophyllene + aromadendrene	19.8	17	15.6	146	14	38
17	*cis*-*p*-menth-2-en-1-ol	3.3	79	1.8	127	?[a]	200
18	*cis*-piperitol	1.4	77	—	—	—	—
19	α-terpineol + viridiflorene	2.1	42	1.5	117	10	19
20	piperitone	—	—	1.3	172	121	58
21	*trans*-piperitol	2.6	111	1.2	111	181	247
22	δ-cadinene	1.0	32	1.8	69	?[a]	98
23	4-phenylbutanone	1.5	12	—	—	—	—
24	$C_{15}H_{26}O$	1.1	63	—	—	—	—
26	γ-eudesmol	1.2	84	2.3	44	18	129
27	α-eudesmol	1.1	85	1.2	63	49	215
28	β-eudesmol	1.6	84	1.2	79	60	333

[a] Solvents comprised >60% of area and peaks could not be accurately defined.

TABLE 3. MAJOR COMPONENTS (>1.0%) OF STEAM-VOLATILE OIL FROM *E. melliodora* AND CONCENTRATION IN DIGESTA FROM DIFFERENT PARTS OF THE GUT OF THE BRUSHTAIL POSSUM

Peak number (Figure 1C, D)	Identification	Experiment 1		Experiment 2			
		Leaf (% composition)	Feces (% leaf)	Leaf (% composition)	Percentage leaf		
					Stomach	Cecum	Feces
2	*iso*valeraldehyde	2.0	124	—	—	—	—
3	α-pinene	7.5	101	3.8	96	83	19
5	limonene	4.9	64	—	—	—	—
6	1,8-cineole	63.1	5	56.7	99	29	10
8	*p*-cymene	2.2	70	1.2	153	143	48
9	terpinolene	—	—	4.2	69	144	95
13	α-terpineol	1.7	62	2.1	180	143	120
—	unknown	—	—	1.1	130	281	220
—	unknown	—	—	2.0	—	—	—
19	$C_{15}H_{26}O$	1.8	87	5.1	—	—	—
—	unknown	—	—	1.6	24	28	—

