Asian Bull Elephants: Flehmen-Like Responses to Extractable Components in Female Elephant Estrous Urine

Abstract. *Flehmen-like responses (urine tests) are one of the characteristic behavioral reactions of male Asian elephants* (Elephas maximus) *to cow elephants in estrus. Components of the urine of estrous cow elephants were extracted with organic solvents and partially purified by chromatography and shown to evoke Flehmen-like responses when they were presented to adult bulls.*

We have demonstrated that components (or a component) of the urogenital sinus secretions and urine of female Asian elephants (*Elephas maximus*) in estrus evoke demonstrable, repeatable Flehmen-like responses in male Asian elephants. The Flehmen response, or grimace, has been described in many mammals and usually consists of the male of the species contacting urine or other excretions with its lips or nose and subsequently bringing a sample of the excretion into contact with the vomeronasal organ (*1*). Most tetrapods possess a discrete vomeronasal organ or vomeronasal area, and sensations mediated by this sensory system are presumably discrete from nasal olfaction (*2*). Sexually stimulating odors are evidently among the odors received by the vomeronasal organ (*3*).

In the male elephant, the Flehmen-like response (termed the urine test) (*4*), is composed of several characteristic actions (Fig. 1). The tip of the trunk—a highly innervated and elongated muscular fusion of the upper lip and nose (*5*)—detects and then contacts directly any potentially stimulating wet spot (Fig. 1). After several sniffs and aspirations, if sufficient odor stimulus is present, the trunk tip is curled toward the mouth and inserted into a small recess in the dorsal anterior part of the mouth. The trunk tip presses against the twin orifices of the vomeronasal organ (Fig. 2), directly transferring a sample of the liquid or volatile compounds. A characteristic 3- to 8-second pause occurs at the termination of about 50 percent of Flehmen-like responses.

Results from an 8-year study at the Washington Park Zoo, Portland (*7, 8*), indicate that the number of Flehmen-like responses made by a bull elephant after it has touched the urine or urogenital orifice of a female (*6*) is inversely related to the female's serum progesterone levels and is correlated with occurrence of penile erections. When a female is approaching estrus (about every $3\frac{1}{2}$ months), the number of Flehmen-like responses increases dramatically and remains elevated until the cow goes out of

