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VOLATILE CONSTITUENTS OF DOG (*Canis familiaris*) AND COYOTE (*Canis latrans*) ANAL SACS

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Abstract—The volatile organic compounds from the anal sac secretions of male and female dogs and coyotes were examined using gas chromatography and gas chromatography–mass spectrometry. Short chain (C_2–C_6) acids and trimethylamine were major constituents. Changes in the type and abundance of the volatiles were examined across state of estrus, species, and gender. No consistent difference in the pattern of volatiles was detected that was indicative of estrus state or gender. Dogs displayed larger amounts of all constituents. The anal sac secretions of a third carnivore, the cat, were examined to see if they contained trimethylamine: none was found.

Key Words—anal sacs, canids, GC-MS, chemical communication, metabolic profiling.

INTRODUCTION

Although various biological functions have been ascribed to the odorous

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secretions from the anal sacs of canids, none has been definitely established. Qualitative observations of dog (*Canis familiaris*) behavior have suggested to some authors that these anal sac secretions may function in sexual attraction (Donovan, 1967), individual recognition, territorial demarcation (Baker, 1962), and alarm or defense (Donovan, 1969). However, scientific reports suggest that canids mark mainly with urine (Kleiman, 1966), and that anal sac secretions from estrous females elicit no more investigatory behavior from male dogs than those from diestrous females (Doty and Dunbar, 1974a).

No rigorous studies have been conducted with coyotes (*Canis latrans*); however, anal sac secretions often constitute a major portion of coyote lures (Young and Jackson, 1961), suggesting that the secretion may have attractive properties. Although little is known about population dynamics and/or the role of odor in the reproductive biology of the coyote, such information is important since it may assist in developing coyote management techniques (Cain, 1972).

The anal sac secretion of dogs is reported to consist of 88% water with proteins, lipids, and inorganic substances secreted into the sac by surrounding glands (Montagna and Parks, 1948). The color, the odor, and the rate at which the secretion accumulates vary from dog to dog, and appear not to depend upon the extent of exogenously produced early androgenization (Doty and Dunbar, 1974b).

The chemistry and microbiology of anal sac secretions from the red fox (*Vulpes vulpes fulva L*), have been studied (Albone and Fox, 1971; Albone and Eglinton, 1974). They established the presence of C_2–C_5 aliphatic acids, and 4-methylvaleric acid as well as phenylacetic, 3-phenylpropionic, *p*-hydroxyphenylacetic, and *p*-hydroxyphenylpropionic acids in the secretions from this canid. Trimethylamine was also identified as a constituent of these secretions. The nature of these constituents suggested that they may be formed by flora in the sac utilizing available substrates (Albone and Eglinton, 1974).

The purpose of this study was to determine if changes in the nature and abundance of volatiles in the secretions from the anal sacs of beagles and coyotes occur across species, gender, or state of estrus. The work of Donovan (1967) suggested that dogs in estrus possess odors, attractive to conspecifics, in their anal sac secretions. Preliminary data suggested to us that trimethylamine was found only in the anal sacs of female beagles and coyotes. Consequently, we determined the amount of this compound in each sample. Albone and Fox (1971) reported this amine as a constituent of female red fox anal sacs. In order to determine if it is found in glandular secretions of a noncanid, secretions from the anal sacs of the cat (*Felis domesticus*) (Greer and Calhoun, 1966) were examined for trimethylamine. These secretions have been shown to contain large amounts of the C_2–C_5 acids discussed above (Berüter, unpublished, 1972).

METHODS AND MATERIALS

Sixteen pure-bred beagles and 14 coyotes used in this study were 2–5 yr old and were housed at the Denver Wildlife Research Center, Denver, Colorado. Two of the female coyotes were born in captivity while the remainder were trapped in Colorado or Texas. Each animal was housed individually in an outdoor, concrete-lined run containing weather-proofed wooden shelters. The animals were all maintained under routine dog colony procedures with dry dog food (Wayne "Krumettes")[5] and water provided ad libitum. The nutritional status and health of the animals used were excellent for the duration of the study.

The secretions were collected by two procedures. The first (Method I) was performed on coyotes only after anesthetization using a 4% thioseconal solution (to effect). The beagles were not anesthetized. The sac contents were then directly expressed into beakers held over the sac orifices. After collection, the contents of the anal sacs were washed into a glass vial using 1.0 ml of 0.15 M saline. The vials were then frozen at −18°C and sealed. Using Method I, samples were collected from each animal at 2–4 wk intervals over a period of 4 mo. Almost every attempt to express secretions from coyote anal sacs yielded little or no material (see Table 1). Consequently, secretions were aspirated from the sacs using 0.3–0.5 ml of 0.15 M saline. The aspirated material was placed in vials, frozen, and sealed. The difficulty in obtaining anal sac secretions from coyotes via direct expression led to development of a second collection procedure (Method II), designed to collect equal volumes of aspirated sac contents from both species.