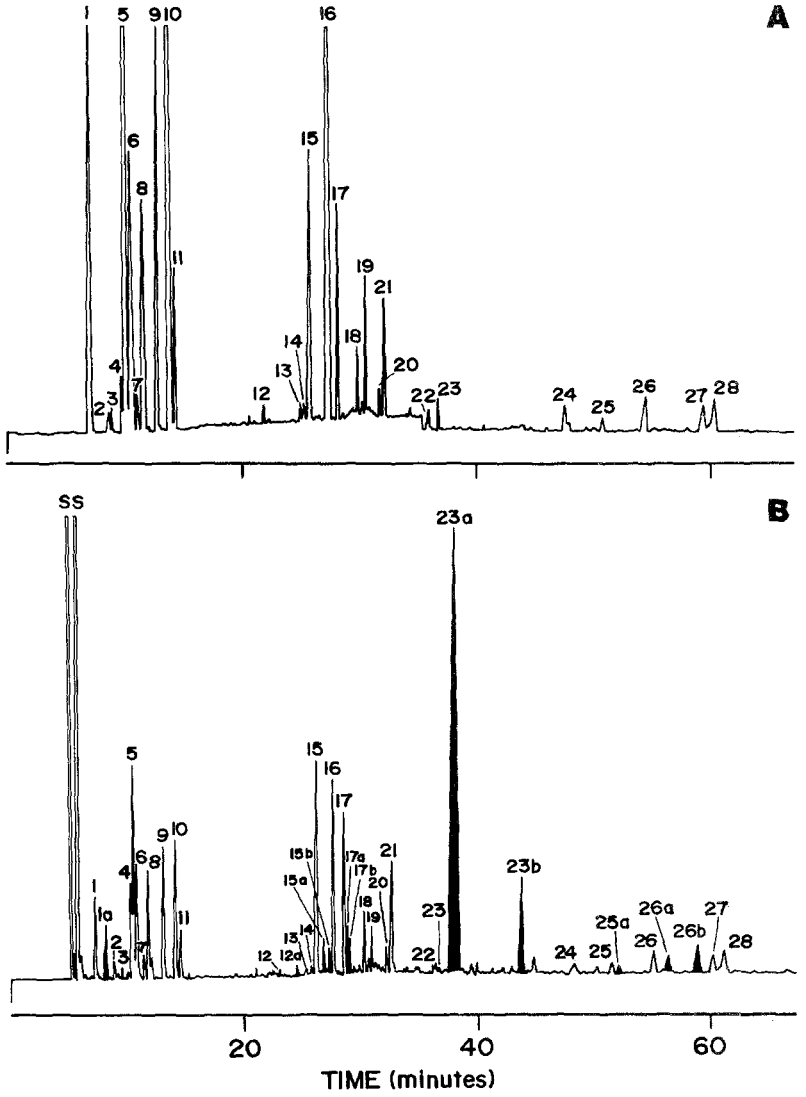

FIG. 1. GLC trace of steam-volatile essential oils from (A) *E. radiata* foliage, (B) feces from greater gliders fed *E. radiata* foliage, (C) *E. melliodora* foliage, and (D) feces from brushtail possums fed *E. melliodora* foliage. Shaded peaks represent feces oil components not present in leaf oils. Peaks not identified in Tables 2 and 3 are as follows: *E. radiata*/greater glider: 2, β-pinene; 3, sabinene; 4, myrcene; 7, limonene; 12, $C_{10}H_{18}O$; 12a, menthone; 13, linalool; 14, unknown; 15a, menthyl acetate; 15b, unknown; 17a, menthol, 17b, alloaromadendrene; 23a, an octane diol dibutyrate; 23b, globulol; 25, $C_{15}H_{26}O$; 25a, $C_{15}H_{24}O$; 26a, $C_{15}H_{28}O$; 26b, thymol. *E. melliodora*/brushtail possum: 4, myrcene; 7, γ-terpinene; 10, terpinen-4-ol; 11, caryophyllene; 11a, b, unknown; 12, pinocarveol; 14, bicyclogermacrene; 15, *cis*-mentha-1(7),8-dien-2-ol; 16, *trans*-mentha-1(7),8-dien-2-ol; 16a, an octane diol dibutyrate; 16b, unknown; 16c, 17, 18, 19a, $C_{15}H_{26}O$; 20, $C_{15}H_{24}O$. "S" represents solvent.

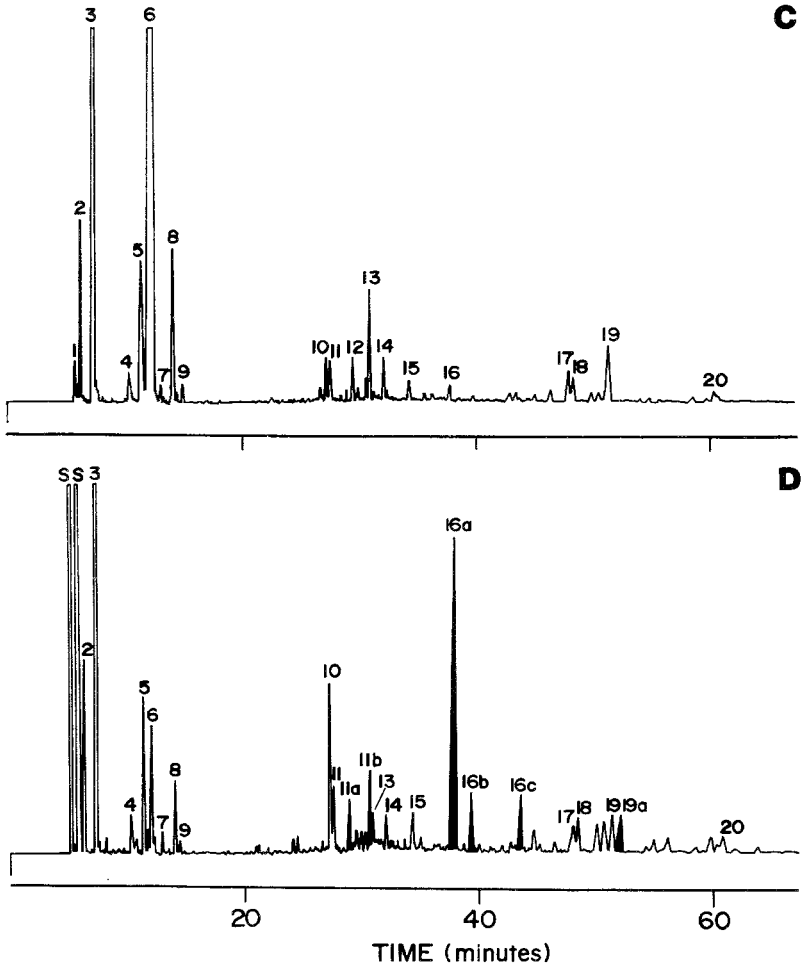

FIG. 1. Continued.

