X-linked hypophosphatemia contains a second parathyroid hormone–insensitive phosphate-transport system. We also suggest that this component of phosphate transport in kidney is responsive directly to calcium, perhaps in a manner analogous to that documented in canine kidney by Lavender and Pullman (*14*). This would account for the well-known effect of hypercalcemia on tubular reabsorption of phosphate in X-linked hypophosphatemia (*4*).

CLAUDE ARNAUD
Department of Endocrine Research, Mayo Clinic, Rochester, Minnesota 55901

FRANCIS GLORIEUX
CHARLES SCRIVER
deBelle Laboratory for Biochemical Genetics, McGill University–Montreal Children's Hospital Research Institute, Montreal 108, Quebec

References and Notes

1. R. Winters, J. Graham, T. F. Williams, V. McFalls, C. Burnett, *Medicine* **37**, 97 (1958); T. F. Williams, R. W. Winters, C. H. Burnett, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds. (McGraw Hill, New York, ed. 2, 1966), p. 1179.
2. L. V. Avioli, T. F. Williams, J. Lund, H. F. DeLuca, *J. Clin. Invest.* **46**, 1907 (1967).
3. E. B. Olson and H. F. DeLuca, *Science* **165**, 405 (1969); D. R. Fraser and E. Kodicek, *Nature* **228**, 764 (1970); M. F. Holick, H. K. Schnoes, H. F. DeLuca, *Proc. Nat. Acad. Sci. U.S.* **68**, 803 (1971).
4. D. Fraser, J. Leeming, E. Cerwenka, *Helv. Paediatr. Acta* **14**, 497 (1959); W. Falls, N. Carter, R. Rector, D. Seldin, *Ann. Intern. Med.* **68**, 553 (1968).
5. H. Earp, R. Ney, M. Gittelman, R. Richman, H. DeLuca, *N. Engl. J. Med.* **283**, 627 (1970).
6. B. R. Robertsen, R. C. Harris, D. J. McClure, *Am. J. Dis. Child.* **64**, 948 (1942); C. E. Dent, *J. Bone Jt. Surg.* **34B**, 266 (1952); G. Fanconi and R. Girardot, *Helv. Paediatr. Acta* **7**, 14 (1952); B. Frame and R. W. Smith, Jr., *Am. J. Med.* **25**, 771 (1958); R. W. Winters and J. B. Graham, *Pediatrics* **25**, 932 (1960).
7. J. Rey and J. Frezal, *Rev. Fr. Etud. Clin. Biol.* **11**, 771 (1966).
8. D. Fraser, D. Geiger, J. Munn, P. Slater, R. Jahn, E. Liu, *Am. J. Dis. Child.* **96**, 460 (1958); F. Glorieux, C. Arnaud, C. Clow, H. Goldman, T. Reade, C. R. Scriver, *Clin. Res.* **18**, 726 (1970).
9. C. West, J. Blanton, F. Silverman, N. Holland, *J. Pediatr.* **64**, 469 (1964); H. Harrison, F. Lifshitz, A. D. Johnson, *Am. J. Dis. Child.* **112**, 290 (1960); C. Nagant de Deuxchaisnes and S. Krane, *Am. J. Med.* **43**, 508 (1967).
10. C. Arnaud, H. Tsao, T. Littledyke, *J. Clin. Invest.* **50**, 21 (1971).
11. The criteria for diagnosis of X-linked hypophosphatemia were: proven hypophosphatemia for age on a morning blood sample after overnight fast; absence of hypocalcemia and hyperaminoaciduria in the untreated state; no male-to-male transmission; female clinical phenotype not more severe than male in same pedigree.
12. Tubular reabsorption of phosphate (TRP) was measured after an overnight fast. A timed urine collection lasting about 180 minutes was obtained, and venous blood was obtained at the end of the period. Phosphorus and creatinine determinations were made and the TRP value was calculated. TRP was also determined with inulin clearance rates to monitor GFR on several occasions. Inulin and creatinine clearances were comparable in the same subject.
13. E. Reiss, J. Canterbury, M. Bercovitz, E. Kaplan, *J. Clin. Invest.* **49**, 2146 (1970).
14. A. R. Lavender and T. N. Pullman, *Am. J. Physiol.* **205**, 1025 (1963).
15. Supported by grants from the Medical Research Council of Canada (MT-1085), the Department of National Health and Welfare (DPG 647-7-643), and NIH (NIH-AM-12302). F.G. is a national Genetics Foundation trainee in genetics. We thank Mrs. Terry Reade, Mr. Peter Lamm, and Dr. H. Goldman of the deBelle Laboratory, and Miss H. S. Tsao, Miss Judith Hess, Miss Karen Laakso, and Miss Julianna Bishoff of the Mayo Clinic for their excellent technical assistance. This is publication No. 231 from the McGill University–Montreal Children's Hospital Research Institute. Send reprint requests to C.S.