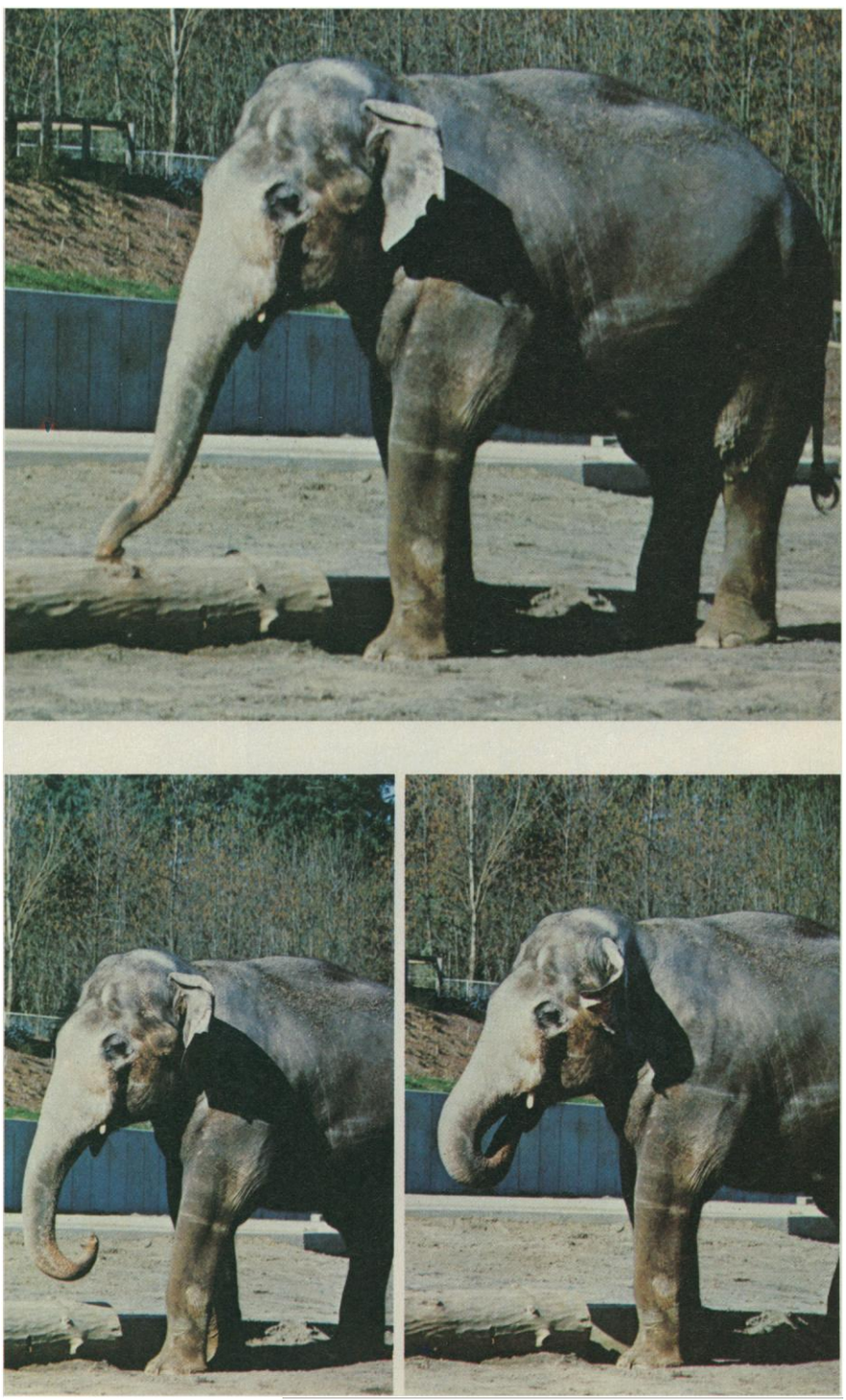

Fig. 1. Packy, an Asian bull elephant, gives a Flehmen-like response to a test solution. (Top) Detection and pickup of a sample of the solution. The elephant stops, turns its trunk tip toward the scent, places its trunk tip on the test spot, and picks up some of the solution. (Left) Curling of the trunk tip. The mouthward curl of the trunk tip proceeds until the trunk is flattened and doubled back upon itself (a gesture clearly distinguishable from eating or drinking actions). As the trunk tip is inserted into the mouth, it is bent at about a 70° angle before being pressed on the vomeronasal organ. (Right) Contact with vomeronasal organ. The lower lip aids in positioning the trunk tip directly on the twin orifices of the vomeronasal organ. The entire Flehmen-like sequence takes place in seconds and may occur repetitively.

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heat (*7*, *8*). This suggests that cow elephants secrete a pheromone that attracts male elephants and signals that the cow is in estrus (*9*).

For our experiments the bull elephant is routinely permitted to roam alone and unrestrained in a sandy yard (0.3 hectare) that is partially covered and is used by male and female elephants. On entering the yard, the bull inspects the entire area, showing special interest in damp spots. Urine spots are often sniffed several times, and Flehmen-like responses result if sufficient concentrations of specific stimulatory components are present. The principal bull used in our study, Packy, is unique in the Western Hemisphere as a breeding bull elephant. Born at the Washington Park Zoo in 1962, he has successfully inseminated several female elephants, producing seven calves, five more than any other living Asian bull in the Western Hemisphere (*10*). Such breeding behavior in captivity is lacking in many Asian male elephants, hence the rarity of elephant births. However, Tunga, a mature (17 years old) former circus bull, resident at the Washington Park Zoo since 1980, was also tested; his Flehmen-like responses to the extracts presented were similar to Packy's.

Urine for study was obtained from six regularly cycling cow elephants during the height of established estrus. Several females were sampled in more than two estrous periods. Between 1 and 50 liters of urine was collected in clean glass containers with special Teflon gaskets. No loss of activity occurred with freezing and thawing, but generally samples were extracted on the day of collection.

Urine samples (1-liter portions) or saline washes from the urogenital sinus (UGS) (3-liter portions) were extracted with one of the following solvents: diethyl ether, dichloromethane, or chloroform. The extract was concentrated to about 0.5 ml and the extracting solvent was gradually replaced with acetone. Complete evaporation of solvent was avoided to ensure retention of (possibly highly volatile) active substances. For bioassay, extracts (in 0.5 to 1 ml of acetone) were suspended in 500 to 1000 ml of nonestrous elephant urine. Control samples, consisting of 1-ml portions of the solvents used during the extraction procedure added to nonestrous urine, were also tested. These samples evoked no Flehmen-like responses. Second-level separation techniques of thin-layer or flash chromatography (*11*) were used in several instances.

