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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6. If a cow does not allow access to the urogenital orifice, the bull may aggressively provoke urination or aspirate back and forth over the cow's urine-spattered feet until he is stimulated to perform the Flehmen-like response.
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9. Such a pheromone could prove useful in the development of an artificial insemination program. Most zoos in the United States keep only female elephants because adult males are difficult to handle and expensive to maintain; as the population of Asian elephant declines and their importation into the United States is restricted, breeding programs will become necessary. Such a pheromone would also be useful in behavioral studies of wild Asiatic elephants and would facilitate the capture of live bulls. Little is known at present about their behavior in spite of their continued economic importance in the lumber industry.
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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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day-old Sprague-Dawley rats were randomly distributed to lactating females. On postnatal days 2 to 5 the pups received physiological saline (NaCl), normal rabbit serum (NRS), or rabbit antiserum to rat prolactin in a single bolus (36 μl per day, intraperitoneally). The antiserum, which was raised against NIH rat prolactin reference preparation RP-2, binds 23 μg of rat prolactin per milliliter in vitro. It does not cross-react with growth hormone, luteinizing hormone, thyrotropin, or arginine vasopressin at concentrations up to 1000 ng/ml, 8.2 ng/ml, 3.1 μg/ml, and 1000 μU/ml, respectively. Neither 10 nor 50 μl of the antiserum added directly to rat plasma significantly decreased plasma renin activity (*9*) or active renin (*10*), indicating that the antiserum contained no demonstrable antirenin activity.

Treatment with the antiserum did not alter growth rate, serum thyroxine concentration, or reproductive function in the animals. When the rats were 14 weeks of age, systolic blood pressure was determined by the tail cuff method (*11*) in a double-blind design. As shown in Fig. 1, the rats that were given antiserum to prolactin had significantly lower blood pressures (mean, 114 mmHg) than the rats that received NaCl or NRS (mean, 125 mmHg) ($P < .001$, unpaired Student's t-test).

To assess the effect of the antiserum on plasma prolactin concentrations, we cannulated all the animals when they were between 14 and 16 weeks of age (*12*). Plasma prolactin levels were determined by radioimmunoassay (*13*) of blood samples drawn every other hour between 9 a.m. and 5 p.m. After the 5 p.m. sampling the rats were subjected to ether stress for 3 minutes, and plasma prolactin was again determined. In male rats the antiserum prevented the diurnal prolactin surge that occurred between 3 and 5 p.m. in NaCl-treated rats (Fig. 2a). Over the 8-hour sampling period, serum prolactin was 10.4 ± 3.4 ng/ml in males treated with NaCl and 5.3 ± 0.9 ng/ml in antiserum-treated males—a difference of 49 percent ($P < .01$). Prolactin levels were also lower in antiserum-treated males after ether stress ($P < .05$) (Fig. 2a). In diestrous females the antiserum had no effect on plasma prolactin (8-hour sampling) but reduced the prolactin response to ether stress in comparison to NaCl-treated rats ($P < .05$) (Fig. 2b). It is possible that alterations in plasma prolactin levels become apparent during other phases of the estrous cycle when spontaneous secretion of prolactin is higher.

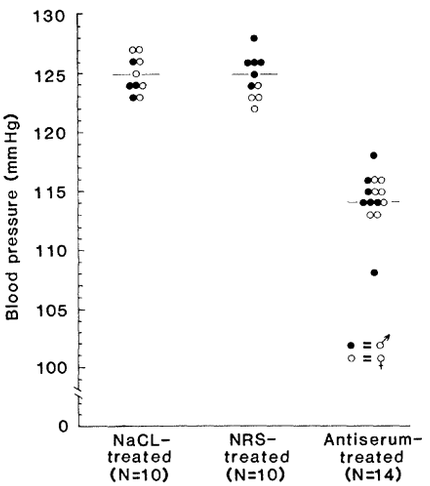

Fig. 1. Systolic blood pressures in conscious 14-week-old rats that received NaCl, NRS, or rabbit antiserum to rat prolactin on postnatal days 2 to 5. Horizontal lines represent group means. Numbers in parentheses indicate animals per group.

Seven male rats treated with NaCl and two males treated with antiserum were decapitated for measurement of pituitary weight and prolactin content (*14*). While there was no difference in mean pituitary weight between groups, prolactin content ranged from 45 to 156 ng/mg (wet weight) in NaCl-treated animals and from 16 to 48 ng/mg in antiserum-treated animals.

