ring naturally in a human population, especially in one of the environments of human evolutionary adaptedness, raises a basic challenge to our understanding of human lactation. Apart from fertility, variables that might be affected by highly frequent nursing include lactation success or failure; infant digestive distress, sleeping patterns, and glucose dynamics; milk composition; and maternal mood and attitudes toward nursing.

MELVIN KONNER
CAROL WORTHMAN
Biological Anthropology Wing, Department of Anthropology, Harvard University, Cambridge, Massachusetts 02038

References and Notes

1. Alaskan Eskimo: M. L. Berman, K. Hanson, I. Hellman, *Am. J. Obstet. Gynecol.* **114**, 524 (1972); Punjab Indian: R. G. Potter, M. L. New, J. B. Wyon, J. E. Gordon, *J. Chronic Dis.* **18**, 1125 (1965); Bangladesh: L. C. Chen, S. Ahmed, M. Gesche, W. H. Mosley, *Popul. Stud. (London)* **28**, 277 (1974); Chile: A. Perez, P. Vela, G. S. Masnick, R. G. Potter, *Am. J. Obstet. Gynecol.* **114**, 1041 (1972); Zambia: R. W. Wenlock, *J. Biosocial Sci.* **9**, 481 (1977); Rwanda: M. Bonte and H. van Balen, *ibid.* **1**, 97 (1969). All but the last two are prospective studies. For review, see F. W. Rosa, *PAG Bull.* **5**, 5 (1975) (U.N. Protein Advisory Group).
2. P. G. Crosignani and C. Robyn, Eds., *Prolactin and Human Reproduction* (Academic Press, London, 1977).
3. J. E. Tyson, in (*2*), pp. 97–108; R. C. Kolodny, L. S. Jacobs, W. H. Daughaday, *Nature (London)* **238**, 284 (1972); P. Hwang, H. Guyda, H. Friesen, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1902 (1971); G. L. Noel, H. K. Suh, A. G. Frantz, *J. Clin. Endocrinol. Metab.* **38**, 413 (1974); J. E. Tyson, H. G. Friesen, M. S. Anderson, *Science* **177**, 897 (1972). This effect is mediated through a neural reflex loop via the fourth to sixth intercostal nerves, the posteromedial and anterolateral hypothalamus, and the median eminence, and probably involves hypothalamic dopamine, the most likely prolactin inhibiting factor [J. S. Tindal, *J. Reprod. Fertil.* **39**, 437 (1974); F. Mena, A. Enjalbert, L. Carbonell, M. Priam, C. Kordan, *Endocrinology* **99**, 455 (1976); J. E. Tyson, in (*2*)].
4. P. Delvoye, M. Demaegd, J. Delogne-Desnoeck, *J. Biosocial Sci.* **9**, 447 (1977).
5. R. Rolland, R. M. Lequin, L. A. Schellekens, F. H. DeJong, *Clin. Endocrinol.* **4**, 15 (1975).
6. S. Jeppson, G. Rannevik, J. I. Thorell, L. Wide, *Acta Endocrinol. (Copenhagen)* **84**, 713 (1977).
7. D. F. Archer and J. B. Josimovich, *Obstet. Gynecol.* **48**, 155 (1976); R. Maneckjee, B. R. Srinath, N. R. Mougdal, *Nature (London)* **262**, 507 (1976); G. Weiss, W. R. Butler, D. J. Dierschke, E. Knobil, *Proc. Soc. Exp. Biol. Med.* **153**, 330 (1976).
8. G. M. Besser and M. O. Thorner, *Pathol. Biol.* **23**, 779 (1975); A. Mroueh and T. M. Siler-Khodr, *J. Clin. Endocrinol. Metab.* **43** (1976). The most compelling evidence for a direct gonadal effect is prolactin suppression of progesterone production by human ovarian granulosa cells in vitro [K. P. McNatty, R. S. Sawers, A. S. McNeilly, *Nature (London)* **250**, 653 (1974); K. P. McNatty, A. S. McNeilly, R. S. Sawers, in (*2*)].
9. A. M. Mroueh and T. M. Siler-Khodr, *Am. J. Obstet. Gynecol.* **127**, 291 (1977); E. S. Canales, G. Forsbach, J. Soria, A. Zarate, *Fertil. Steril.* **27**, 1335 (1976).
10. R. B. Lee, *!Kung San: Men, Women, and Work in a Foraging Society* (Cambridge Univ. Press, New York, 1979). Less nomadic !Kung, able to supplement infant feeding with cow's milk, have a 36-month birth spacing. In the population as a whole, which is becoming sedentary, mean birth spacing was 8 months shorter in 1968 to 1973 than in 1963 to 1968. Age at weaning is also dropping, reaching 24 months in the more settled bands in 1975, with few infants in the traditional bands nursing beyond the age of 3 years.
11. N. Howell, *Demography of the Dobe !Kung* (Academic Press, New York, 1979); in (*12*), pp. 137–151.
12. R. B. Lee and I. DeVore, Eds., *Kalahari Hunter-Gatherers* (Harvard Univ. Press, Cambridge, Mass., 1976).
13. T. Jenkins and G. Nurse, *Health and the Hunter-Gatherer* (Karger, Basel, 1978); L. Marshall, *The !Kung of Nyae Nyae* (Harvard Univ. Press, Cambridge, Mass., 1976).
