other tadpole hemoglobins, Td-1 and Td-2 (Fig. 2c). The origin of hemoglobin Td-2a is open to question, since we have not found it in any of the organ culture extracts.

These results provide evidence that two different erythropoietic sites are simultaneously active in tadpoles, and that the two erythropoietic organs promote differentiation of different red blood cell lines containing different tadpole hemoglobins. We recognize the possibility of a single circulating stem cell which could become lodged in the two organs and develop into the two red blood cell lines under the influence of the different environments in the two organs. Whether there are two types of stem cell or only one, it would be interesting to know what the different factors are in the two organs that promote differentiation of RBC's containing different hemoglobins. The culture system described in this paper should allow these important questions to be investigated.

ROBERT H. BROYLES
MICHAEL J. DEUTSCH

Department of Zoology, University of Wisconsin, Milwaukee 53201

References and Notes

1. C. Baglioni and C. E. Sparks, *Dev. Biol.* **8**, 272 (1963); C. D. Trader and E. Frieden, *J. Biol. Chem.* **241**, 357 (1966); B. Moss and V. M. Ingram, *J. Mol. Biol.* **32**, 481 (1968); S. J. Aggarwal and A. Riggs, *J. Biol. Chem.* **244**, 2372 (1969).
2. H. E. Jordan and C. C. Spiedel, *J. Exp. Med.* **38**, 529 (1923); R. L. Fernald, *Univ. of Calif. Publ. Zool.* **51**, 129 (1942–1947); G. M. Maniatis and V. M. Ingram, *J. Cell Biol.* **49**, 372 (1971).
3. R. H. Broyles and E. Frieden, *Nat. New Biol.* **241**, 207 (1973).
4. G. Flores and E. Frieden, *Science* **159**, 101 (1968); *J. Pharmacol. Exp. Ther.* **174**, 463 (1970).
5. M. J. Deutsch and R. H. Broyles, *Dev. Biol.* **46**, 227 (1975).
6. A. Taylor and J. J. Kollros, *Anat. Rec.* **94**, 7 (1946).
7. R. Rugh, *Experimental Embryology* (Burgess, Minneapolis, Minn., ed. 3, 1962), p. 50.
8. M. Balls, J. D. Simnett, E. Arthur, in *Biology of Amphibian Tumors*, M. Mizell, Ed. (Springer-Verlag, New York, 1969), p. 385.
9. The data in Fig. 1 do not indicate rates of protein synthesis per se, since sizes of the amino acid pools were not determined for the cultures. However, we have determined rates of incorporation of amino acids into the trichloroacetic acid (TCA)-soluble fractions; those data show that the increases in rates of incorporation into protein after 4 days of culture (Fig. 1) are not due to changes in rates of incorporation into the TCA-soluble fractions.
10. G. E. Shambough, III, J. G. Balinsky, P. P. Cohen, *J. Biol. Chem.* **244**, 5295 (1969).
11. J. J. Just and B. G. Atkinson, *J. Exp. Zool.* **182**, 271 (1972).
12. Supported in part by a Cottrell Grant from Research Corporation and by funds from the Graduate School of the University of Wisconsin–Milwaukee. We thank S. Berger for technical assistance.

9 April 1975; revised 27 May 1975

Parathyroid Hormone and 25-Hydroxy Vitamin D_3: Synergistic and Antagonistic Effects on Renal Phosphate Transport

Abstract. *The effect on phosphate excretion of graded doses of parathyroid hormone (PTH) and the biologically active vitamin D_3 metabolite, 25-hydroxycholecalciferol (25-HCC), administered singly and in combination, were studied in the nonexpanded, vitamin D–depleted thyroparathyroidectomized rat. Infusion of 1 unit of 25-HCC per hour for 6 hours induced an antiphosphaturia only when administered with 0.2 unit of PTH per hour, while neither agent alone changed phosphate excretion. A dose of 2.0 units of PTH per hour did not cause phosphaturia unless given with 1 unit of 25-HCC per hour. In pharmacologic dosage (5 units per hour), PTH produced phosphaturia in the absence of the metabolite.*

A number of observations recorded by clinicians as well as basic science investigators over the past 30 years suggest that under certain circumstances both vitamin D and parathyroid hormone (PTH) depend on the presence of the other for the full expression of the characteristic biological effects of either (*1, 2*). For this interdependence to be demonstrated, physiological, as compared to pharmacological, amounts of each agent must be used. Thus, for example, the capacity of PTH to mobilize bone mineral requires small "priming" doses of vitamin D, according to most investigators (*3–5*). Similarly, it is generally agreed that stimulation of the intestinal transport of calcium required physiological amounts of the vitamin (*6*). However, it is also generally recognized that pharmacological doses of the vitamin can act on both gut and bone, leading to an elevation of serum calcium in the absence of PTH (*2, 7, 8*), an observation that has provided the rationale for the therapy of hypoparathyroidism and resistant forms of rickets with massive doses of vitamin D (*9*).