25 March 1971

Effects of Long-Term Reserpine Treatment on Brain Tyrosine Hydroxylase and Behavioral Activity

Abstract. *Treatment of rats with reserpine (for 8 or 9 days) produced a temporally related increase in behavioral activity and in tyrosine hydroxylase activity in the midbrain. Weight loss resulting from such treatment was not sufficient, by itself, to account for either the behavioral or enzymatic changes. The results support the role of catecholamines in behavioral arousal.*

The depletion of brain catecholamines (CA's) by reserpine is believed to play an important role in reserpine-induced sedation (*1*). However, animals treated for extended periods with appropriate doses of reserpine exhibit normal gross behavior (*2*) and, in fact, eventual hyperactivity (*3*); this delayed reversal into hyperactivity occurred when the CA concentrations in the brain were maintained substantially below normal by the long-term drug treatment. Haggendal and Lindqvist (*2*) have attempted to explain this apparent lack of correlation between the concentrations of CA in the brain and the behavior by suggesting that the CA's exist in two pools: (i) a large "storage" pool that is not directly involved in the mediation of neuronal activity and (ii) a smaller pool that more directly influences synaptic function. This hypothesis is based on their finding that after the depletion of the large "physiologically inert" CA pool, reserpine-induced fluctuation in the residual CA concentrations ("functional" pool) correspond to behavioral changes. A number of other findings are consonant with the existence of a relatively small, labile pool that mediates neuronal activity (*4*). This functional pool appears to be maintained primarily by synthesis de novo because newly synthesized CA's have been shown to be released preferentially when adrenergic neurons are activated (*5*).

Although the existence of a small functional pool may partially explain the restoration of normal behavior during long-term treatment with reserpine, it does not by itself explain the hyperactivity that appears after several days of such treatment. However, recently it has been shown that tyrosine hydroxylase, the rate-limiting enzyme in CA biosynthesis (*6*), can be induced in the adrenal medulla and sympathetic ganglia after reserpine treatment (*7*). This elevation of tyrosine hydroxylase activity appeared to be due to an increased synthesis of new enzyme, mediated by the prolonged reflexive increase in sympathetic nerve activity. In addition, in the case of the adrenal medulla, the increase in tyrosine hydroxylase activity, as measured in tissue homogenates, correlates with an increase in CA biosynthesis in the intact animal (*8*). If a similar increase in tyrosine hydroxylase activity occurs in the brain concomitant with the behavioral activation produced by long-term treatment with reserpine, an increase in brain tyrosine hydroxylase and the resulting increase in functional norepinephrine levels (provided that tissue tyrosine levels are maintained such that the enzyme is saturated) would be consonant with the alleged role of norepinephrine in behavioral arousal (*1*, *9*).