14. M. Konner, in *Culture and Infancy*, P. H. Leiderman, S. Tulkin, A. Rosenfeld, Eds. (Academic Press, New York, 1977), pp. 69–109 and 287–328; in (*12*), pp. 217–244.
15. Observations for 15 minutes of infant behavior and caretaker-infant interaction, recorded to the nearest 5 seconds; 45 infants observed at from 1 to 4 age points with six 15-minute observations at each age.
16. Manual nipple stimulation by the infant also occurs. It appears first at 41 weeks, but is rare until the second half of the second year, when it occurs in 14.4 percent of the 15-minute observations, lasting a few seconds to a minute, with or without simultaneous nursing. Manual stimulation of the nipple causes prolactin release, but the efficacy of such brief stimulation is unknown (*3*).
17. Blood (20 ml) was taken on each of 2 days, in 10-ml nonheparinized siliconized Vacutainers, from the antecubital vein, and allowed to clot for 30 minutes. The serum was separated and stored in liquid nitrogen until processing.
18. G. E. Abraham, J. E. Buster, L. A. Lucas, P. C. Corrales, R. C. Teller, *Anal. Lett.* **5**, 509 (1972); G. E. Abraham, R. Swerdloff, D. Tulchinsky, W. D. Odell, *ibid.* **4**, 325 (1971).
19. C. Worthman, "Psychoneuroendocrine study of human behavior," thesis, Harvard University (1978). Specificity, sensitivity, accuracy, and precision of the assay were within accepted limits. The assay consisted of Celite partition chromatography in preparation for radioimmunoassay (RIA). Blanks, standards, and unknowns were extracted in ether, frozen, decanted, dried, taken up in 1 ml of isooctane, and applied to prepared Celite microcolumns. After an 0.5-ml isooctane rinse, the next 3.5 ml (isooctane) were saved for P RIA, the next 14.5 ml was discarded [5.0 ml of isooctane, 6.0 ml of a mixture of benzene and isooctane (30:70), 3.5 ml of a mixture of ethyl acetate and isooctane (15:85)], and the last 3.5 ml [ethyl acetate and isooctane (40:60)] was saved for E_2 RIA. Labeled (high specific activity, ^3H quadruple labeled) steroids were obtained from New England Nuclear. Antiserums, supplied by G. E. Abraham, were S-49#6 (P) and S-52#5 (E_2) (*18*).
20. M. Konner, C. Worthman, M. Shostak, in preparation.
21. For comparable values, see (*2*, pp. 153–159, 225–238, and 245–258). In Western populations follicular phase values are 468 ± 92 for P and 50 ± 20 for E_2; I. Dyrenfurth, R. Jewelewicz, M. Warren, M. Ferin, R. L. Vande Wiele, in *Biorhythms and Human Reproduction*, M. Ferin, F. Halbert, R. M. Richart, R. L. Vande Wiele (Wiley, New York, 1972), p. 171; C. D. West, D. K. Mahajan, V. J. Chavre, C. J. Nabors, F. H. Tyler, *J. Clin. Endocrinol. Metab.* **36**, 1230 (1973). L. A. Van Der Walt, E. N. Wilmsen, and T. Jenkins [*ibid.* **46**, 658 (1978)] have reported that a cross-sectional sample of !Kung San women of childbearing age have low levels of E_2, P, and T as compared to controls (of blacks) in Johannesburg, a result not confirmed by our long-term study of eight cycling women, who showed typical monthly variation in typical ranges. Since Van Der Walt *et al.* did not ascertain the cycling status of their subjects, we suspect that the discrepancy results from a large number of lactating noncycling women among their subjects.
22. One, with seven children and in her late 30's, had short luteal phases (E_2 peak to menses onset) of 12 and 13 days in two cycles.
23. J. Nokin, M. Vekemans, M. L'Hermite, C. Robyn, *Br. Med. J.* **3**, 561 (1972); J. F. Sassin, A. G. Frantz, E. D. Weitzman, S. Kapen, *Science* **177**, 1205 (1972).
24. E. del Pozo, H. Wyss, I. Lancranjan, W. Obolensky, L. Varga, in *Ovulation in the Human*, P. G. Crosignani, Ed. (Academic Press, London, 1976), pp. 297–299; D. L. Foster, *Biol. Reprod.* **17**, 584 (1977); B. Fredricsson, G. Björk, K. Carlström, *Lancet* **1977-I**, 1210 (1977).
25. A. Psychoyos, *Vitam. Horm. (N.Y.)* **31**, 201 (1973). Suckling-induced oxytocin release may also affect implantation.
26. We thank G. Abraham, N. Blurton Jones, I. DeVore, J. Diring, M. Elias, P. English, P. Greene, D. Hamburg, R. Lee, L. Livingston, D. Marshak, K. Roesch, R. Rose, M. Shostak, J. Wurtman, R. Wurtman, and two anonymous referees for help and discussion. Supported by the Harry Frank Guggenheim Foundation and an NSF traineeship to C.W.

