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CHEMICAL ANALYSIS OF TEMPORAL GLAND SECRETIONS COLLECTED FROM AN ASIAN BULL ELEPHANT DURING A FOUR-MONTH MUSTH EPISODE

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Abstract—The temporal glands, modified facial apocrine sweat glands unique to elephants, release collectable secretions during an unusual physiological state termed "musth" in the Asian bull elephant (*Elephas maximus*). Recently we began the characterization of the chemical components of musth, especially in the temporal gland secretions (TGS), and the examination of the role of such secretions as agents for chemical communication between elephants. The present study focuses on possible correlations between testosterone levels in the serum and temporal gland secretions. We were especially interested in possible qualitative and/or quantitative changes in volatile compounds as the testosterone levels varied during a discrete musth period. Quantitative changes in TGS and serum testosterone were determined by radioimmunoassay. Qualitative and semiquantitative changes occurring in volatile composition were studied by high-resolution gas chromatography (fused silica capillary column, on column injection). Compound identification was by nuclear magnetic resonance, gas chromatography–mass spectrometry, and gas chromatography internal standards. Twenty-three major compounds and a number of minor components were identified. Androgen concentrations were correlated with TGS-specific volatiles including benzoic acid, 2-nonanone, 5-nonanol, tetradecanoic acid, and decanoic acid. The latter two compounds and (*E*)-farnesol, a major component of African TGS, demonstrated an inverse relationship to T levels.

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Key Words—Temporal gland secretions, testosterone, dihydrotestosterone, *Elephas maximus*, volatiles, elephant, benzoic acid, 2-nonanone, 5-nonanone, 5-nonanol, tetradecanoic acid, decanoic acid, (*E*)-farnesol.

INTRODUCTION

The function of the temporal gland in Asian bull elephants (*Elephas maximus*) has intrigued scientists for decades. This gland, unique to elephants, is a modified apocrine sweat gland, located in the mid-cheek region (Fernando et al., 1963; Estes and Buss, 1976). Secretions from the gland have been implicated in several aspects of chemical communication (chemocommunication) in African as well as Asian elephants (Buss et al., 1976; Rasmussen et al., 1984; Rasmussen, 1988). The unusual physiological state of musth in the Asian bull elephant is characterized in part by the emission of secretions from the temporal glands (Jainudeen et al., 1972a; Schmidt, 1978); such secretions do not occur in the nonmusth condition. Chemical analyses of the temporal gland secretions (TGS) have demonstrated high testosterone levels (Rasmussen et al., 1984) and a variety of volatile chemical species (Rasmussen, 1988). Identification of species-specific chemicals in the TGS during musth and the changes, both qualititive and quantitative, that occur in the composition of volatile components during the progression of a musth period may help elucidate possible chemocommunicative roles for this gland.

Specific chemicals in the temporal gland secretions of Asian bulls in musth may affect behaviors such as male–male or female–male relationships, perhaps through pheromonal mediation. Previous studies suggested that mainly females respond to musth temporal gland secretions; predominantly flehmen responses were recorded to samples of TGS that had been frozen and then thawed prior to bioassay (Rasmussen, 1988). [In contrast, recent studies have demonstrated that avoidance responses by females and young calves occurred frequently to samples of fresh TGS (Rasmussen, Haight, and Perrin, unpublished).] As preliminary studies comparing the TGS volatiles from Asian bull elephants in musth and those from an Asian bull elephant whose serum testosterone had been artificially lowered during musth (Rasmussen, 1988) suggested that serum T levels might correlate with volatile compounds, we recorded, as described in this paper, the chemical changes occurring in the TGS during an entire musth episode in an individual captive Asian bull elephant maintained on a constant diet. Serum testosterone was assayed in randomly obtained samples. TGS samples were collected almost daily and testosterone measured.

Concurrently, volatile components from the temporal gland secretions were identified and described. We chose to analyze components of TGS that are less volatile than the headspace volatiles for several reasons. (Headspace compo-

nents refer to those compounds that, in a closed chamber, volatilize and subsequently can be directly analyzed by gas chromatography (GC) or trapped in hexane prior to GC analysis.) First, our observations had indicated that components of less volatility are important in elephant-to-elephant communication involving flehmen responses and palatal pit area contact responses (Rasmussen, 1988), and second, we lacked, at the time of this study, the equipment and personnel time for headspace analyses. That an investigation of the moderate volatiles might be instructive was suggested by the flehmen responses of the female elephants, especially their strong responses to samples containing cholesterol (Rasmussen 1988).