Male Sprague-Dawley rats (250 to 275 g) were injected intraperitoneally with reserpine (0.5 mg/kg) at 24-hour intervals for 9 days, or with distilled water for 1, 2, or 6 days and reserpine (0.5 mg/kg) for the remaining 8, 7, or 3 days, respectively. Control animals were injected with distilled water for 9 days. Behavioral activity of crossovers in a free-field situation (*10*) was determined during a 1-hour interval, 23 hours after each injection. In order to obtain a stable level of responding, all animals were exposed to the experimental chambers for hourly intervals during the 5 days immediately prior to the onset of drug administration (*11*).

In agreement with previous findings, long-term treatment with reserpine pro-

duced an initial decrease in activity followed by hyperactivity (Fig. 1). Animals receiving distilled water followed by three injections of reserpine exhibited a significant decrease in activity on the final day of testing.

Tyrosine hydroxylase activity in the midbrain was determined in different rats receiving the same schedules of reserpine treatment. The rats were decapitated 23 hours after the last injection, and their midbrains were quickly removed and homogenized in 0.005*M* potassium phosphate buffer at *p*H 7.0. The homogenates were then centrifuged at 50,000*g* for 20 minutes, and the supernatants (containing over 90 percent of the total enzyme activity) were assayed for tyrosine hydroxylase activity by a modification of the method of Nagatsu *et al.* (*12*). Portions of the supernatants were also used to estimate protein (*13*). The tyrosine hydroxylase activity in the midbrains of animals after long-term treatment with reserpine (8 to 9 days) was significantly increased when compared to controls (Fig. 2), whereas the enzyme activity after 3 days of reserpine treatment was not significantly different from controls. The increase in tyrosine hydroxylase activity was observed at a time when hyperactivity was exhibited but not at a time concomitant with the earlier reserpine-induced decrease in behavioral activity. After 7 days of reserpine treatment the mean enzyme activity was increased, although the increase was not significant. This reflects the behavioral results in that after such treatment some animals exhibit hyperactivity whereas others remain depressed.

In addition, we have evidence that reserpine treatment results in a similar change in tyrosine hydroxylase activity in the caudate, although the time course of the effect differs. The level of activity of tyrosine hydroxylase in the caudate was increased by about 5 to 10 percent on days 7 and 8 and by 25 percent on day 9. Because it has been reported (*14*) that the midbrain contains primarily adrenergic cell bodies and the caudate primarily adrenergic nerve endings, the apparent disparity in the time course of the reserpine effect on tyrosine hydroxylase of these two regions may reflect movement of newly synthesized enzyme from the site of assembly to the nerve terminals.

This increase in tyrosine hydroxylase activity may reflect a specific compensatory response to the prolonged depletion of CA in the brain or to the consequent changes in neuronal activity resulting from such depletion (*15*). However, one major obstacle to such an interpretation is the fact that a precipitous weight loss occurred in rats receiving long-term treatment with reserpine (*16*). Thus, whereas control rats continued to gain weight throughout the duration of the experiment, reserpine-treated animals declined in weight to approximately 60 percent of their weight prior to the injections. Therefore, reserpine-induced starvation could have been responsible for either the increase in behavioral responsivity, tyrosine hydroxylase activity, or both. In order to determine the degree to which weight loss contributed to the increase in enzymatic and behavioral activities, rats were deprived of food for 9 days, during which time their behavioral activity was monitored as described. Starvation for 9 days resulted in approximately the same rate of weight loss as observed in reserpine-treated animals, although the weights at 9 days were generally lower for the food-deprived group. A parallel group of food-deprived animals was used for determination of tyrosine hydroxylase activity in the midbrain. Control animals were tested with both deprived groups for comparison. The results showed that deprivation produced a marked decrease in behavioral activity (Fig. 1) and no significant change in tyrosine hydroxylase activity in the midbrain (Fig. 2). It appears, therefore, that reserpine-induced weight loss is not, by itself, sufficient to produce the increase in behavioral and enzymatic activities (*17*).