9 March 1979; revised 21 August 1979

Bronchodilatation: Noncholinergic, Nonadrenergic Mediation Demonstrated in vivo in the Cat

Abstract. *The composite vagus nerve was stimulated during intravenous infusion of 5-hydroxytryptamine in cats subjected to pharmacologic autonomic blockade with atropine, propranolol, and phentolamine. Bronchial caliber, as assessed by changes in pulmonary resistance, demonstrated a marked dilatation, and dilatation could still be demonstrated after preliminary treatment with reserpine. By stimulating the component branches of the vagus nerve, it was determined that the parasympathetic branch is responsible for this phenomenon.*

Although the physiologic role of bronchial smooth muscle is still speculative (*1*), the neural control of its tone is a subject of active research because of the importance of this muscle in disease. Traditionally, bronchial smooth muscle tone has been thought to be mediated through the autonomic nervous system. Stimulation of parasympathetic cholinergic fibers in the vagus nerve clearly causes bronchoconstriction; stimulation of sympathetic adrenergic fibers through β receptors is generally found to be bronchodilatating (*2*), although stimulation through α-adrenergic receptors, if present, would result in bronchoconstriction (*3*). Vagally mediated bronchodilatation has been reported occasionally (*3*) and dismissed as either anomalous or the result of stimulation of stray sympathetic dilator fibers (*4*).

A noncholinergic, nonadrenergic inhibitory nervous system that mediates bronchial smooth muscle relaxation has been reported (*5*). Evidence for such a system is based on pharmacologic and anatomic observations in vitro; the proposed neural mediator is adenosine triphosphate (*6*). This "purinergic" nervous system is thought to serve several autonomic functions, especially the relaxation that precedes a normal peristaltic wave in the intestinal tract (*7*). Since the lung, like the gut, has endo-