The remaining females (six NaCl-treated and five antiserum-treated) were bred with normal males to determine whether female fertility was affected by the antiserum. All the females conceived and delivered litters of 9 to 12 pups, suggesting that the antiserum does not significantly impair the integrity of the hypothalamic-pituitary-ovarian axis. The blood pressure nadir during pregnancy in NaCl-treated females (92 ± 2 mmHg) was similar to that in antiserum-treated animals (91 ± 3 mmHg), suggesting that pregnancy eliminates the effects of the antiserum. Consonant with this, pup growth, an indication of lactation, was not impaired in the offspring of the rats that received antiserum, and blood pressures increased normally in both groups of females and were similar after the weaning of their litters. Loss of the antiserum's effects during and following pregnancy may result from estrogen stimulation of lactotroph numbers and activity (*15*).

In this study a drug-free method was used to induce functional hypoprolactinemia in male and virgin female rats. The effect of antiserum to prolactin on pro-

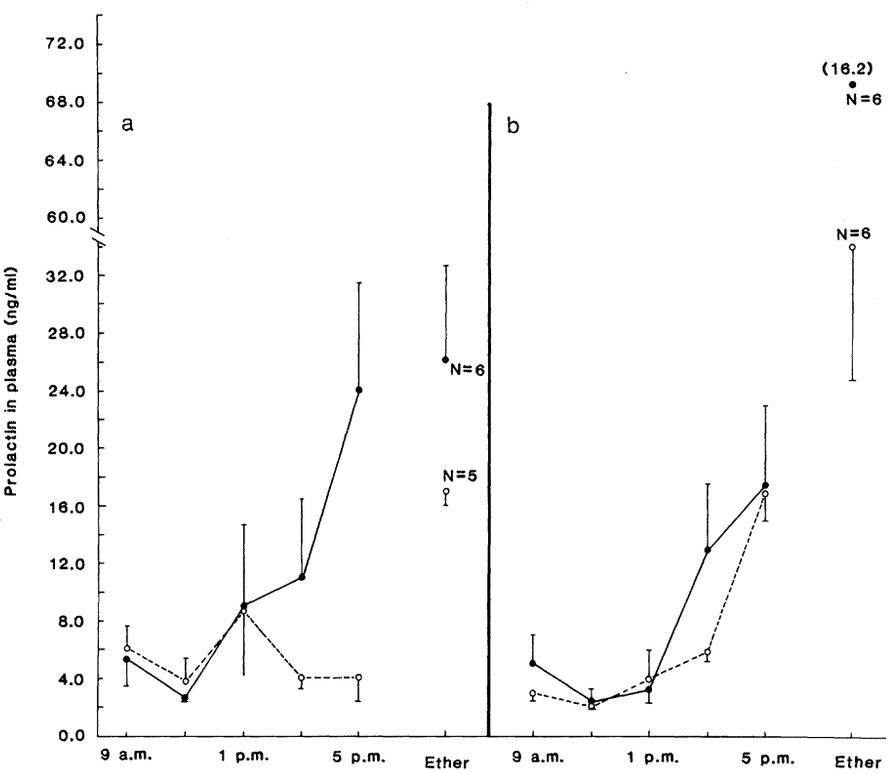

Fig. 2. Eight-hour and ether-stimulated prolactin concentrations in 14- to 16-week-old male (a) and female (b) rats that received NaCl (●) or rabbit antiserum to rat prolactin (○) on postnatal days 2 to 5. Values are means ± standard errors; the number in parentheses is the standard error for that mean.

lactin levels in males was similar to that observed following a hypotensive dose of the dopamine agonist lergotrile mesylate (*2, 12*). These data suggest that endogenous prolactin is involved in blood pressure regulation in the rat.