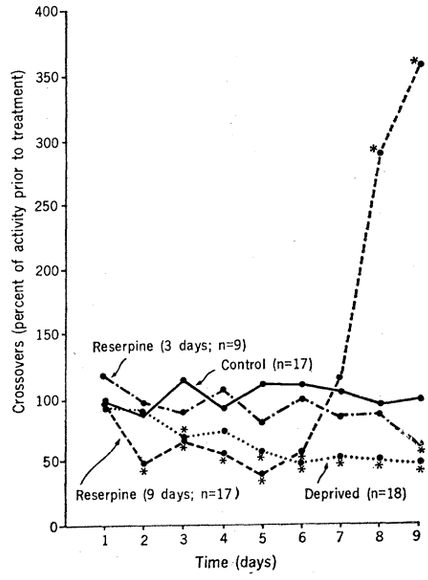

Fig. 1. Effects of two schedules of reserpine treatment or food deprivation on gross locomotor activity (crossovers) of rats measured in 1-hour intervals for 9 days. Hyperactivity was produced by long-term treatment with reserpine (8 to 9 days) after an initial period of behavioral depression. Food deprivation resulted only in decreased activity. Asterisk (*) indicates $P < .05$ by the Mann-Whitney U test.

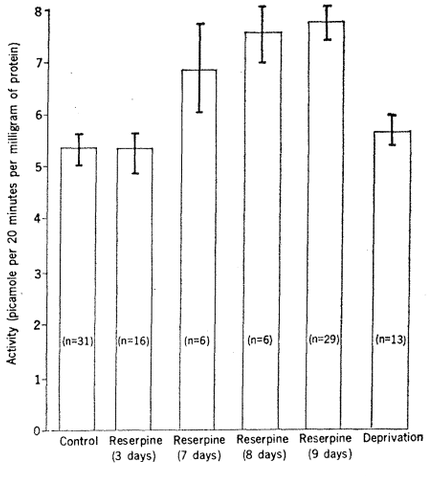

Fig. 2. Effects of four schedules of reserpine treatment or food deprivation on the tyrosine hydroxylase activity in the midbrain of rats. Each bar represents the mean ± standard error of the mean (brackets) for the indicated number of observations. Only 8 to 9 days of long-term treatment with reserpine induced a significant increase in enzyme activity ($P < .05$ by the Mann-Whitney U test).

Our study demonstrates the existence of a temporal correlation between the prolonged depletion of CA by long-term treatment with reserpine and the elevation of behavioral activity and of tyrosine hydroxylase activity in the midbrain and in the caudate. Although the precise nature of this relation is not presently clear, the rate of synthesis or degradation, or both, of brain tyrosine hydroxylase may be alterable by a variety of pharmacological agents and physiological stimuli, perhaps analogous to adaptive enzymatic changes elucidated in peripheral tissues (*18*). Our results are consistent with the role of norepinephrine in behavioral arousal because the increase in brain tyrosine hydroxylase may result in an increase in the relatively small stores of functional norepinephrine despite the reduction of the total levels of this amine produced by long-term treatment with reserpine.

DAVID S. SEGAL, JOHN L. SULLIVAN III
RONALD T. KUCZENSKI
ARNOLD J. MANDELL
Department of Psychiatry,
University of California,
San Diego, La Jolla 92037