For the bioassay, 500- to 1000-ml experimental or control samples at 38°C were placed on clean concrete or on a log immediately before the bull was admitted to the yard. A double-blind observer (who did not observe sample placement and was not informed about the samples until after the test) recorded the responses of the bull for 1 hour or until the test site was checked by the bull five times. One test session was conducted per day, with one control and one experimental sample being tested per session.

The control urine samples as well as the experimental samples were located by the bull elephant usually within 1 to 5 minutes after his entry into the yard. The Flehmen-like response (Fig. 1) was observed in Packy between 3 and 15 times in response to 31 different samples of urine from estrous females for a total of 191 responses (Tables 1 and 2). The second bull, Tunga, in eight tests averaged 5.25 Flehmen-like responses per test (Table 2). The response to nonestrous urine was different; the urine spots were checked briefly but repeatedly.

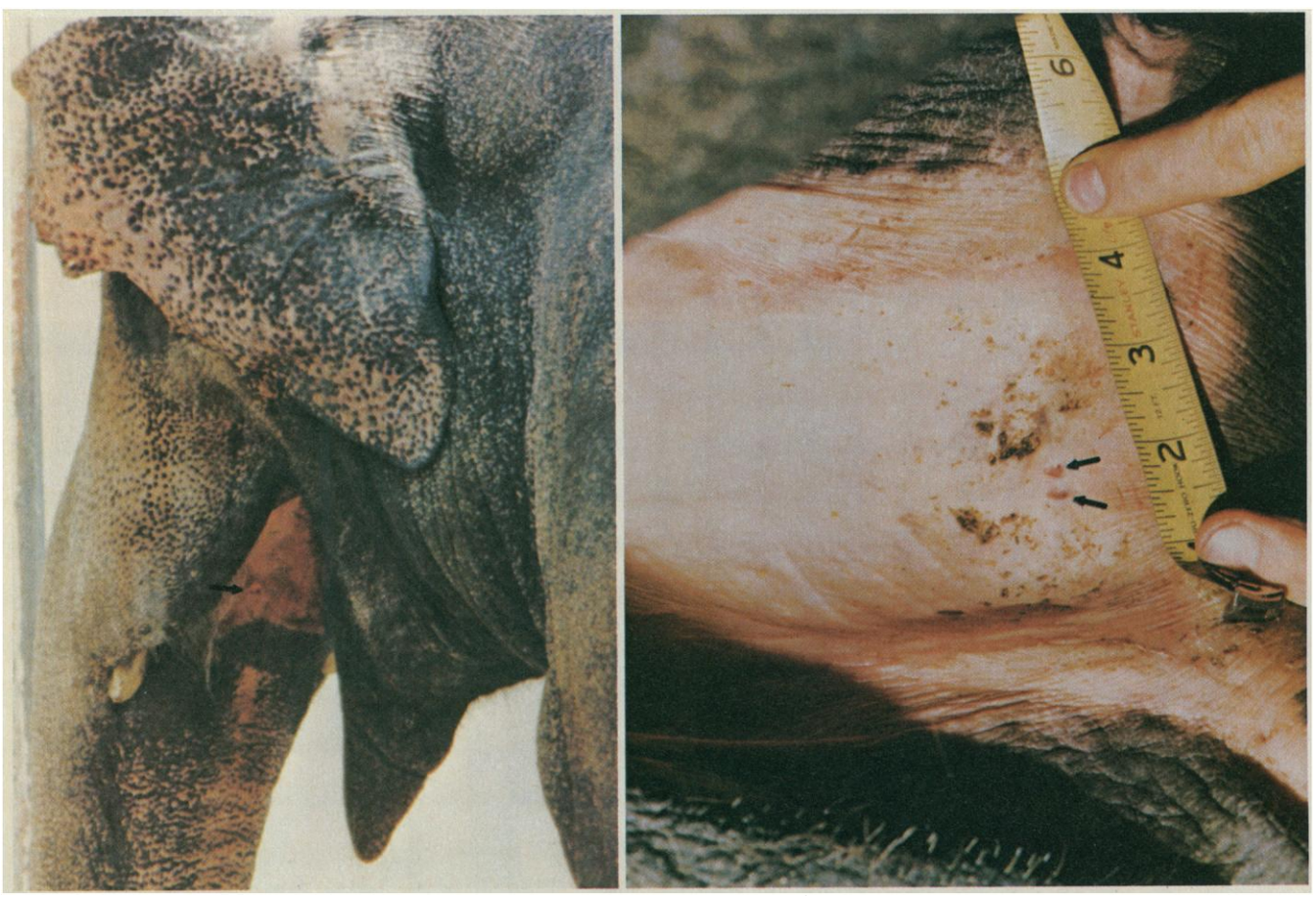

Fig. 2. The paired orifices of the elephant vomeronasal organ are in the dorsal anterior roof of the mouth, rostral to the nasal area. (Left) The two openings are visible in the open mouth of Packy. (Right) A closeup view of the two openings, indicated by arrows.