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Morphologic Effect of Dimethyl Sulfoxide on the Blood-Brain Barrier

Abstract. *Dimethyl sulfoxide (DMSO) opens the blood-brain barrier of mice to the enzymatic tracer horseradish peroxidase. A single injection of horseradish peroxidase in 10 to 15 percent DMSO into the tail vein along with 10 to 15 percent DMSO delivered intraperitoneally allowed horseradish peroxidase to fill the extracellular clefts throughout the brain within 2 hours. In the absence of DMSO, peroxidase failed to enter brain parenchyma except through the circumventricular organs. Opening of the blood-brain barrier by DMSO is reversible. Dimethyl sulfoxide stimulated the pinocytosis of horseradish peroxidase by the cerebral endothelium; the peroxidase was then directed to lysosomal dense bodies for degradation. Vesicular transport of horseradish peroxidase from the luminal to the abluminal wall of the endothelial cell was not observed. Dimethyl sulfoxide did not alter the morphology of endothelial cells or brain parenchyma.*

Dimethyl sulfoxide (DMSO) is an aprotic solvent with widespread laboratory use as a cryopreservative and, at higher concentrations, in the solubilization of biological membranes (*1*). The use of this drug in the treatment of cerebral infarction, brain swelling, and spinal cord injury is controversial (*2*). These conditions often exhibit abnormalities of blood-brain barrier function, but the effect of DMSO on the blood-brain barrier is unknown. The ability of DMSO to open the blood-brain barrier has been the subject of conflicting reports (*3, 4*). We know of no morphologic study of DMSO action on the blood-brain barrier. Although the blood-brain barrier normally prevents many blood-borne, large molecular weight, fat-insoluble substances from entering the cerebral extracellular fluid, opening of the blood-brain barrier can be induced by a number of experimental manipulations and pathological conditions, including seizures, systemic hypertension, hypervolemia, and the intracarotid injection of hyperosmotic substances (*5*). A safe chemical means for reversibly opening the blood-brain barrier would be of value in the treatment of brain tumors and central nervous system infections. We now report the reversible opening of the blood-brain barrier of the mouse to the intravenously administered protein tracer horseradish peroxidase (HRP) when the animals are injected intravenously and intraperitoneally with DMSO.

A total of 80 female white mice weighing 25 to 30 g were used. Injections of HRP (Sigma, type VI) (1 mg per gram of body weight) dissolved in 0.25 ml of saline or in 0.25 ml of DMSO (Tera Pharmaceuticals) at concentrations of 2, 3, 5, 10, 15, 20, or 30 percent were given as a bolus into the tail vein of unanesthetized, but restrained, mice. Some mice were also given intraperitoneal injections of 10 to 30 percent DMSO 5 to 30 minutes before the HRP injection (*6*). The brains from all mice were fixed by perfusion with a mixture of aldehydes (*7*) 2 hours after intravenous injection of HRP (*8–10*).

In mice given an intraperitoneal injection of 0.5 ml of DMSO in combination with an intravenous injection of HRP in 0.25 ml of DMSO, best results without adverse physical or behavioral effects were obtained when 10 to 15 percent DMSO was used. Dense HRP reaction product was distributed homogeneously throughout most of the forebrain, brainstem, and cerebellum (Fig. 1a). In the absence of DMSO, leakage of blood-borne HRP into the brains of control mice occurred only at sites normally containing fenestrated capillaries (*10*), such as the choroid plexus and circumventricular organs (Fig. 1b). At the ultrastructural level in brain sections from mice exposed to 10 to 15 percent DMSO, peroxidase reaction product filled the extracellular clefts of the neuropil. The membranes and general morphology of neurons, glia, and microvascular endothelial cells appeared to be unaltered. Peroxidase was never observed free in the cytoplasm of these cells, an indication that the plasma membranes remained intact. The numbers of HRP-labeled endocytotic vesicles, vacuoles, and elongated tubules in endothelial cells of DMSO-treated mice were greater than the numbers of similarly labeled structures in the cerebral endothelial cells of control animals. No concentration of labeled vesicles or tubules was seen adjacent to the abluminal surface of endothelial cells. In control animals, the HRP-labeled endocytotic structures were channeled to endothelial dense bodies, which were shown by acid-hydrolase cytochemistry to be lysosomes (*7*). Such HRP-labeled dense bodies were also present in endothelial cells of brains exposed to DMSO, but their concentration was not noticeably greater than that in controls. Neither intraperitoneal injection of DMSO with intravenous administration of HRP in saline nor intravenous injection of HRP in 0.25 ml of DMSO alone opened the blood-brain barrier to peroxidase.

Peroxidase staining of brain sections from mice given HRP in saline intravenously 2 to 24 hours after being given 10 to 15 percent DMSO intravenously and intraperitoneally was identical to that seen in brain sections from control mice. Opening of the mouse blood-brain barrier by DMSO, therefore, may be a transient event.

All mice given DMSO intravenously exhibited brief hind-limb muscle twitching and hematuria. No morphological changes were observed on gross or mi-

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