References and Notes

1. J. J. Schildkraut and S. S. Kety, *Science* **156**, 21 (1967); J. J. Schildkraut, *Amer. J. Psychiat.* **122**, 509 (1965).
2. J. Haggendal and M. Lindqvist, *Int. J. Neuropharmacol.* **3**, 59 (1964); ———, B. E. Roos, *Acta Physiol. Scand.* **69**, 95 (1967).
3. J. H. Pirch and R. H. Rech, *Psychopharmacologia* **12**, 115 (1968); J. H. Pirch, *ibid.* **16**, 253 (1969).
4. A. Alousi and N. Weiner, *Proc. Nat. Acad. Sci. U.S.* **56**, 1491 (1966); F. L. Lee, N. Weiner, U. Trendelenburg, *J. Pharmacol. Exp. Ther.* **155**, 211 (1967); L. T. Potter, J. Axelrod, I. Kopin, *J. Biochem. Pharmacol.* **11**, 254 (1962).
5. G. C. Sedvall, V. K. Weise, I. Kopin, *J. Pharmacol. Exp. Ther.* **159**, 274 (1968); I. Kopin, G. R. Breese, K. R. Krauss, U. K. Weise, *ibid.* **161**, 271 (1968); M. J. Besson, A. Cheramy, P. Feltz, J. Glowinski, *Proc. Nat. Acad. Sci. U.S.* **62**, 741 (1969).
6. M. Levitt, S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Ther.* **148**, 1 (1965).
7. R. A. Mueller, H. Thoenen, J. Axelrod, *ibid.* **169**, 74 (1969); A. J. Mandell and M. Morgan, *Nature* **227**, 75 (1970).
8. W. Dairman and S. Udenfriend, *Mol. Pharmacol.* **6**, 350 (1970).
9. D. S. Segal and A. J. Mandell, *Proc. Nat. Acad. Sci. U.S.* **66**, 289 (1970).
10. Activity was measured in a soundproof chamber (45 by 45 by 28 cm). The floor of the chamber was divided into quadrants, and an index of gross activity was obtained from the number of crossovers each animal made from one quadrant to another. The data were automatically recorded.
11. Although the time course of behavioral activation during long-term reserpine treatment had been demonstrated previously, female rats were used as subjects and activity was measured in a circular activity maze. Because female rats exhibit activity fluctuations corresponding to estrous cycling, and because activity measures are notorious for their lack of agreement, our behavioral study was undertaken in order to examine the generality of the reserpine-induced hyperactivity and in order to determine the optimal times for enzyme determination under our particular experimental conditions.
12. T. Nagatsu, M. Levitt, S. Udenfriend, *J. Biol. Chem.* **239**, 2910 (1964).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 265 (1959).
14. K. Fuxe, *Acta Physiol. Scand.* **64** (Suppl.), 37 (1965).
15. It is possible that a negative feedback loop exists between postsynaptic neurons and adrenergic cell bodies. In addition, the tyrosine hydroxylase activity in the central nervous system may be influenced by adrenergic stimulation in a manner similar to that reported in peripheral sympathetic structures (*7*). Thus the initial decrease in central adrenergic activity produced by reserpine-induced CA depletion would be expected to reduce negative feedback, subsequently increasing the levels of tyrosine hydroxylase.
16. Nutritional state has been shown to influence liver and muscle enzyme [see W. E. Knox, V. H. Auerback, E. C. C. Lin, *Physiol. Rev.* **36**, 164 (1956)].
17. Also examined was the possibility that reserpine and starvation interact to produce the increase in behavioral activity and tyrosine hydroxylase activity, that is, reserpine-induced starvation may alter the pattern of metabolites of subsequently administered reserpine. Results indicate that rats that were starved and then injected with reserpine (0.5 mg/kg, for two consecutive days) exhibited neither the behavioral nor enzymatic changes produced by long-term reserpine treatment. Whereas behavior was further depressed by the injection of reserpine, midbrain tyrosine hydroxylase activity was unaltered.
18. O. Greengard, *Enzymol. Biol. Clin.* **8**, 81 (1967).

19. Supported by grant MH-18065 from the National Institute of Mental Health.

17 December 1970; revised 3 May 1971

Microdroplets and Water Drop Freezing

In his report Cheng (*1*) referred to the appearance of numerous microdroplets in the vicinity of a freezing water drop as a "newly observed phenomenon." In fact, microdroplets of this kind have been observed and reported previously (*2, 3*). Dye and Hobbs (*3*) showed that they occur by ejection from the freezing water drop and also by the condensation of water vapor in regions of comparatively high supersaturations in the vicinity of the freezing drop.