The interdependence of PTH and vitamin D in the renal tubules has been much less clearly defined. Opinion is divided as to whether small amounts of vitamin D are necessary for PTH to bring about phosphaturia (*5, 10, 11*) or not (*3, 5, 12*). The opposing views on this matter seem related to two principal circumstances: (i) much less attention has been directed toward an evaluation of vitamin D effects on the kidney than on the other two target organs, bone and gut (*13*); and (ii) supraphysiological amounts of one or both agents may have been used in the pertinent studies. We now report the effects on the urinary excretion of phosphate of the single and combined administration of graded doses of the biologically active metabolite of vitamin D_3, 25-hydroxy vitamin D_3 [25-hydroxycholecalciferol (25-HCC)] (*14*), and of PTH.

Female weanling rats deficient in vitamin D (Holtzman Company, Madison, Wisconsin) were fed a diet containing 0.400 percent calcium and 0.426 percent phosphorus, supplemented with vitamins A, B, C, E, and K (Teklad Mills, Chagrin Falls, Ohio). Matched control rats were given exactly the same diet, except that approximately 25 international units (I.U.) of vitamin D per day was added. From each new shipment of 24 to 30 rats, 3 to 5 animals were selected at random and killed after 3 to 4 weeks on the D-deficient diet, so that serum calcium and phosphorus could be determined. Groups of rats in which the mean calcium did not fall below 6.5 mg per 100 ml of serum were not used. Thyroparathyroidectomy (TPTX) was performed by electrocautery under light anesthesia (ether), a bladder catheter was inserted, and perfusion of the tail vein was begun. The animals were then placed in a restraining cage and urines were obtained with a fraction collector at intervals of 1 to 2 hours. The technique was a modification of that originally described by Cotlove (*15*) as adapted by Rasmussen and his co-workers (*4, 5*).

The perfusion mixture contained 4 percent glucose, and 10 m*M* calcium, 20 m*M* sodium, 2.5 m*M* potassium, and 5m*M* magnesium, as the respective chloride salts. A priming dose of inulin (approximately 0.2 ml of a 10 percent solution) was given. The sustaining solution, containing inulin (5 mg/ml) was administered at 1.3 ml per hour with a constant infusion pump so that volume expansion of the animals would be avoided. After a 14- to 16-hour equilibration period and a 4- to 6-hour control period, either 25-HCC (*16*), suspended in propylene glycol, or a highly purified preparation of PTH (Wilson Laboratories, lot 156552), dissolved in normal saline solution, or both, was administered at a rate of 0.023 ml per hour. Control animals received only the vehicle used for the 25-HCC (propylene glycol). Groups of five to eight animals were given doses of PTH from 0.2 to 5 units per hour or 25-HCC, 1 to 100 units per hour, or a combination of these agents. The animals were killed after 6 hours of drug or hormone infusion (total perfusion period, 26 hours), or both, and blood was obtained by aortic puncture. Urinary inulin was stable in most animals after the equilibration period of 14 to 16 hours; the data from those rats in which wide fluctuations in urinary inulin excretion were observed were discarded. Urine