During 40 tests with nonestrous urine a single Flehmen-like response was recorded only nine times, for an average of 0.23 response per test. Extracts of estrous urine, resuspended in nonestrous urine in 28 tests, evoked 109 Flehmen-like responses (data for both elephants combined), an average of 3.8 per test—that is, fewer than 5.9 responses per test with whole estrous urine but clearly greater than the < 1 average of nonestrous urine extract, nonestrous urine, and all other controls (Tables 1 and 2).

That extracts of estrous urine evoke fewer Flehmen responses than whole estrous urine suggests that (i) our extraction procedure is incomplete; (ii) one or more of the active components are lost during processing; (iii) the solvents used mask the active components; or (iv) components of nonestrous urine provide contradictory signals to the male elephant. Saline irrigations of the UGS of estrous cows and extracts of these also evoked Flehmen-like responses (Table 1). As extracts of estrous urine were further purified the total number of Flehmen-like responses in a 1-liter equivalent increased from an average of 3.8 in crude extract to 5.5 in extracts purified by preparative thin-layer chromatography and 5.3 in extracts purified by flash chromatography (Table 2).

Males of several mammalian species, including a marsupial, are attracted by olfaction to receptive females; these species include wombats, deer mice, rats, golden hamsters, goats, rams, cattle, horses, dogs, deer, white rhinos, and New World monkeys (*12, 12a, 13*). Estrus is detected by olfaction of female urine in rams, male dogs, and male rats (*12a, 14*). In male hamsters the vomeronasal organ is used during sexual behavior (*15*). Males of certain Artiodactyla

Table 1. The number of Flehmen-like responses made by male Asian elephants per hour. All the samples tested were 1-liter equivalents. Extracted urine refers to the urine left over after organic solvent extraction. Estrous urine extract refers to the organic solvent–extractable components resuspended in nonestrous urine for bioassay. The number of assays is indicated in parentheses. Neither Packy nor Tunga showed any Flehmen-like responses to the control samples consisting of the solvents (1 ml of acetone, methylene chloride, chloroform, ether, or hexane) added to nonestrous urine. Controls for the UGS samples, which consisted of saline washes from the UGS of nonestrous females, evoked no Flehmen-like responses.

Nonestrous urine		Estrous urine		Extracted nonestrous urine*	Extracted estrous urine		Nonestrous urine extract*	Estrous urine extract		UGS		Active fraction of extract semipurified by	
Packy	Tunga	Packy	Tunga		Packy	Tunga		Packy	Tunga	Wash*†	Extract of wash*	Thin-layer chromatography*	Flash chromatography*
1 (7)	1 (2)	15 (1)		0 (8)	1 (4)	1 (1)	0 (8)		8 (1)	8	4	8 (1)	9 (1)
0 (28)	0 (3)	14 (1)			0 (10)	0 (2)		(1)†	7 (1)			7 (1)	8 (1)
		12 (2)						6 (2)				4 (1)	7 (1)
		10 (1)						5 (2)	5 (2)			3 (1)	6 (1)
		9 (1)						4 (4)					5 (1)
		8 (1)	8 (1)					3 (9)					4 (5)
		(1)‡	7 (2)					2 (6)					3 (1)
		6 (4)											
		5 (6)											
		(1)§	5 (2)										
		4 (6)	4 (1)										
		(3)§											
		3 (3)	3 (2)										

*Data for Packy only. †Saline wash of UGS of estrous cow elephant. ‡Penile erection observed. §Heated at 100°C for 15 minutes.

Table 2. Statistical data derived from results in Table 1.