Selected samples were analyzed by gas chromatography–mass spectrometry (GC-MS). This selection was based on the serum and TGS testosterone levels and the variety of volatiles demonstrated by GC. Direct or inverse quantitative relationships between androgen levels and the concentrations of several specific TGS volatiles were noted over the time of the musth period.

METHODS AND MATERIALS

Procurement of TGS Samples

Biological samples were obtained from a 22-year-old Asian bull (Tunga) housed at the Washington Park Zoo (WPZ) facilities in Portland, Oregon. One hundred one sequential TGS samples were collected from this bull at 24-hr intervals during a single four-month-long musth episode in 1986 (June 3–October 7, 1986). The total time for the musth period was 127 days: 87 days of continual secretion, followed by a 10-day hiatus, and then 30 additional days of secretion. Although the procurement of daily TGS was dangerous, it was accomplished with relative safety because of the WPZ's restraining crush, which limited bull movement during sample collection (Rasmussen et al., 1984; Acknowledgments). Specially designed wide-bore Erlenmeyer flasks with flattened openings were placed directly over the cleaned orifice of the temporal gland and pressure was firmly applied to obtain the secretion. Sufficient pressure was applied to ensure that the sample secretions were ejected into the flask. This simulated the effect of behaviors normally exhibited by musth bulls in captivity and in the wild, during which they shove their heads against objects with sufficient force to eject material from the gland 0.5–1.0 m. Samples were all fresh, flowing secretions. The bull soon learned to associate no unpleasantness with the procedure, and samples were obtained routinely. Samples were frozen in 1-ml aliquots in liquid nitrogen and stored at $-20°C$ until analysis. Whenever possible, concurrent serum samples were obtained by the method described by Hess et al. (1983). All serum and TGS samples were obtained between 9–11 AM.

Sample Analyses

Radioimmunoassay of T and DHT. Serum and TGS aliquots (500 μl) were extracted with redistilled diethyl ether and the extract sequentially chromatographed on two different Sephadex LH-20 chromatographic columns prior to analyses by radioimmunoassay (RIA) (Resko et al., 1973, 1980). The first column (1.0 g LH-20, elution phase hexane–benzene–methanol, 62:20:13 v/v) separated neutral and phenolic steroids. The second column (2.5 g LH-20, elution phase, hexane–benzene–methanol, 85:15:5 v/v) isolated progesterone (P), dihydrotestosterone (DHT), and testosterone (T).

Extraction and chromatographic losses were monitored by adding known amounts of tritiated authentic steroids to the elephant serum or TGS and processing in parallel with the samples for assay. Respective recoveries following the final chromatographic step were DHT—72.0% and T—70.0%. Water blanks were also processed in parallel to provide solvent–method blanks for each steroid (DHT, 18.6 ± 9.8; and T, 3.5 ± 1.9 pg). Reported values were corrected for both procedural losses and method blanks before correcting for aliquots assayed. Each sample was diluted with 500 μl of ethanol after chromatography and assayed at three or four different volumes. The reported values are the average concentration calculated from aliquots whose values fell between the 5–95% binding limits of the standard curve following a logit-log transformation. Further details of the procedures are listed in Rasmussen et al. (1984).

Gas Chromatography. One-milliliter aliquots of TGS from each sample day were extracted by hexane, dichloromethane, acetonitrile, and chloroform. The dichloromethane fraction contained most of the organics, which were further studied; they were filtered through sodium sulfate for water removal prior to GC analysis.

Samples were analyzed by on-column capillary column GC. If, after initial analysis, few compounds were detected, the sample was concentrated 10-fold.

Prepared sample extracts (0.5 μl) were injected directly by silica needle onto a fused silica column (DB-1, 60 m), using helium as the carrier gas, at a flow rate of 30 cm/sec. Flame ionization detector (FID) was utilized with makeup gases of O_2 (40%) and N_2 (60%) mixture. The oven of the Hewlett-Packard 5790A gas chromatograph was programmed to initiate a warming sequence at 35°C, increasing at a rate of 6°/min. The sequence required 48 min for completion and attained a maximum temperature of 323°C.

Standards during the on-column capillary column GC analyses included compounds identified in TGS by GC-MS (Wheeler et al., 1982; Rasmussen et al., 1986; Rasmussen, 1988). Standards were utilized both separately and as internal standards. The areas of all peaks were determined using an HP 5792 integrator. All samples were run in triplicate; the data were analyzed statistically for run-to-run reproducibility of 3%.