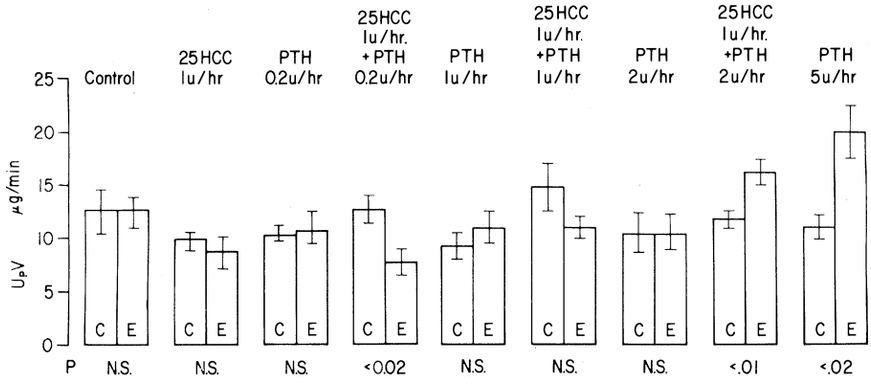

Fig. 1. Effects of graded doses of PTH and 25-HCC, alone and in combination, on absolute phosphate excretion (UpV). The combined administration of 0.2 unit of PTH per hour and 1 unit of 25-HCC per hour reduced UpV by approximately 40 percent, while there was no change from control (C) to experimental (E) phase of the studies for the control animals or those given each agent singly. Likewise, neither 1 to 2 units of PTH per hour nor 1 unit of PTH + 1 unit of 25-HCC per hour changed UpV, but the addition of 1 unit of 25-HCC per hour to 2 units of PTH per hour resulted in a phosphaturia. However, 5 units of PTH per hour incremented phosphate excretion without the necessity for the presence of the vitamin D metabolite.

and blood were analyzed for inulin and phosphate by automated adaptations of the methods of Davidson and Sackner (*17*) and of Fiske and Subbarow (*18*), respectively; serum calcium was determined by a modification of the method of Gitelman (*19*). Statistical analyses were performed by Student's t-test.

After 3 to 4 weeks on the vitamin D–deficient diet, mean serum calcium was 5.93 ± 0.06 mg per 100 ml, while that in the D-replete animals averaged 10.34 ± 0.06 mg per 100 ml ($P < .001$). Serum phosphorus was not significantly altered by the diet (with D, 9.29 ± 0.11 mg per 100 ml; D-deficient, 9.08 ± 0.13 mg per 100 ml; $P > .20$). The weights of the D-depleted rats began to diverge from those of the D-fed animals at about 2 weeks, and weight gain had virtually ceased in the D-deficient rats at 3 weeks. Weight at the time of diet institution in the weanlings was not different in the two groups: with D, 61.7 ± 1.3 g; D-deficient, 61.9 ± 1.4 g ($P > .90$); but after 3 to 4 weeks on their respective diets, the mean weight for 50 D-fed rats was 148.6 ± 1.2 g, while that for 50 D-depleted animals was 114.4 ± 1.5 g ($P < .001$).

In analyzing the effects of the various maneuvers on urinary phosphate excretion, each animal was used as its own control. The mean excretion data for two to three consecutive urine collection periods just prior to the beginning of drug or hormone infusion (or both) were compared to the mean values from two consecutive periods after 5 to 6 hours of perfusion. Urinary phosphate excretion was unchanged in the control rats from the control situation (12.5 ± 2.1 μg/min) to the experimental phase (12.3 ± 1.6 μg/min; $P > .90$; Fig. 1). Neither the infusion of 25-HCC at 1 unit per hour (control, 9.8 ± 1.0; experimental, 8.7 ± 1.4 μg/min; $P > .50$) nor the administration of PTH at 0.2 unit per hour (10.4 ± 0.6 to 10.8 ± 1.4 μg/min; $P > .70$) altered phosphate excretion significantly. However, when given in combination, a fall in phosphate excretion of 39.5 percent occurred: 12.4 ± 1.2 to 7.5 ± 1.3 μg/min; $P < .02$. The combined effects of PTH and 25-HCC did not occur immediately, but appeared to begin at 3 to 4 hours, although the change in absolute phosphate excretion did not reach statistical significance until 5 to 6 hours after the infusion started.

Neither the infusion of 1 unit of PTH per hour (control, 9.3 ± 1.3; experimental, 10.7 ± 1.5; $P > .50$) nor the addition of 1 unit of 25-HCC per hour (14.6 ± 2.4 to 10.8 ± 1.0 μg/min; $P > .10$) resulted in any consistent variation in absolute phosphate excretion (Fig. 1). Similarly, 2 units of PTH per hour was without effect (10.3 ± 1.9 to 10.3 ± 1.6 μg/min; $P > .90$). However, when 1 unit of 25-HCC was infused along with 2 units of PTH per hour, a phosphaturia developed (control, 11.7 ± 0.5; experimental, 16.0 ± 1.1 μg/min; $P < .01$). Moreover, a larger dose of PTH (5 units per hour) was capable of effecting a phosphaturia without the necessity for 25-HCC to be present (10.0 ± 1.1 to 19.0 ± 2.5 μg/min; $P < .02$).