In Method II, all animals were first anesthetized with a 4% thioseconal solution (to effect) and then 0.15 ml of 0.15 M saline were injected into the opening of each sac (left and right) using a blunt, stainless steel, 20-gauge needle attached to a glass syringe. After 10 min, the saline plus secretion were withdrawn by the same syringe, transferred to individual glass vials which were then frozen in liquid nitrogen and sealed. Two collections were made within 2 mo of each other using Method II.

In both procedures, a number of saline blanks of 1.0 ml volume were prepared after being exposed to the collection room atmosphere. All samples and blanks were frozen at −18°C until shipment to Philadelphia.[6]

The gender, state of estrus, and number of samples obtained from each animal using Methods I and II are shown in Table 1. Vaginal cytology was used to determine the state of estrus.

Before gas chromatography (GC) analysis was performed, each tube was

[5] Use of trade names does not imply a United States Government endorsement.

[6] Samples were shipped packed in dry ice via air freight and never defrosted until analysis. Samples collected by Method I were shipped at three separate intervals in the 4-mo period. Those from Method II were shipped within 2 wk of collection.

TABLE 1. GENDER, STATE OF ESTRUS, AND THE NUMBER OF SAMPLES OBTAINED FROM EACH ANIMAL USING METHODS I AND II[a]

Animal		Method I					Method II	
		Sample No.						
		1	2	3	4	5	1	2
Beagle ♀	1	E	Ps	—	—	—	—	—
	2	E	P	—	—	—	An	M
	3	Pr	M	—	—	—	An	M
	4	E	E	—	—	—	—	—
	5	Pr	E	P	—	—	—	—
	6	Pr	E	E	P	—	—	—
	7	Pr	—	—	—	—	—	—
	8						An	M
	9						—	M
	10						—	M
Coyote ♀	1	Pr	Pr[b]	Pr[b]	Pr[b]	E	M	An
	2	Pr	—	—	—	—	—	—
	3						M	An
	4						M	An
	5						—	An
	6						—	An
		Number of samples						
Beagle ♂	1	2					1	1
	2	2					1	1
	3	2					—	—
	4	—					1	1
	5	—					—	1
	6	—					—	1
Coyote ♂	1	5[b]					—	—
	2	3[b]					—	—
	3	4[b]					1	1
	4	4[c]					—	—
	5	—					1	1
	6	—					1	1
	7	—					—	1
	8	—					—	1

[a] E represents estrus; Ps, pseudopregnant; P, pregnant; Pr, proestrus; M, metestrus; An, anestrus.
[b] Anal sac contents aspirated using saline.
[c] Two of four samples were aspirated.

cracked open and a 5 μl aliquot removed for injection. The remaining secretion was transferred to a screw-top vial with a Teflon-lined cap and refrozen at -10°C. Analysis of several samples after 3–4 mo under such storage conditions showed no decrease in the levels of volatiles.

The four cats used in the study were housed at the Monell Center. Each animal was individually quartered in a $2\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{1}{2}$ ft stainless steel cage having a 12-in-wide shelf and a "kitty litter" box. A 12-hr light–12-hr dark cycle was used in the cat quarters. Eight ounces of dry Purina Cat Chow was provided each day, with water provided ad libitum. Two intact cats and two castrates (one from each gender) were used as donors. The ovariectomized female was given 50 μg/day of estradiol benzoate.

Anal sac secretions from the cats were obtained via Method I (i.e., direct expression). Due to the viscosity of the secretion it had to be washed into the bottom of glass vials using 0.5 ml of 0.15 M saline. The vials were then sealed and frozen (-10°C) until analysis (3 days later). One sample from each cat was used for analysis.

Analyses were performed with a Perkin-Elmer 990 gas chromatograph equipped with a flame ionization detector (FID). The gas chromatograph was fitted with a 6 ft $\times$ 0.25 in ID or a 12 ft $\times$ 2 mm ID stainless-steel column each packed with Porapak Q-S. The 6-ft column was operated isothermally at 215°C with a 40-ml/min helium flow. All samples were chromatographed on this column. The 12-ft column was programmed from 100 to 215°C at 4°/min using a 50-ml/min helium flow. Mass spectra were routinely obtained using a Perkin-Elmer 990 GC interfaced to a Hitachi/Perkin-Elmer RMU-6L mass spectrometer. High resolution spectra were run on a CEC-21-110B high resolution mass spectrometer interfaced with a Varian-Aerograph model 600 GC located at the Mass Spectrometer Laboratory at MIT. Identifications were confirmed by comparison of mass spectra and GC retention times with those from commercially available samples. Quantitation of volatile constituents was performed using samples collected with Method II. Except for trimethylamine (see below) individual constituent amounts were determined by first calculating their response factors with standard solutions and then calculating peak areas from the chromatograms.