GC-MS. After the screening analyses by GC, representative extracts from samples demonstrating different testosterone levels were further analyzed by GC-MS. While quantitative comparisons were obtained through peak integration routines (HP 5792), identification of the specific profile constituents was accomplished by GC-MS. Gas chromatographic conditions for the GC-MS analyses were identical to those used for the routine GC analyses. When necessary, trimethylsilyl derivatives were prepared. For the initial studies, we utilized a Finnegan model 4000 mass spectrometer interfaced with an InCOS data system. Subsequently, the analyses were performed on a VG 7070 E-HF double-focusing mass spectrometer system using electron impact ionization at 70 eV. The mass spectrometer was equipped with an 11/250 data system. The NBS library of chemical compounds was utilized in computer searches involving compound identification. Numerous peaks were resolved. However, in this study, only compounds resolved in the initial GC-MS analysis with a greater than 80% purity and meeting the standard of the computer matching criteria of 90% were further studied, and obvious contaminants were ignored. Criteria for authentication included GC retention times and mass spectrometry. All compounds in Tables 3 and 4 (below) were verified by matching spectra information and relative retention times with those of authentic compounds. Particular attention was paid to two compounds: (*E*)-farnesol, previously identified as a major constituent of African elephant TGS (Wheeler et al., 1982), and 4-methylphenol, identified in African elephant TGS, Asian bull elephant TGS, and urine extracts of both male and female Asian elephants (Rasmussen et al., 1986). Other phenols (4-ethylphenol, 2-*n*-propylphenol, and 4-*n*-nonylphenol) reported in the temporal gland secretion from captive Asian bull elephant TGS (Rasmussen, 1988) also were monitored, as were additional ketones, aliphatic acids, and alcohols.

RESULTS

Testosterone and Dihydrotestosterone

Serum. Although it was possible to obtain only a few serum samples during the 1986 musth episode, the nine serum samples collected had significantly higher testosterone (T) levels during the first part of the musth episode than toward the end. The T concentrations ranged from 12.71 ng/ml during late musth to 125.7 ng/ml during early musth (Table 1). During the first two months of the musth episode, the average serum concentrations were 70.4 ng/ml ($\pm$13.5), $N = 6$, declining in a single sample during the third month of musth to 26.7 ng/ml, with the minimal level of 12.7 ng/ml achieved during late musth (fourth month). DHT levels in the serum declined from 7.98 to 1.41 ng/ml over the same time period. Serum data expressed as T/DHT ratios from this discrete

TABLE 1. CHANGES IN LEVELS OF SERUM STEROID HORMONES OF AN ASIAN BULL ELEPHANT DURING MUSTH

Days in musth episode	Testosterone (ng/ml)	Dihydrotestosterone (ng/ml)	Ratio T/DHT
Day 16	125.70	7.98	16.0
Day 24	51.83	3.70	14.0
Day 31	39.8	2.95	13.6
Day 42	42.54	3.55	12.0
Day 53	89.35	5.58	16.0
Day 59	70.87	3.54	20.0
Day 72	73.80	3.11	23.7
Day 82	26.67	3.33	8.0
Day 110	12.71	1.41	9.0
Mean	59.25	3.91	14.7
SE	11.5	0.62	4.9
Range	12.71–125.7	1.41–7.98	8.0–23.7

musth episode exhibited differences related to the time into the musth period, with ratios varying from 23.7 to 8.0.

Temporal Gland Secretions. Testosterone levels in the temporal gland secretions varied from 13.6 ng/ml to a high of 2.7 μg/ml (Table 2). Dihydrotestosterone levels varied from 3.28 ng/ml to 2.9 μg/ml (Table 2).

Temporal Changes in TGS-T and DHT during Musth. Notably, TGS testosterone levels were significantly higher during the initial phase of the musth episode than in the latter stages (Table 2). Average T levels in the TGS for the first two months (June and July, days 1–59) were 921.6 ng/ml; TGS-T was low for several days between the two clusters of high TGS-T levels, June 15–26, days 13–24, and July 14–31, days 42–59. During the third month (August, days 60–87) TGS-T level averages were 162.0 ng/ml and during the last month of the musth episode (September and October, days 88–117), 36.6 ng/ml (Table 2). During the first half of the musth period, 87% of the samples exhibited T levels above 300 ng/ml. During the latter half of the musth episode, 92% were lower than 300 ng/ml.