In Table 1 we present the data for serum calcium and phosphorus obtained at the end of the experiment, and the calculated glomerular filtration rate (GFR) (the clearance of inulin) for each experimental group, compared to the control animals. There were no changes in GFR. Serum phosphate rose in the rats given 0.2 unit and 1 unit of PTH per hour, suggesting a bone effect of the hormone without its phosphaturic action. In the group given 0.2 unit, this elevation was associated with a slight fall in serum calcium, presumably reflecting deposition in bone.

In order to determine whether there was a dose of 25-HCC which could act on the kidney of the D-depleted TPTX rat without the presence of PTH, several dosages of the metabolite (5, 25, and 100 units per hour) were administered. Neither the 5-unit dose (control, 13.3 ± 2.0; experimental, 11.3 ± 2.4 μg/min; $P > .50$) nor the 25-unit dose (10.7 ± 1.4 to 11.1 ± 2.0 μg/min; $P > .70$) altered urinary phosphate excretion. However, a mild but significant phosphaturia did result from the administration of 100 units of the metabolite per hour: 9.5 ± 0.9 to 11.9 ± 1.6 μg/min; $P < .05$. In the latter group, serum phosphate rose significantly (to 10.36 ± 0.55 mg per 100 ml; $P < .02$) and serum calcium fell slightly (to 5.02 ± 0.14; $P < .001$), again suggesting that this massive dose of the metabolite affects the bones.

In contrast to our results with the dog (*13, 20*) and in agreement with the work of Popovtzer *et al.* (*21*), small ("permissive") doses of PTH were required for the antiphosphaturic effect of 25-HCC to become evident (Fig. 1). The reason for this differ-

Table 1. Serum phosphorus and calcium values, and glomerular filtration rate in control and experimental animals. N, number of studies per group.

Dose	Amount (unit/hour)	N	Serum phosphate (mg/100 ml)	Serum calcium (mg/ml)	Glomerular filtration rate (ml/min)
None		6	7.65 ± 0.79	6.17 ± 0.19	0.90 ± 0.06
25-HCC	1	7	9.09 ± 0.44	6.04 ± 0.47	1.07 ± 0.08
PTH	0.2	5	10.57 ± 0.46*	5.11 ± 0.22*	1.06 ± 0.14
25-HCC	1				
+PTH	0.2	6	6.46 ± 0.22	6.31 ± 0.22	0.85 ± 0.04
PTH	1	5	10.68 ± 1.02†	7.22 ± 0.87	1.04 ± 0.21
25-HCC	1				
+PTH	1	5	7.47 ± 0.35	6.45 ± 0.61	0.87 ± 0.11
PTH	2	6	9.44 ± 1.02	5.54 ± 0.38	0.82 ± 0.10
25-HCC	1				
+PTH	2	6	8.37 ± 0.54	5.83 ± 0.37	0.92 ± 0.14
PTH	5	5	8.88 ± 0.68	6.05 ± 0.34	0.88 ± 0.09

*$P < .02$ †$P < .05$.

ence is not obvious from the available data, but potential explanations include species difference and the possibility that 25 units of 25-HCC in the dog may have represented a dose greater than physiologic. The achievement of vitamin D depletion and its absence in our previous dog experiments may have been important, as suggested by earlier workers (*8, 10*). The time lapse which characterized the development of reduced phosphate excretion in response to the combined administration of 25-HCC at 1 unit per hour and PTH at 0.2 unit per hour (whereas neither agent was effective alone) lends additional credence to the concept that at this minimal dosage level both substances are required for the biological effect of the metabolite to be expressed. This delay could reflect either the time required for further metabolic conversion or the necessity for transcription and new protein formation to occur, or both. The necessity for the presence of PTH most likely results from the hormone's ability to act as a trophic agent (*22*) for the conversion of 25-HCC to that derivative of the vitamin which is directly active at the renal tubular cell level. This hypothesis would also explain the lack of effect of large doses (up to 25 units per hour) of the metabolite in the absence of PTH. The phosphaturia which occurred with massive doses of 25-HCC may well have reflected alterations induced in bone (*23*) or in the renal tubular cell (*24*) which, at this dosage level, may not require further metabolism of the 25-HCC (*23*).