Statistical test	Nonestrous urine*	Estrous urine		Extracted nonestrous urine†	Extracted estrous urine		Nonestrous urine extract†	Estrous urine extract		Active fraction of extract semipurified by	
		Packy	Tunga		Packy	Tunga		Packy	Tunga	Thin-layer chromatography†	Flash chromatography†
Total number of responses	9	191	42	0	4	1	0	84	25	22	58
Total number of tests	40	31	8	8	14	3	8	24	4	4	11
Average	0.23	6.11	5.25	0	0.29	0.33	0	3.50	6.25	5.50	5.27
Range	0 to 1	3 to 15	3 to 8	0	0 to 1	0 to 1	0	2 to 7	5 to 8	3 to 8	3 to 9
Mean‡	0.23	6.16	5.25	0	0.29	0.33	0	3.50	6.25	5.50	5.27
Standard deviation	0.42	3.25	1.90		0.47	0.58		1.41	1.50	2.38	1.95
Standard error	0.07	0.58	0.67		0.13	0.34		0.29	0.75	1.19	0.59

*Data for Packy and Tunga combined. †Data for Packy only. ‡Statistical tests [based on the Welsh test (*28*)] at the 90 percent confidence limit showed a difference of 5.7 ± 0.8 between estrous and nonestrous urine, 5.2 ± 2.8 between estrous urine extract and extracted estrous urine, 4.98 ± 0.73 between the extract somewhat purified by thin-layer chromatography and extracted estrous urine, and 3.56 ± 0.45 between the extract somewhat purified by flash chromatography and extracted estrous urine.

species exhibit the Flehmen response to the urine of females in estrus (*16*). Although the total number of Flehmen responses increased during the rutting season in black-tailed deer (*17*), the numbers of responses to male urine and estrous and nonestrous female urine were almost equal (*18*). Male house cats exhibit more Flehmen responses to urine from estrous than nonestrous females (*19*). Trace amounts of compounds have been identified in urine and vaginal secretions from female hamsters (dimethyl disulfide) (*20*), dogs (methyl parahydroxylbenzoate) (*21*), and monkeys (five aliphatic acids) (*22, 23*) that elicit sexual responses in males, including a vomeronasal-mediated response in hamsters (*12a, 13, 14, 19*). Increased mounting frequencies occurred in male rhesus monkeys presented with synthetic mixtures of the identified acids (*24*).

Female elephants have a true UGS, and male elephants touch females most frequently in the urogenital area (*25*). Males have apparently functional vomeronasal organs (*26*) and respond frequently with the Flehmen-like response to females in estrus in the wild (*25, 27*). In previous experiments, both Packy and Tunga responded similarly to females in estrus at the Washington Park Zoo (*7, 8*). The results described herein indicate that in Asian elephants the estrous state, and probably the receptivity of the female, is revealed by a substance or substances that can be extracted from the urine or UGS secretions, and that this substance is detected by the male using a stereotypical Flehmen-like response involving the vomeronasal organ.

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Neonatal Treatment with Antiserum to Prolactin Lowers Blood Pressure in Rats

Abstract. *Prolactin administration reportedly increases blood pressure in rats and rabbits. To study the effects of prolactin deficiency on blood pressure, rats were given saline, normal rabbit serum, or rabbit antiserum to rat prolactin on postnatal days 2 to 5. Both males and females given antiserum had significantly lower blood pressure at 14 weeks than rats given saline or normal rabbit serum. Blood pressure differences between females given antiserum and females given saline disappeared during and following pregnancy. The antiserum also lowered the concentration of prolactin in plasma 49 percent in males and decreased the prolactin response to ether stress in both sexes. These results suggest that endogenous prolactin is involved in blood pressure regulation.*

Administration of the adenohypophyseal hormone prolactin has been reported to increase blood pressure in rabbits (*1*) and rats (*2*) and in mesenteric vascular preparations from rats (*3–5*), suggesting that prolactin is involved in blood pressure regulation in mammals. Furthermore, elevated concentrations of circulating prolactin have been reported in spontaneously hypertensive rats (*6, 7*) and in humans with essential hypertension (*8*). However, the role of prolactin in the pathophysiology of hypertension is controversial. While suppression of circulating prolactin by dopaminergic agents is associated with a decrease in blood pressure in normotensive (*2*) and hypertensive (*7, 8*) individuals, it is thought that drug-induced alterations in central dopaminergic activity are the primary events resulting in the blood pressure changes and that lower concentrations of circulating prolactin merely reflect enhanced central dopaminergic activity.

We sought to determine whether changes in blood pressure accompany long-term suppression of prolactin independent of any hypotensive effect of dopaminergic drugs. Pooled litters of 2-

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