DHT levels exhibited similarly high values during the first half of the musth period (Table 2). Mean values from June and July, days 1–59, and the first half of August, days 60–75 (1007.4 ng/ml), were significantly higher than values from the next month (177.1 ng/ml) and the last month (75.9 ng/ml) (Table 2). DHT values were often higher than T levels (Figure 1).

T/DHT Ratio in TGS. T and DHT were generally elevated or lowered to the same degree throughout the four-month musth episode, resulting in T/DHT ratios that varied only between 0.16 and 2.8, except for two peaks of high T/

TABLE 2. SUMMARY OF TEMPORAL GLAND SECRETION STEROID HORMONE LEVELS DURING 1986 MUSTH EPISODE, JUNE–OCTOBER

	Testosterone (ng/ml)	Dihydrotestosterone (ng/ml)
First two months		
June 3–July 31		
Day 1–Day 59		
59 days, 45 samples		
Mean	921.6	1007.4
SE	91.9	95.0
Range	282.9–2781.3	128.6–2949.2
Third month		
August 1–August 28		
Day 60–Day 87		
28 days, 28 samples		
Mean	162.0	177.1
SE	29.1	33.1
Range	24.2–504.9	24.6–619.5
Last month		
September 8–October 7		
Day 88–Day 117		
29 days, 28 samples		
Mean	36.6	75.9
SE	4.3	11.1
Range	13.6–104.8	12.1–262.7

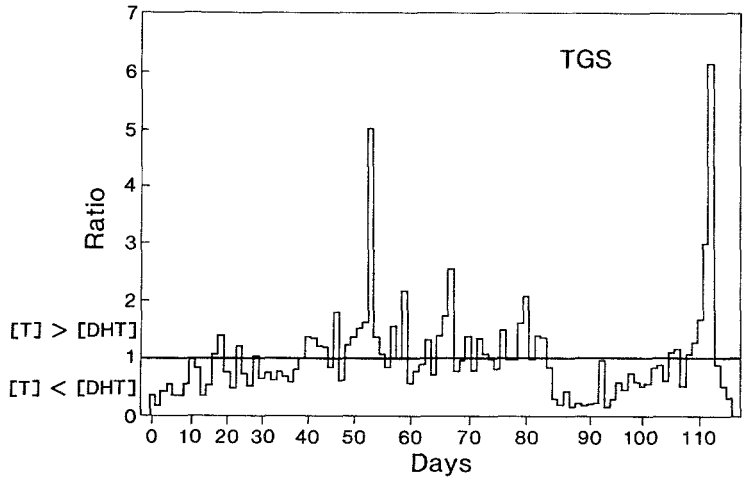

FIG. 1. Ratio of testosterone (T) to dihydrotestosterone (DHT) in the temporal gland secretions of an Asian bull elephant during a four-month musth episode. [T] > [DHT] means T concentration greater than DHT; [T] < [DHT] means DHT concentration greater than T. The solid line indicates the ratio of 1 or when the concentrations are equal. The horizontal axis is the days into the musth period.

DHT ratios (Figure 1). These TGS T/DHT ratios were lower compared to serum T/DHT ratios (8.0–23.7) (Tables 1 and 2).

Highest TGS/S ratios of testosterone were recorded between days 24 and 59 of the musth episode. This period of very high TGS-T levels also demonstrated the highest concentrations of 5-nonanol and 2-nonanone, as well as several other ketones. Several phenols were also relatively high just prior to this period (Figure 2).

Gas Chromatography and Mass Spectrometry

Dichloromethane extracted the most volatiles when compared to other solvents tested using capillary column gas chromatography, and only these results are reported.

GC-MS analysis was conducted on 30 extracts of samples of Asian bull elephant temporal gland secretion. Twenty-three major compounds and 16 minor compounds were identified. Table 3 lists all the compounds identified, together with their molecular weight, molecular ion, and mass ions in order of GC retention time. Eight compounds were detected in all musth TGS samples: 5-nonanol or 2-nonanol, 4-methylphenol, phenylacetic acid, 2-nonanone, octanoic acid, 4-*n*-nonylphenol, tetradecanoic acid, and testosterone (Table 4). Fifteen compounds were identified in more than five samples: phenol, benzoic acid, phenylpropionic acid, 2-hydroxyacetophenone, hexanoic acid, 3-octen-2-one, 4-ethylphenol, decanoic acid, 4-hexenoic acid, 3-nonen-2-one, pentadecanoic acid, hexadecanoic acid (palmitic acid), (*E*)-farnesol (3,7,11 trimethyl-1,6,10 dodecatrien-3-ol), dotriacontanol, and 3-ethyl-3-hydroxy-5-α-androstan-17-one.