As to the necessity for some form of the vitamin being present if PTH is to effect a phosphaturia, the existent experimental observations are conflicting. Harrison and Harrison described only an equivocal alteration in phosphate excretion after PTH in D-depleted animals, whereas repletion with 500 units of vitamin D 18 hours prior to hormone injection restored the (substantial) phosphaturic effect of the PTH (*5*). Similar conclusions were reached by Suh, Frasier, and Kooh, on the basis of improved renal responsiveness to PTH after vitamin D_2 administration in a patient with pseudohypoparathyroidism (*10*). Rasmussen and his colleagues originally reported that vitamin D was not required for the production of a phosphaturia by PTH (*3, 5*) although they later demonstrated that vitamin D–repleted rats did exhibit greater phosphate excretion in response to a standard dose of hormone, as was the case also when D-deficient animals were fed a high calcium diet (*5, 25*). An important reason, as well as the most likely explanation for this apparent discrepancy between their studies and ours presented here, is the fact that the dose of PTH used by Rasmussen's group (5 μg per hour, approximately equivalent to 10 units per hour of our hormone preparation) must be considered to be above the physiologic range.

JULES B. PUSCHETT, WILLIAM S. BECK, JR.
Allegheny General Hospital and
University of Pittsburgh,
Pittsburgh, Pennsylvania 15212
ADAM JELONEK
Institute of Pediatrics, Krakow, Poland

References and Notes

1. H. Rasmussen and H. F. DeLuca, *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* **53**, 108 (1963); H. F. DeLuca, in *Parathyroid Hormone, Thyrocalcitonin and Related Drugs*, G. Peters, Ed. (Pergamon, Oxford, 1970), p. 101.
2. H. F. DeLuca, H. Morii, M. J. Melancon, Jr., in *Parathyroid Hormone and Thyrocalcitonin, Proceedings of 3rd Parathyroid Conference*, R. V. Talmadge and L. F. Belanger, Eds. (Excerpta Medica, New York, 1968), p. 448.
3. C. Arnaud, H. Rasmussen, C. Anast, *J. Clin Invest.* **45**, 1955 (1966).
4. H. C. Harrison, H. E. Harrison, E. A. Park, *Am. J. Physiol.* **192**, 432 (1958); H. Morii and H. F. DeLuca, *Am. J. Physiol.* **213**, 358 (1967); J. H. P. Jonxis, *Helv. Paediatr. Acta* **33**, 491 (1959); R. Steendijk, *Acta Paediatr. Scand.* **53**, 105 (1964); D. Fraser, S. W. Kooh, C. R. Scriver, *Pediatr. Res.* **1**, 425 (1967); C. Marnay and Y. Raoul, *Comptes Rendus* **153**, 1949 (1959).
5. H. E. Harrison and H. C. Harrison, *Metabolism* **13**, 952 (1964).
6. E. B. Olson, Jr., H. F. DeLuca, J. T. Potts, Jr., in *Parathyroid Hormone and Thyrocalcitonin, Proceedings of 3rd Parathyroid Conference*, R. V. Talmadge and L. F. Belanger, Eds. (Excerpta Medica, New York, 1968), p. 448; H. E. Harrison and H. C. Harrison, *J. Biol. Chem.* **188**, 8370 (1951); R. Nicolaysen, *Acta Physiol. Scand.* **22**, 260 (1951); R. H. Wasserman, *The Transfer of Calcium and Strontium Across Biological Membranes* (Academic Press, New York, 1963), pp. 211–228; H. C. Harrison and H. E. Harrison, *Endocrinology* **86**, 756 (1970).
7. F. Albright and E. C. Reifenstein, Jr., *The Parathyroid Glands and Metabolic Bone Disease* (Williams & Wilkins, Baltimore, 1948), pp. 35–40 and 217–220.
8. H. E. Harrison, H. C. Harrison, F. Lifshitz, *Proceedings of 3rd Parathyroid Conference*, R. V. Talmadge and L. F. Belanger, Eds. (Excerpta Medica, New York, 1968), p. 445.
9. E. Yendt, in *Parathyroid Hormone, Thyrocalcitonin and Related Drugs*, G. Peters, Ed. (Pergamon, Oxford, 1970), p. 139; L. Avioli, *Am. J. Med.* **57**, 34 (1974).
10. S. M. Suh, D. Fraser, S. W. Kooh, *J. Clin. Endocrinol. Metab.* **30**, 609 (1970).
11. H. E. Harrison and H. C. Harrison, *J. Clin. Invest.* **20**, 47 (1941).
12. R. L. Ney, W. V. W. Au, G. Kelly, I. Radde, F. C. Bartter, *ibid.* **44**, 2003 (1965).
13. J. B. Puschett, J. Moranz, W. S. Kurnick, *ibid.* **51**, 373 (1972).
14. H. F. DeLuca, *Am. J. Med.* **57**, 1 (1974).
15. E. Cotlove, *J. Appl. Physiol.* **16**, 764 (1961).
16. 25-HCC was provided by Dr. J. Hinman, Upjohn Co., Kalamazoo, Michigan.
17. W. D. Davidson and M. A. Sackner, *J. Lab. Clin. Med.* **62**, 351 (1963).
18. C. H. Fiske and Y. Subbarow, *J. Lab. Chem.* **66**, 375 (1925).
19. H. Gitelman, *Anal. Biochem.* **18**, 521 (1967).
20. J. B. Puschett, P. C. Fernandez, I. T. Boyle, R. W. Gray, J. L. Omdahl, H. F. DeLuca, *Proc. Soc. Exp. Biol. Med.* **141**, 379 (1972).
21. M. M. Popovtzer, J. B. Robinette, H. F. DeLuca, M. F. Holick, *J. Clin. Invest.* **53**, 913 (1974).
22. H. Rasmussen, M. Wong, D. Bikle, D. B. P. Goodman, *ibid.* **51**, 2505 (1972); M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673 (1972); D. R. Fraser and E. Kodicek, *Nature (Lond.)* **241**, 163 (1973).
23. C. L. Trummel, L. G. Raisz, J. W. Blunt, H. F. DeLuca, *Science* **163**, 1450 (1969).
24. J. B. Puschett, W. S. Beck, Jr., A. Jelonek, P. C. Fernandez, *J. Clin. Invest.* **53**, 756 (1974).
25. H. Rasmussen and P. Bordier, *The Physiological and Cellular Basis of Metabolic Bone Disease* (Williams & Wilkins, Baltimore, 1974), pp. 248–249.
26. Supported in part by PHS grants AM 14708, AM 17575, and 5S01-RR-05709, by a grant-in-aid from the Health Research Services Foundation, Pittsburgh, Pennsylvania, and by institutional research funds from the Allegheny General Hospital (886), Pittsburgh, and the VA Hospital, Philadelphia, Pennsylvania. J.B.P. was a clinical investigator of the Veterans Administration and A.J. was supported by the American Research Hospital in Poland, Indiana, and was sponsored by the Maternal and Child Health Service of the Department of Health, Education, and Welfare. We thank D. Sylk, K. Loy, M. Kuhrman, H. Gaudiosi, D. Gallo, and C. Thomas for their assistance in the performance of the chemical analyses.