RESULTS

The chromatogram shown in Figure 1 was obtained from a female beagle but is representative of the chromatographic pattern from both genders of the two species. However, there was a great deal of variation in the amounts of volatiles found in individual samples. Samples from males or females of both species could be volatile rich or poor from one collection

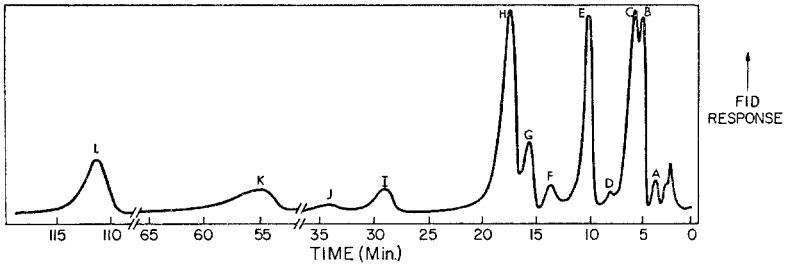

FIG. 1. Chromatogram produced by the volatile materials from a female beagle anal sac. Peaks B through H were attenuated (× 5) to keep them on scale. Flame ionization detector response is plotted on the ordinate. Peak A = ethanol, B = trimethylamine, C = acetic acid and acetone (see Table 2), D = 2-methylpropanal, E = propionic acid, F = 2- and 3-methylbutanal (see Table 2), G = isobutyric acid, H = *n*-butyric acid, I = 2-methylbutyric and isovaleric acids, J = *n*-valeric acid, K = 4- and 2-methylvaleric acids (see Table 2), and L = 2-piperidone. Because acetic acid has a low response in a FID, it does not appear larger than peaks E and H.

TABLE 2. COMPOUNDS IDENTIFIED IN THE DOG AND COYOTE ANAL SAC SECRETIONS

Chromatographic peak	Compound	Molecular weight	Characteristic ions seen in the mass spectrum[a]
A	Ethanol	46	*m/e* 46, 45, and 31
B	Trimethylamine	59	*m/e* 59, 58, 42, and 30
C	Acetic acid and	60	*m/e* 60, 45, and 43
	Acetone[b]	58	*m/e* 58 and 43
D	Isobutanal	72	*m/e* 72, 43, 41, 29, 27
E	Propionic acid	74	*m/e* 74, 73, 57, and 45
F	2- and 3-Methylbutanal[c]	86	*m/e* 86, 71, 58, 57, 44, 41, 29
G	Isobutyric acid	88	*m/e* 88, 73, 45, 43, and 41
H	*n*-Butyric acid	88	*m/e* 88, 73, 55, and 60
I	2-Methylbutyric acid and		*m/e* 87, 74, 57, and 41
	iso-valeric acid	102	*m/e* 87, 60, 43
J	*n*-Valeric acid	102	*m/e* 73 and 60
K	4-Methylvaleric acid and		*m/e* 87, 60, and 43
	2-Methylvaleric acid[b]	116	*m/e* 87, 74, and 43
L	2-Piperidone	99	*m/e* 99, 98, 70, 69, and 30

[a] Most intense ion underlined.
[b] Tentatively identified by mass spectrometric fragmentation patterns.
[c] Both compounds have identical retention times on Porapak Q-S, and ions for both are present in the mass spectra.

to the next. Although male and female beagles displayed far larger amounts of volatiles than their coyote counterparts, no consistent differences were found in the nature and abundance of one or more of the volatiles across estrous state, gender, or species.

All of the compounds identified are listed in Table 2. Owing to the large amounts of acids present in the samples and their tailing on Porapak Q-S, combination GC–high resolution mass spectrometry of several samples was needed to identify peaks D and F as aldehydes.

Acetic acid was generally the most abundant acid present in the secretion. In the sample which produced the chromatogram in Figure 1 its concentration was $\simeq 6$ μg/μl of sample. Other acids in this sample were present in these amounts: propionic (0.84 μg/μl); isobutyric (0.1 μg/μl); *n*-butyric (0.70 μg/μl); isovaleric/2-methylbutyric (0.17 μg/μl); *n*-valeric (0.016 μg/μl); and 4-methylvaleric/2-methylvaleric (0.25 μg/μl).