The volatiles, as separated on GC during four representative periods during the musth episode, are depicted in Figure 2. These four selected composite chromatograms are representative of TGS samples containing various T concentrations during the progression of the musth period. Fluctuations of note in concentrations of TGS components are apparent. This especially includes various phenols, several alcohols (predominantly farnesol), several aliphatic and carboxylic acids, ketones, and steroids.

DISCUSSION

The musth period of Asian bull elephants is characterized by radical behavioral changes, including an increase in aggressiveness and dominance displays (Schmidt, 1978, 1989), and by physiological and biochemical events including temporal gland secretions, elevated serum testosterone (Jainudeen et al., 1972b; Rasmussen et al., 1984), and TGS testosterone levels in the microgram range (Rasmussen, 1988). Distinct differences in the TGS volatiles from Asian bulls in musth and a bull whose musth cycle was artificially terminated for manage-

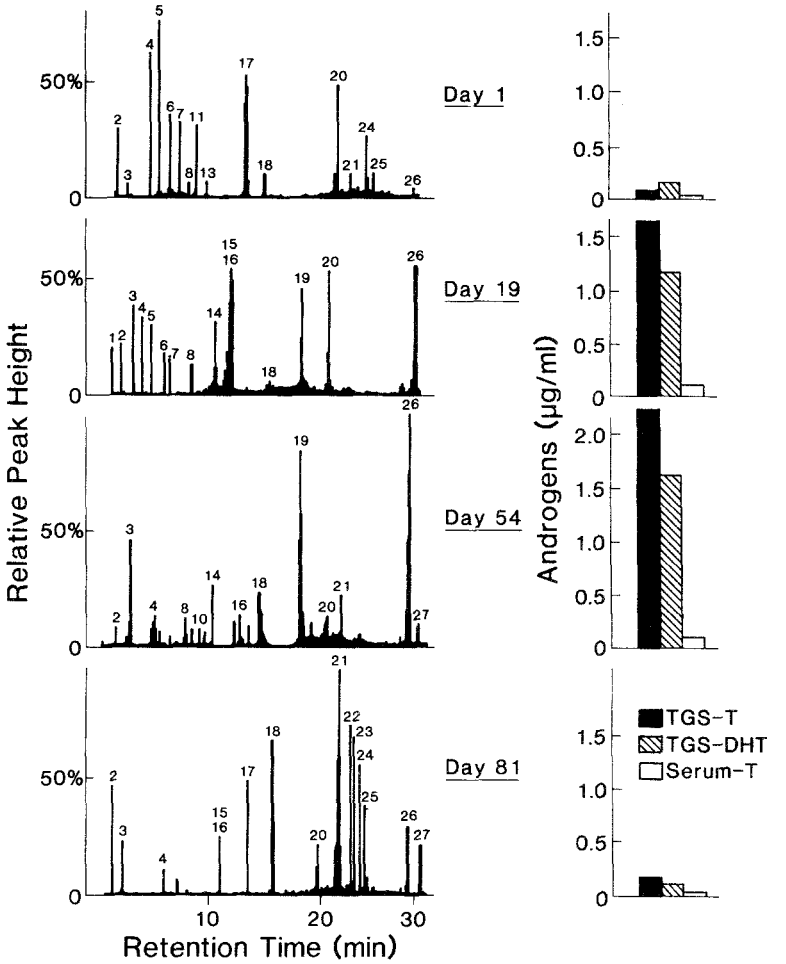

FIG. 2. Depicted in the major graph on the left are the fluctuating concentrations of volatiles in temporal gland secretions of an Asian bull elephant during a discrete musth period. (peak 26, day 54 (graph 3) is set at 100%). The four graphs are composite chromatograms combining specific separation runs. Compounds include: 1, cyclohexanone; 2, phenol; 3, 5-nonanol; 4, 4-methylphenol; 5, benzoic acid; 6, phenylacetic acid; 7, phenylpropanoic acid; 8, 2-hydroxyacetophenone; 10, 2-nonanone; 11, octanoic acid; 13, 3-octen-2-one; 14, 4-ethylphenol; 15, 2-*n*-propylphenol; 16, 4-*n*-propylphenol; 17, decanoic acid; 18, 4-hexenoic acid; 19, 3-nonen-2-one; 20, 4-*n*-nonylphenol; 21, tetradecanoic acid; 22, pentadecanoic acid; 23, hexadecanoic acid; 24, farnesol; 25, dotriacontanol; 26, testosterone; 27, 3-ethyl-3-hydroxy-5α-androstan-17-one. The smaller graph on the right depicts the concentrations of temporal gland secretion testosterone (TGS-T), temporal gland secretion dihydrotestosterone (TGS-DHT), and serum testosterone (Serum-T).