24 February 1975; revised 14 June 1975

Behavioral Characterization of *d*- and *l*-Amphetamine: Neurochemical Implications

Abstract. *Various doses of* d- *and* l-*amphetamine affect the temporal pattern of rat behavior in the following ways: First, the patterns of activity produced by* d- *and* l-*amphetamine are similar but out of phase; that is, the response to* d-*amphetamine has a relatively shorter latency whereas the effects of* l-*amphetamine persist for longer periods of time. Second,* d-*amphetamine is approximately five times as potent as* l-*amphetamine in its effects on both the total amount of locomotor activity and the duration of stereotypy. Both amphetamine-induced locomotion and stereotypy may be mediated by the same neurochemical mechanisms.*

Recent reports (*1*) have suggested relative potencies for *d*- and *l*-amphetamine that differ significantly from earlier estimates (*2*). The later results indicate that *d*-amphetamine is approximately four to six times more potent than *l*-amphetamine with respect to various indices of dopamine (DA) function in brain areas where DA is the predominant catecholamine (CA), and that the two isomers of amphetamine are approximately equal in their effects on norepinephrine (NE) mechanisms in brain regions where NE is the predominant CA. Similarly, conflicting results have been reported for the relative potency of *d*- and *l*-amphetamine in eliciting stereotypy (which refers to the continuous repetition of behaviors such as sniffing, licking, and gnawing). Scheel-Kruger (*3*) found that, rather than being approximately equivalent to *l*-amphetamine in eliciting stereotypy, *d*-amphetamine is four to six times more potent. These discrepancies may be due, at least in part, to an incomplete characterization of the effects produced by amphetamine. In most studies the time pa-