Experiment 1. E. radiata oil was complex, consisting primarily of terpinen-4-ol (20%), *p*-cymene (11%), α-phellandrene (9%) and γ-terpinene (8%). *E. melliodora* oil was much simpler, being dominated by 1,8-cineole (63%) with smaller amounts of α-pinene (7%), and limonene (4%). Both oils contained only small amounts of sesquiterpenes (3–5%).

Only minor amounts of oil were recovered from the feces of both species. However, these oils were more complex than those from the corresponding leaves. The oil from the brushtail possum feces was notable for the almost complete absence of 1,8-cineole (3% vs. 63% in the leaf). In the oil from the feces of the greater glider, the peaks representing terpinen-4-ol, *p*-cymene, α-

pinene, and α-phellandrene were greatly reduced, and no oil component passed through the gut without some apparent digestion.

In both species, some peaks appeared in the oil from the feces but did not occur in the oil from the leaves. While many of these were minor (e.g., Figure 1B, peaks 12a, 15a, 17a), peak 23a was the largest component (21%) of the oil from the greater glider feces. The same component appeared in the oil of the brushtail possum feces (Figure 1D, peak 16a) as 14% of the total oil. Infrared spectra of the two feces oils showed large absorptions at 1740 cm^{-1} and 1160 cm^{-1}, characteristic of an ester function. The strength of these bands, absent in the leaf oils, suggested that they belonged to the large extra peaks in the GLC traces of both feces oils. The mass spectra of these GLC peaks indicated that the highest mass ion was at *m/z* 243; however, chemical ionization mass spectrometry showed a (MH^+) ion at *m/z* 287, and accurate mass measurement of this ion resulted in a mass of 287.2236, indicating a formula of $C_{16}H_{31}O_4$ (287.2220) for the (MH^+) ion.

Alkaline hydrolysis of a sample of the oil from the greater glider feces resulted in the elimination of this significant extra GLC peak, lending support to the suggestion that it was an ester. From the base-soluble fraction, after acidification, solvent extraction, and methylation, a GLC trace was obtained which contained a peak, the mass spectrum of which is suggestive of an octane diol, which fits the solubility characteristics of the compound. The mass spectrum was similar (but not identical) to that of 2-ethylhexane-1,3-diol. A small amount of methyl butyrate was also detected in this fraction.

It appears from these results that the unknown peak in the feces oil from both folivores is a dibutyrate ester of an octane diol. In fact, the mass spectrum of 2-ethylhexane-1,3-dibutyrate was similar (but not identical) to that of the natural material. The natural material had a shorter retention time (on FFAP) than either 2-ethylhexane-1,3-dibutyrate or diisobutyrate, suggesting greater branching in the natural material.

Experiment 2. Details of the yield and percentage composition of the major components of the steam-volatile oils recovered from different parts of the digestive tracts of the two species are given in Tables 1–3. Although the yields of oils from the leaves were notably higher in this experiment than in experiment 1, the yield of oil from the feces was similar. On the other hand, while the percentage composition of the oils from leaves was similar to that in experiment 1, those isolated from the feces were different. For example, the octane diol dibutyrate found in experiment 1, although present in this sample, comprised only 9% of the oil from greater glider feces and 3% of that from brushtail possum feces.

Experiment 3. The essential oil peaks on the GLC trace of cyclohexane-soluble material from expired air of greater gliders in preliminary experiments were identified by their mass spectra. This also indicated that some of the other

peaks represented aliphatic straight-chain hydrocarbons resulting from impurities in the cyclohexane solvent.

No peaks representing essential oils were apparent in the GLC traces of the air samples from the leaf alone in the chamber, from greater gliders feeding on the leaf in the chamber, or from the greater gliders alone in the chamber. Recoveries of evaporated terpenes in the two runs with *E. radiata* essential oils were 28% and 35%.

DISCUSSION

Some workers (e.g., Von Rudloff, 1975) have criticized steam distillation as a means of extracting essential oils because of the possibility of inducing artifactual rearrangements of components of the oils. This was unlikely to have been a serious problem in the present study. Lassak (unpublished) has shown that the steam-volatile essential oil of the leaves of *E. dives*, a close relative of *E. radiata* (Ladiges et al., 1983) is chromatographically identical to that extracted from individual oil glands with a fine capillary needle.