Mass spectra obtained of peaks C, I, and K consistently showed that these peaks contained two compounds, as listed in Table 2. Peak C consisted almost entirely of acetic acid; however, no effort was made to distinguish the ratios of the two acids comprising I and K. Peak F undoubtedly consisted of the two methylbutanals, since ions for both were present and the GC retention times were identical on our column. Peak L (2-piperidone), as seen in Figure 1, had an extremely long retention time, and unless it was present in large concentrations it was difficult to detect during routine GC assays owing to peak spreading.

Measurement of trimethylamine in our samples was complicated by a co-elution phenomenon in which this amine co-eluted with acetic acid below a 25:1 weight ratio of acid to amine. In samples where no amine was seen in the GC trace, it was searched for by continuously obtaining mass spectra every 5 sec as acetic acid eluted into the ion source of the mass spectrometer. By comparison of the abundance of m/e 58 in these samples to known injected amounts of amine and acetic acid mixtures we found that as little as 30–50 ng of amine could be detected in the presence of up to 10 μg of acetic acid.

The male–female difference in the amounts of trimethylamine was not statistically significant. This was shown by a 2(sex) $\times$ 2(species) $\times$ 2(left sac–right sac) analysis of variance with repeated measures on the third factor for the data from collection Method II, second collection. The analysis did indicate a statistically significant species difference with respect to the amine ($F = 5.65$, $df = 1/16$; $P < 0.05$). Typically, the amounts of amine found in the beagle anal sacs was from 0.14 μg to 0.70 μg/μl of secretion (estimated, as discussed above). On the average these amounts were 10 times more than the amount of amine found in the coyotes and may be a reflection of the lower amounts of secretion found in coyote's anal sacs.

No trimethylamine was detected in the samples obtained from the cats.

In addition, we have found that the two aldehydes listed in Table 2 as well as 2-piperidone were present in the cat anal sac secretions. No evidence for any of the compounds found above was seen in the blanks.

DISCUSSION

As noted above, no consistent changes indicative of the estrous state could be found in the patterns of volatiles from the beagle or coyote anal sacs. In addition, no one compound or ratio of volatiles appeared to be characteristic of gender. Species differences do, however, exist in that the beagles had greater amounts of volatiles than the coyotes. The amount of volatiles from the same animal appeared to be variable, using both collection methods. Because of this variability, subtle changes in the patterns of volatiles may have gone undetected. Doty and Dunbar (1974a and 1974b) found that the color, odor, and secretory rate of beagle anal sac secretion appears to be independent of hormonal status of the animal. In addition, urine and vaginal secretions from estrous females, but not anal sac secretions, elicited attraction by sexually experienced male beagles. Consequently, our results are in agreement with this behavioral study.

The anal sacs of dogs have been shown to possess numerous apocrine tubules and sebaceous glands in the tissue lining them (Montagna and Parks, 1948). Histochemical studies have shown that these secrete proteins, carbohydrates, and lipids into the sacs (Montagna and Parks, 1948). Short chain aliphatic acids, trimethylamine, ethanol, and acetone may all arise via microbial action on proteins, carbohydrates, and lipids (Wood, 1961; Sokatch, 1969; Hayward and Stadtman, 1960; Holdeman and Moore, 1972; Thimann, 1963). The indigenous microflora of the fox anal sac, when incubated in vitro, produce the C_2–C_5 acids (Albone and Eglinton, 1974). In addition, Gorman et al. (1974) have shown that the series of short chain aliphatic acids present in the anal sacs of the Indian mongoose (*Herpestes auropunctatus*) are products of bacterial metabolism. Hence, the presence of the available substrates, in addition to the volatiles formed, strongly suggests a microbiological origin for these compounds.

The short chain aliphatic acids are widely distributed in biological fluids. In addition to the three canids discussed above, they have also been reported to be constituents of cats' anal sacs (Berüter, unpublished, 1972), guinea pig (*Cavia porcellus*) perineal glands (Berüter et al., 1974), Beagle and coyote vaginal secretions (Preti, Muetterties, Furman, Kennelly, and Johns, unpublished results, 1973), rhesus monkey vaginal secretions (Michael et al., 1971), and human vaginal secretions (Michael et al., 1974; Preti and Huggins, 1975).

Trimethylamine has been confirmed as present in the anal sac secretions of three canid species: dog, coyote, and red fox. It was not detected in the four secretion samples from cats' anal sacs and was not reported as present in these secretions from the lion (Albone and Eglinton, 1974) or the Indian mongoose (Gorman et al., 1974), which are noncanid carnivores. In addition, the perineal gland of the guinea pig, a site of volatile acid production and storage, does not contain the amine. These findings suggest that the amine will not be found in all glands where volatile acids are produced and/or stored.

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