TABLE 3. COMPOUNDS DETECTED BY GC-MS IN MALE ASIAN ELEPHANT[a] TEMPORAL GLAND SECRETION

Compound	RT	MW	*m/z* (relative abundance)
Dimethyl disulfide	7	94	94(100), 79(74), 47(60)
Dimethyl sulfone	7.3	94	94(50), 79(100)
Cyclohexanone	7.5	98	98(22), 70(30), 69(30), 55(100), 45(58)
Trimethylcyclohexane (1,3,5)	9.0	126	126(20), 111(50), 69(100), 57(70), 55(75), 41(80)
PHENOL	10	94	94(100), 66(16), 65(14), 39(8)
5-NONANOL	11	144	144(4), 87(65), 69(100), 41(35)
2-Nonanol	11	144	144(4), 69(10), 55(10), 45(100)
4-METHYLPHENOL	12.0	108	108(81), 107(100), 90(15), 79(43)
BENZOIC ACID	13.4	122	122(75), 106(5), 105(100), 77(70), 51(45), 50(40)
PHENYLACETIC ACID	13.8	136	136(35), 92(10), 91(100)
PHENYLPROPANOIC ACID	13.9	150	150(40), 105(10), 104(45), 95(5), 91(100)
2-HYDROXYACETOPHENONE	14.1	136	136(5), 122(15), 106(5), 105(100), 77(55), 51(10)
Toluene	14.3	92	92(50), 91(100), 64(10), 39(5)
2-NONANONE	14.4	142	142(5), 71(20), 59(20), 58(100), 57(15), 43(85)
5-Nonanone	14.4	142	142(10), 85(90), 58(75), 57(100), 41(40)
3-Nonanone	14.4	142	142(5), 114(35), 72(50), 58(80), 43(100)
HEXANOIC ACID	14.5	116	116(5), 87(10), 73(40), 60(100)
OCTANOIC ACID	15	144	144(2), 116(5), 101(10), 73(75), 60(100), 55(30), 43(50), 41(40)
3-OCTEN-2-ONE	15.3	126	126(15), 11(60), 55(100), 43(80), 41(45)
Cyclododecanone	15.5	182	182(5), 139(5), 11(25), 71(50), 58(50), 55(90), 42(100)
4-*n*-ETHYLPHENOL	16	122	122(31), 108(5), 107(100), 77(5)
2,3-Dihydroindole	16.5	119	119(60), 118(100), 117(20), 91(20)
2-*n*-Propylphenol	17	136	136(40), 121(100), 122(5), 107(15), 91(5), 77(7)
4-*n*-Propylphenol	18	136	136(35), 122(5), 121(100), 107(10), 79(45), 78(5), 77(35)
DECANOIC ACID	19	172	172(5), 129(40), 73(90), 71(30), 60(100), 58(45), 56(40), 44(50), 42(50)
4-hydroxyacetophenone	20	136	136(40), 121(100), 94(40), 65(36)
4-HEXENOIC ACID	21	114	114(50), 72(20), 69(30), 68(40), 60(50), 56(100), 41(50)
3-NONEN-2-ONE	23	140	140(5), 125(55), 98(30), 71(30), 55(100), 43(100), 41(35)
8-Nonen-2-one		140	140(3), 82(25), 71(30), 58(60), 43(100)
5-Nonen-2-one		140	140(3), 82(30), 60(30), 55(30), 43(100)
4-*n*-NONYLPHENOL (and isomers)	26	220	220(5), 192(5), 164(10), 149(45), 134(100), 135(10), 122(50), 107(55)
tridecanoic acid	27	214	214(10), 171(15), 129(30), 115(15), 73(100), 60(100), 57(50), 55(50), 43(80), 41(70)
TETRADECENOIC ACID	29	228	228(20), 185(20), 129(30), 73(100), 71(30), 69(30), 60(90), 57(55), 55(60), 43(85), 41(65)
PENTADECANOIC ACID	30	242	242(30), 198(15), 185(5), 129(30), 74(100), 71(30), 69(80), 60(90), 57(60), 55(60), 43(95), 41(80)
HEXADECANOIC ACID	30.5	254	254(20), 236(10), 84(40), 69(70), 55(100), 43(60), 41(90)