Using the mean intake and dry-matter digestibility figures for greater gliders and brushtail possums fed *E. radiata* and *E. melliodora*, respectively (44 g/kg body mass$^{0.75}$/day and 58% in greater gliders and 36 g and 51% in brushtail possums; Foley, 1984), it can be calculated that greater gliders apparently digested 97% of the essential oils of *E. radiata* while brushtail possums apparently digested 96% of *E. melliodora* essential oils. Using similar techniques, Eberhard et al. (1975) found that koalas apparently digested 70–97% of the essential oils of the leaves of *E. punctata*. Similarly, Southwell et al. (1980) found only traces of essential oil in the feces of brushtail possums dosed with 5 ml of purified oil components (*p*-cymene and 1,8-cineole) daily for five days. Igimi et al. (1974) detected only 10% of the ^{14}C label in the feces of rabbits fed [^{14}C]*d*-limonene. That components of these essential oils are readily absorbed is not surprising in view of their low molecular weight and high lipid solubility. The important question is where are they absorbed?

The apparent interaction between Cr-EDTA and essential oils in the gut, discovered in preliminary experiments, meant that the site of absorption could not be accurately ascertained. However, analysis of oils from different parts of the gut showed that the quantity of oil in the stomach contents was only 49% of what would be expected, on the basis of digesta mass, in brushtail possums and 59% in greater gliders. Similar discrepancies have recently been observed in the rumen contents of mule deer (*Odocoileus hemionus*) (Cluff et al., 1982) and stomach ingesta of pygmy rabbits (*Brachylagus idahoensis*) (White et al., 1982). There are two possible explanations for this. First, lipid-soluble material such as essential oils could be rapidly absorbed across the mucosa of the stomach of both ruminants and hindgut fermenters (Cook et al., 1952, Alexander

and Chowdhury, 1958). Igimi et al. (1974) have shown that there is rapid disappearance of [^{14}C]*d*-limonene from the rat stomach after dosing by stomach tube. Similarly, Narjisse (1981) was unable to detect monoterpenes in the rumen contents of goats 3 hr after direct infusions.

Alternatively, volatile oils may be lost during mastication of the leaf. If this is the case, it is surprising that the percentage loss from stomach contents was greater in the brushtail possum than in the greater glider, since mastication in the greater glider produces finer particles (Gipps, 1980; Foley, 1984). However, the coarse grinding action of brushtail possum teeth may be more effective in disrupting oil glands than the fine cutting action of greater gliders (Gipps, 1980).

The results of experiments designed to measure losses of essential oils during mastication by greater gliders suggested that this was of only minor importance. Although preliminary qualitative experiments had detected terpenes arising from expired breath, no traces of oils were detected in the quantitative experiment. This was unexpected since several steps were taken to maximize the recovery of oil components. This involved decreasing the rate of air flow through the chamber, bulking samples from three animals, and distilling the cyclohexane through longer packed columns. Although recoveries of standards evaporated in the chamber averaged only 32%, the losses measured during mastication cannot explain the low concentration of oil in the stomach contents of the greater gliders relative to that ingested.

Using a similar collection system (but with diethyl ether), White et al. (1982) found that twice as much monoterpene was trapped when *Artemisia tridentata* foliage was in a chamber with pygmy rabbits compared with *Artemisia* alone. Nevertheless, this represented only a minor proportion (0.5%) of the total fraction "missing" from the stomach contents. No measurements of the efficiency of the collecting apparatus were made in the experiments of White et al. (1982), but even assuming that this was only 10%, losses during mastication of *Artemisia* would still account for only 5% of the volume of oil missing from the stomach. It would seem that in both the White et al. (1982) and the present study, although losses through mastication can occur, they are of minor quantitative importance, and absorption from the stomach must be the principal avenue of loss.

Further absorption must take place in the small intestine, since the amount of terpene reaching the hindgut was of the order of only 1% of that ingested by both greater gliders and brushtail possums. There would thus seem to be little chance of major interaction with the microbial ecosystem in the hindgut. On the other hand, it is interesting that a major unknown feces peak was found in both the greater glider and brushtail possum. No examination was made of the feces of animals fed noneucalypt diets in the present experiments, but Southwell et al. (1980) did not detect any similar metabolite in the feces of brushtail pos-

sums fed a diet of fruit or fruit supplemented with 1,8-cineole, *p*-cymene, or α-pinene.