Cheng suggested that this phenomenon might provide a mechanism for charge generation in thunderstorms. The same theory was postulated by Mason (*4*). However, considerable care must be taken in extrapolating observations and measurements made on large water drops freezing in the laboratory to the behavior of small droplets in natural clouds. In particular, it has been shown (*3, 5*) that it is important to ensure that the water drops are in thermal and solution equilibrium with the environmental air before they are nucleated. Drops that are nucleated before attaining these conditions are more likely to eject both ice particles and microdroplets during freezing, thereby separating electric charges, than are cloud droplets that are generally very close to equilibrium with their environment. Cheng does not give any information on the condition of the water drops in his experiments prior to their nucleation. However, it is unlikely that they were in equilibrium with the environmental air since they probably nucleated at their surfaces before their interiors had fallen to the temperature of the environment. Moreover, the freezing behavior of cloud droplets is certainly not simulated in the laboratory by the freezing of water drops on glass slides. It is essential that the drops be either freely suspended or in free fall, so that they are ventilated in the proper manner (*6*). It has yet to be shown that drops freezing under conditions similar to those in natural clouds eject microdroplets.

PETER V. HOBBS*

Cloud Physics Group,
Atmospheric Sciences Department,
University of Washington,
Seattle 98105

References and Notes

1. R. J. Cheng, *Science* **170**, 1395 (1970).
2. L. G. Kachurin and V. I. Bekryaev, *Dokl. Akad. Nauk SSSR* **130**, 57 (1960).
3. J. E. Dye and P. V. Hobbs, *J. Atmos. Sci.* **25**, 82 (1968).
4. B. J. Mason, *Proc. Int. Conf. Cloud Phys.* (1968), p. 657.
5. D. A. Johnson and J. Hallett, *Quart. J. Roy. Meteorol. Soc.* **94**, 468 (1968).
6. P. V. Hobbs and A. J. Alkezweeny, *J. Atmos. Sci.* **25**, 881 (1968); J. L. Brownscombe and N. S. C. Thorndike, *Nature* **220**, 687 (1968).

7. Work supported by grant G A 17381 from the Atmospheric Sciences Section, National Science Foundation.

* On leave of absence at the Scott Polar Research Institute, University of Cambridge, Cambridge, England.

26 January 1971; revised 26 March 1971

Several investigators (*1–3*) have reported the appearance of the particles, the formation of spicules, and the occurrence of fragmentation during the freezing of a water drop. The new observations and measurements that I made (*4*) while freezing a supercooled water drop, under the condition of thermal equilibrium with the environment, are as follows: (i) photomicrographic evidence (Fig. 1) of numerous droplets, ranging from less than 1 to 20 μm, ejected from the surface of a freezing water drop; (ii) the duration of ejection of the microdroplets, in this case, about 50 seconds for a 1-mm water drop; (iii) the time-temperature relationship of the freezing water drop; (iv) reversals of the vapor pressure gradient during the freezing period; (v) the electrical properties of the ejected microdroplets; (vi) in addition to one or a few large spicules as mentioned by others (*1–3*), a large number of miniature spicules (Fig. 1) forming between cracks on the ice surface during the freezing period; and (vii) photomicrographic evidence (Fig. 1)

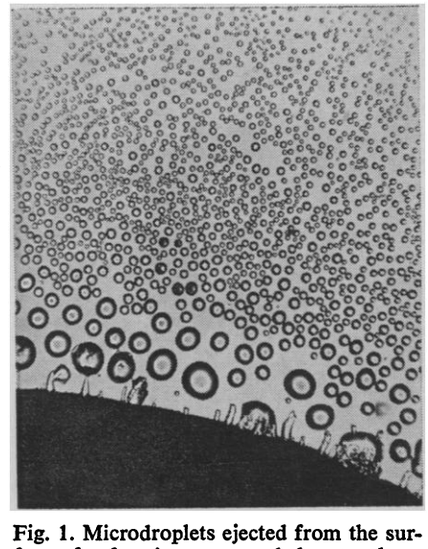

Fig. 1. Microdroplets ejected from the surface of a freezing supercooled water drop.