TABLE 3. Continued

Compound	RT	MW	*m/z* (relative abundance)
(*E*)-FARNESOL (3,7,11-trimethyl-1, 6,10-dodecatrien-3-ol)	29-31 -32	222	222(5), 207(2), 204(15), 191(10), 189(5), 162(10), 93(40), 69(100), 41(80)
DOTRIACONTANOL	34	466	97(10), 85(40), 71(50), 69(45), 58(100), 43(100)
TESTOSTERONE (17-β-hydroxy-4-androsten-3-one)	36	288	288(35), 246(40), 228(5), 204(20), 147(30), 125(100), 109(30), 107(35), 105(40), 95(20), 93(35), 91(50), 55(20), 41(55)
3-ETHYL-3-HYDROXY-(5-α)-ANDROSTAN-17-ONE	36	318	318(5), 300(10), 272(5), 218(5), 190(10), 161(20), 147(40), 135(40), 121(40), 119(40), 107(50), 105(80), 93(95), 91(100), 82(75), 80(80), 78(40), 67(70), 55(80), 53(40), 41(40)

[a] CAPITALS: compound in all TGS samples; CAPITALS: compound in many TGS samples; lower case: compound in some TGS samples; RT = retention time; MW = molecular weight.

ment reasons have previously been reported (Rasmussen, 1988). These variations were sufficient to suggest that changes in TGS volatiles might be correlated to changes in physiology and behavior during musth. Our intimate access to healthy and behaviorally normal bull elephants has afforded us a unique oppor-

TABLE 4. COMPOUNDS IDENTIFIED IN TGS ASIAN BULL ELEPHANTS IN MUSTH -ORDERED PER INCREASING RETENTION TIME-

In all TGS samples	In more than five TGS samples
5-Nonanol	Phenol
4-Methylphenol	Benzoic acid
Phenylacetic acid	Phenylpropionic acid
2-Nonanone	2-Hydroxylacetophenone
Octanoic acid	Hexanoic acid
4-*n*-Nonylphenol	3-Octen-2-one
Tetradecanoic acid	4-Ethylphenol
Testosterone	Decanoic acid
	4-Hexenoic acid
	8-Nonen-2-one
	Pentadecanoic acid
	Hexadecanoic acid
	Farnesol
	Dotriacontanol
	Testosterone
	3-Ethyl-3-hydroxy-5α-androstan-17-one

tunity to analyze the biochemical variations which characterize a normal musth period in an Asian bull elephant.

Of special interest were androgen levels. The temporal patterns of T and DHT during an individual musth episode were distinctive in both the temporal gland secretions and serum. In the TGS, the significantly higher T and DHT shown during the first half of the musth episode were striking. The range of TGS-T concentrations was wide (13.6 ng/ml–2.8 μg/ml). In the serum, wide-ranging T concentrations (12.7–125.7 ng/ml) were also characteristic. These musth serum values were of considerably wider range than previously reported values (Jainudeen et al., 1972b; Rasmussen et al., 1984). In addition, previously, Jainudeen et al. (1972a,b) reported variations in serum T levels during premusth, full musth, and postmusth sampling of about a dozen different bulls. Our report is the first to monitor the chemical changes which occur during a complete musth episode in a single Asian bull elephant.

DHT concentrations in both the serum and the TGS of Asian bull elephants in musth were remarkably high. Other reported exceptions to the characteristically low serum DHT in mammals include rhesus monkeys (Resko et al., 1980) and ferrets (Rieger and Murphy, 1977).

Bull Asian elephants in musth also have elevated serum T/DHT ratios as compared to other male mammals including humans, bats, and dogs (Fiorelli et al., 1976; Bernard, 1986; Tremblay et al., 1972; Folman et al., 1972). Superficially, these high ratios appeared similar to the relatively high ratios observed in some mammals during the breeding season or during rut. For example, mean T/DHT serum ratios in breeding male ferrets ranged from 1 to 4 (Rieger and Murphy, 1977) and in breeding badgers from 11.4 to 17.5 (Maurel et al., 1981). Examination of our data from three studies (this study; Rasmussen et al., 1984; Rasmussen, 1988) demonstrated that three Asian bull elephants, during five successive musth periods, had serum T/DHT ratios ranging from 8.0 to 58.3. [Ratios in nonmusth serum were generally twofold lower; toward the end of the long 1986 musth period, low ratios of 8.0 and 9.0 occurred in serum (Table 1).] Two significant differences appeared when the elephant data were compared with those derived from studies of rutting or breeding animals: serum T levels were strikingly high in the musth Asian bull elephant, and T/DHT ratios were high. In breeding badgers, the high T/DHT ratio occurred when both T and DHT concentrations were low (Maurel et al., 1981). By contrast, high total DHT levels in the elephant were coupled with the extremely high T levels to produce a high/high situation.