The fact that the major unknown feces peak was detected only in or distal to the cecum suggests that it is a product of microbial metabolism. Although the unknown compound was nonterpenoid and most likely a dibutyryl ester of an octane diol, it may have resulted from microbial fermentation of terpenes. For example, Joglekar and Dhavalikar (1969) isolated the 10-carbon compound 3,7-dimethyl-1,7-octane-diol from the fermentation of citral by a soil pseudomonad. Similarly, Bhattacharyya and Dhavalikar (1965) isolated the nonterpenoid anhydride of 2-nonene-2,3-dicarboxylic acid from the *Aspergillus niger* fermentation of a number of different terpenes such as camphene, β-santalene, longifolene, caryophyllene, and δ-cadinene. They suggested that the anhydride was formed by a metabolic shift in which the normal oxidative process in the mold would have been impaired to such a degree that excess pyruvate and acetate were channeled into the formation of the anhydride.

New feces peaks could also arise by absorption and subsequent biliary excretion. Eberhard et al. (1975) suggested that biliary excretion would be important, together with urinary excretion, in dealing with those compounds greater than mol wt 150 (i.e., monoterpenoids and sesquiterpenoids). The amount of digesta in the small intestine of both species was too small to recover any oil, and the gallbladders of both species contained only a minor amount of bile. Igimi et al. (1974) found that 25% of ^{14}C from ingested [^{14}C]*d*-limonene in rats was excreted in the bile within 14 hr. However, since only 5% of the dose was eventually excreted in the feces, much of the biliary excretion must have been fermented or reabsorbed lower in the gut and excreted in the urine. In the present study, it is likely that the majority of oil ingested by both species was absorbed, detoxified, and excreted in the urine. Southwell et al. (1980) found several novel products in the urine of brushtail possums fed fruit and isolated terpenes. Future studies using labeled terpenes would be necessary to identify the pathways of detoxification and to quantify the routes of excretion of ingested essential oils.

The possibility that dietary essential oils could have deleterious effects on gut microorganisms has been raised by several authors (e.g., Freeland and Janzen, 1974; Bryant and Kuropat, 1980). This possibility is based on the work of Nagy et al. (1964), Nagy and Tengerdy (1968), and Oh et al. (1967, 1968), who found that some sagebrush *Artemisia* and Douglas fir terpenes could inhibit fermentation in the rumen of deer. However, this work has been challenged (Welch et al., 1981, 1982; Welch and McArthur, 1979) on the ground that the volumes of oil used to demonstrate microbial inhibition were unrealistically high in relation to the amounts normally expected to be ingested. Also, the in vitro systems did not allow for absorption of the oil. Oh et al. (1967) found that microbial inhibition occurred at an essential oil concentration of 1.2% of

deer rumen fluid. This is about 20 times greater than the concentration of oil found in the hindgut of the greater glider and the brushtail possum in this study.

On the other hand, Sadler (1983) found that pure compounds and ether dilutions down to 10^{-4} of 1,8-cineole, *d*-limonene, terpinen-4-ol, and α-terpineol inhibited the growth of cellulolytic bacteria which had been previously cultured on *Eucalyptus viminalis* leaf in vitro. Similarly, while ether extracts of *E. viminalis* and *E. blakelyi* inhibited both "adapted" and "nonadapted" cellulolytic bacteria, extracts of *E. radiata* did not differ from controls even though these leaves (from the same batch as those used in experiment 3) contained substantial proportions of terpinen-4-ol and α-terpineol. Thus antimicrobial effects of essential oils may be due to synergistic effects of particular components (see also Akimov et al., 1977). Andrews et al. (1980) suggested that the antimicrobial action of terpenes results from disruption of cytoplasmic membranes and that gram-negative organisms are more resistant than gram-positive microbes. Nothing is known of the occurence of each of these groups in the hindgut of greater gliders and brushtail possums, although London (1981) found the cecal flora of the koala to be predominantly gram-positive.

The results from the present study indicate that in both the greater glider and the brushtail possum the microbial population in the hindgut is largely, although not completely, protected from the deleterious effects of *Eucalyptus* essential oils by their absorption mainly from the stomach and small intestine. However, the metabolic cost of detoxifying absorbed oils in the liver may limit the range of eucalypt species that these folivorous marsupials can utilize as a sole source of nutrients. It is suggested that future studies of leaf choice by arboreal folivores should take account of the levels of primary nutrients as well as allelochemicals in selected and rejected plant species.

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