0036-8075/80/0215-0791$00.50/0 Copyright © 1980 AAAS

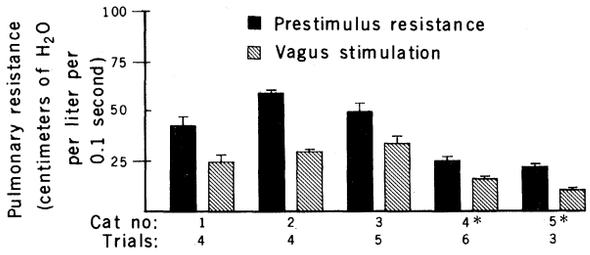

Fig. 1. Effect of combined parasympathetic and cervical sympathetic stimulation during a state of increased bronchial smooth muscle tone. Bars show the mean pulmonary resistance ± standard error for the number of trials indicated for each cat. Pulmonary flow resistance was measured both before and at the peak effect of neural stimulation. All five cats were treated with atropine, propranolol, and phentolamine and then infused with 5-hydroxytryptamine. Cats 4* and 5* received reserpine 24 hours before this treatment.

dermic origins, it is not surprising that studies in vitro have demonstrated noncholinergic, nonadrenergic mediation of relaxation of bronchial smooth muscle in guinea pigs and man (*8*). Our study demonstrates and quantifies such a system in the cat in vivo.

Cats anesthetized with α-chloralose and urethane (60 and 300 mg/kg, respectively) and paralyzed with Pavulon (1.0 mg/kg) were intubated and artificially ventilated. We then measured lung volume with a pressure-compensated volume-displacement plethysmograph (*9*), transpulmonary pressure as the difference between the tracheal and esophageal pressures (which estimates pleural pressure), and flow with a Fleisch pneumotachograph (size 0). Pulmonary flow resistance was determined with the technique of forced sinusoidal oscillations (*10*) at the endotracheal tube and was measured as the ratio of the amplitude of the component of transpulmonary pressure in phase with flow to the amplitude of flow (< 0.5 liter/sec).

Stimulating electrodes were placed around the isolated vagus nerves and then around either the parasympathetic or sympathetic branches. The stimulus (30 Hz, 0.5 msec) was adjusted from 10 to 40 V for maximum effect. The cats' autonomic nervous systems were blocked cholinergically with atropine (0.3 mg/kg) and adrenergically with propranolol (2.0 mg/kg) and phentolamine (2.0 mg/kg), all given intravenously. Twenty-four hours before receiving this treatment, two of the cats were adrenergically depleted with reserpine (4.0 mg/kg intramuscularly). Prior to stimulation, we increased baseline tone with an infusion of 5-hydroxytryptamine (20 to 100 μg/kg-min).

Electrical stimulation of the vagus nerves while smooth muscle tone was high resulted in bronchodilatation in spite of the pharmacologic blockade of the known autonomic neural mediators (Fig. 1). The response was consistent and pronounced, with an approximately 40 percent reversal of the induced bronchoconstriction. Furthermore, since reserpine depletes nerve endings containing both adrenergic and 5-hydroxytryptamine-containing vesicles (*11*), a role for either of these nerve pathways in mediation of this dilatation can be dismissed. Before propranolol, stimulation of both the sympathetic and parasympathetic branches of the vagus nerve caused a substantial fall in pulmonary resistance in the presence of 5-hydroxytryptamine-induced bronchoconstriction. After propranolol, a fall in resistance was produced only by stimulation of the parasympathetic branches. Thus by stimulating the component branches of the vagus nerve (Fig. 2), we determined that the parasympathetic branch is responsible for the bronchodilatation, indicating the nonadrenergic nature of the mediation of this phenomenon.

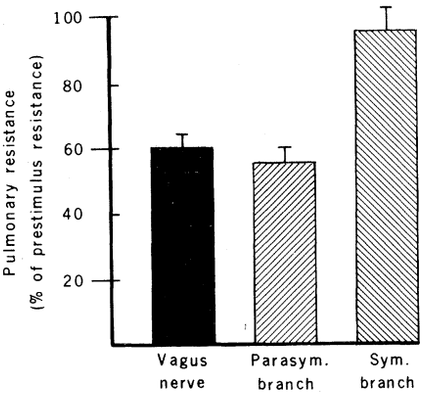

Fig. 2. Identification of the neural connection responsible for noncholinergic, nonadrenergic bronchodilatation in cats. The animals were treated with atropine, phentolamine, and propranolol and then infused with 5-hydroxytryptamine. (Prior to blockade with phentolamine and propranolol, stimulation of the sympathetic branch results in substantial bronchodilatation.) Bars represent the means ± standard error for the changes in pulmonary resistance, expressed as a percentage of the average prestimulus resistance of four cats. First the vagus nerve was stimulated, then each of its component branches in turn. These results show that the bronchodilatation is neither cholinergic nor adrenergic in nature.