In the TGS a higher ratio of T/DHT was seen during the heaviest behavioral musth and time of maximum secretion, June 11–August 24, than during either very early musth or most days toward the end of the musth period (Table 2). TGS samples obtained before 1986 were biased as most were gathered during times of moderate to heavy musth.

In addition, the TGS/serum ratio of testosterone changed during the musth period, becoming very high during "heaviest" musth when the most unpredicable and aggressive behaviors occur, usually concurrently with copious excretion of odoriferous glandular products. The greatest variety and concentration of volatiles, as well as the highest levels of testosterone and dihydrotestosterone, were noted in temporal gland secretion at this time.

Concurrent analysis of the steroid hormones of serum and TGS, and of the organic-extractable volatile TGS components over time demonstrated some definitive changes in these chemical species as the T content fluctuated. Our TGS data suggest that steroid hormonal levels correlate with the composition and amounts of TGS volatiles, chiefly phenols, aliphatic acids, carboxylic acids, alcohols, and ketones during musth in Asian bull elephants. Three striking pattern changes in TGS volatiles were noted during the course of the musth episode: (1) a high proportion of benzoic acid and certain substituted carboxylic acids in early musth; (2) a predominance of ketones during the time of heavy musth (40–70 days); and (3) Similarity of patterns from very early musth and very late musth TGS, especially with regard to long-chain aliphatic acids and two higher alcohols: dotriacontanol and farnesol (Figure 2).

Such moderately volatile compounds deposited in the environment could serve as chemical communicators to neighboring elephants, perhaps affecting long-term relationships such as dominance hierarchy by conveying less immediate, longer-lasting information relative to the hormonal and/or physiological status of the depositor. Processing may occur, as with other stable organics, via the vomeronasal organ. Because of the frequency of flehmen responses by females to moderately aged (2–24 hr) or frozen/thawed TGS, we had hoped to identify organic-solvent-extractable molecules, perhaps steroidal in nature. No single compound appeared to dominate among the volatiles, and bioassays of identified compounds only demonstrated the novel substance response among both the bulls and the cows. Probably the bioactive compounds are either in very low concentration or exist in combinations or bioactivity resides in more volatile compounds.

Recent behavioral response data from both female and male Asian elephants revealed that olfactory responses and avoidance behaviors (defined as deliberate nonapproach to areas that in normal situations would be approached, such as piles of bananas or apples) were of higher frequency toward newly collected, fresh (less than 1 hr) TGS as compared to frozen/thawed TGS or to aged TGS (Rasmussen, Haight, and Perrin, unpublished); in contrast frozen TGS or 1-day-aged TGS elicited a higher proportion of flehmen responses among the total responses observed (Rasmussen, 1988). The more volatile, ephemeral compounds, probably received by the olfactory system, may serve as immediate informants of the presence of a nearby, potentially aggressive bull, as evidenced by the consistency and rapidity with which cows and calves avoid such odors.

Such observations support the concept of the temporal gland as a multifunctional gland.

At present it cannot be stated that T levels influence levels and composition of volatiles in Asian bull elephants in musth, but only that the concentrations of selected volatiles in the temporal gland secretions change concurrently with altered serum testosterone levels. More direct relationships between T levels, released volatiles, and aggression and dominance ranking have been described in pigs, where pheromones and androgens affected aggression (Parrott et al., 1985), and in male golden hamsters, where endogeneous androgens were associated with changes in dominance status (Drickamer et al., 1978). In mice, critical T-dependent, aggression-eliciting characteristics of urinary pheromones were identified (Duvall et al., 1978). In male pine voles a urinary volatile appeared to have an endocrine dependency (Boyer et al., 1989). Our data suggest, in a similar fashion, that the levels of certain volatiles may be under endocrine control and that some of the TGS volatiles of Asian bull elephants in musth may function in chemocommunication, perhaps in the context of conveying information to the older, dominant bulls that younger, mature bulls are present and perhaps ready to challenge, as suggested by Schmidt (1989). The variation seen in T and DHT levels and ratios over time during musth suggest these steroid hormones may either play a role in influencing the events of musth or reflect the chemistry of musth.

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