Our data clearly indicate that in cats there is a potent bronchodilating mechanism that is controlled centrally through fibers in the vagus nerve. The question arises as to whether these fibers are preganglionic or postganglionic. The presence of many ganglia (*12*) close to where the nonadrenergic inhibiting system exerts its effects suggests that the fibers are preganglionic. (If so, neurotransmitters released both at the ganglion and at the ends of the postganglionic fibers are inhibited neither by atropine nor by α- and β-adrenergic inhibitors.) On the other hand, the inhibition of the bronchodilatory response with hexamethonium (*13, 14*) suggests that the vagus nerve fibers are of a postganglionic nature.

What role this mechanism might play in bronchial hyperreactivity or asthma is unknown. Certain states of bronchial hyperreactivity could be caused or exacerbated by inactivity or malfunction of the bronchodilating mechanism. Since the mechanism can now be quantified in vivo, it should be possible (i) to elucidate its role in hyperreactive states and (ii) to identify the neurotransmitter and other agents that may enhance or inhibit its smooth muscle-relaxing function.

CHARLES G. IRVIN
ROBERT BOILEAU
JACQUES TREMBLAY
RICHARD R. MARTIN
PETER T. MACKLEM
Meakins Christie Laboratories, McGill University, Montreal, Canada H3A 2B4

References and Notes

1. P. T. Macklem, *Physiol. Rev.* **51**, 368 (1971); W. M. Thurlbeck and N. S. Wang, in *Respiratory Physiology*, J. G. Widdicombe, Ed. (Butterworth, London, 1974), ser. 1, vol. 2, p. 4.
2. J. A. Nadel, in *Bronchial Asthma*, E. B. Weiss and M. S. Segal, Eds. (Little, Brown, Boston, 1976), p. 155.
3. J. G. Widdicombe and G. M. Sterling, *Arch. Intern. Med.* **126**, 311 (1970).
4. M.-B. Daly and C. P. Hebb, *Pulmonary and Bronchial Vascular Systems* (Arnold, London, 1966), p. 179.
5. G. Burnstock, *Pharmacol. Rev.* **24**, 509 (1972); R. A. Coleman, *Br. J. Pharmacol.* **48**, 360 (1973); ——— and G. P. Levy, *ibid.* **52**, 167 (1974).
6. G. Burnstock, *J. Exp. Zool.* **194** (No. 1), 103 (1975).
7. J. Richardson, *J. Pediatr. Surg.* **10**, 875 (1975).
8. R. F. Coburn and T. Tomita, *Am. J. Physiol.* **224**, 1075 (1973); J. Richardson and J. Beland, *J. Appl. Physiol.* **41**, 764 (1976); J. Richardson and T. Bouchard, *J. Allergy Clin. Immunol.* **56**, 473 (1975).
9. J. Mead, *J. Appl. Physiol.* **15**, 736 (1960).
10. M. Goldman, J. Knudson, J. Mead, N. Peterson, J. Schwaber, M. Wohl, *ibid.* **28**, 113 (1970); C. Irvin and J. Dempsey, *Respir. Physiol.* **35**, 161 (1978).
11. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, New York, 1975), p. 557.
12. D. G. Silva and G. Ross, *J. Ultrastruct. Res.* **47**, 310 (1974).
13. M. O'Donnell and L. Diamond, *Am. Rev. Respir. Dis.* **119** (No. 4), 342 (1979).
14. S. E. Chesrown, C. S. Venugopalan, W. M. Gold, J. M. Drazen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1111 (1979).

21 May 1979