8. C. G. Gross, C. E. Rocha-Miranda, D. B. Bender, *J. Neurophysiol.* **35**, 95 (1972); R. Desimone and C. G. Gross, *Brain Res.* **178**, 363 (1979); T. Sato, T. Kawamura, E. Iwai, *Exp. Brain Res.* **38**, 313 (1980).
9. D. J. Braitman and W. A. Wilson, *Soc. Neurosci. Abstr.* **2**, 1068 (1976); E. T. Rolls, S. J. Judge, M. K. Sanghera, *Brain Res.* **130**, 229 (1977); C. G. Gross, D. B. Bender, G. L. Gerstein, *Neuropsychologia* **17**, 215 (1979).
10. A. Mikami and K. Kubota, *Brain Res.* **183**, 65 (1980).
11. The sample button subtended approximately 8° and was entirely transilluminated with colored light. Its luminance, with color-alone samples, was about 13.5 cd/m^2. The area covered by the symbol in color-symbol combinations was calculated to be the same for all symbols, such that the averaged luminance of the button, whatever the combination, was about 30 cd/m^2. Intensity and colorimetric characteristics were determined with a Pritchard Spectra photometer. The luminances of the sample stimuli used by any given animal were carefully equalized and periodically checked. Deviations from the mean for different colors or color-symbol combinations in no case exceeded 0.1 log unit. Wavelength was varied by the use of Cinemoid Color filters; dominant λ was about 620 nm for red, 590 nm for yellow, 530 nm for green, and 480 nm for blue.
12. J. M. Fuster, *Science* **133**, 2011 (1961).
13. Very few cells with differential delay activity could be tested on color-symbol combinations. The results of this part of our experiments, which is continuing, are still inconclusive.
14. J. M. Fuster and J. P. Jervey, in preparation.
15. B. Seltzer and D. N. Pandya, *Brain Res.* **149**, 1 (1978).
16. C. G. Gross, D. B. Bender, C. E. Rocha-Miranda, *Science* **166**, 1303 (1969).
17. Recording unit responses in untrained animals would not provide suitable control of the memory factor, since in such animals it is not possible to duplicate conditions of attention and perception of the sample stimulus as in the memory task.
18. In the acute preparation, some IT cells have been noted to react to visual stimuli with excitatory discharge that outlasts the stimulus by many seconds [C. G. Gross, personal communication; C. G. Gross, D. B. Bender, M. Mishkin, *Brain Res.* **131**, 227 (1977)].
19. M. Mishkin, in *Brain and Human Behavior*, A. G. Kaczmar and J. C. Eccles, Eds. (Springer, New York, 1972), p. 187; A. Sahgal and S. D. Iversen, *Brain Res.* **146**, 341 (1978).
20. R. Desimone, J. Fleming, C. G. Gross, *Brain Res.* **184**, 41 (1980).
21. Supported by NSF grant BNS 76-16984 and National Institute of Alcohol Abuse and Alcoholism grant AA3513. J.M.F. was the recipient of a research scientist award (MH 25082). We thank R. H. Bauer for behavioral preparation of two of the animals that were also used in another study. We also thank G. E. Mount and J. P. Thomas for their assistance in photometric determinations. B. Bergerson, M. Dunst, D. Riley, and R. Smith provided valuable technical help.

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Peripherally Administered Reduced Pterins Do Enter the Brain

Abstract. *The content of tetrahydrobiopterin in rat brain was doubled by peripherally administered tetrahydrobiopterin, with the natural* l *diastereoisomer more effective than the unnatural* d *configuration. The model pteridine, 6-methyltetrahydropterin was ten times more efficient than tetrahydrobiopterin in crossing the blood-brain barrier, and striatal concentrations of 6-methyltetrahydropterin remained elevated for 2 hours, declining with a half-life of 3 hours. While no evidence for a specific uptake mechanism for concentrating 6-methyltetrahydropterin in cells containing tetrahydrobiopterin was detected, the pterin was found in its presumed site of action, the nerve terminal. Replacement therapy with reduced pterins may therefore be effective in the treatment of the neurological disorders associated with the variant forms of hyperphenylalaninemia that result from defects in the biosynthesis or metabolism of tetrahydrobiopterin within the central nervous system.*

A hyperphenylalaninemia in which neurological disorders persist despite dietary control of phenylalanine levels has been described (*1*). This condition, which is produced by defects in the phenylalanine hydroxylase system other than in phenylalanine hydroxylase itself, is characterized by a deficiency of dihydropteridine reductase (*2*) or by a defect in the biopterin biosynthetic pathway leading to a deficiency of tetrahydrobiopterin (BH_4) (*3*). Because the reductase and BH_4 are also necessary for the activity of tyrosine and tryptophan hydroxylases (*4*), this condition is further characterized by a lack of catecholamines and serotonin in the peripheral and central nervous systems (*5*). The variant forms of hyperphenylalaninemia are currently treated by restriction of phenylalanine intake and administration of dopa and 5-hydroxytryptophan, the hydroxylated amino acid precursors of catecholamines and serotonin, respectively, in conjunction with peripheral aromatic amino acid decarboxylase inhibition (*5*, *6*). Although administration of BH_4 might also appear to be a reasonable therapy (*2*), the reports that the blood-brain barrier is impermeable to peripherally administered BH_4 (*7*) made it seem unlikely that this treatment would prevent the neurological damage that characterizes these diseases. We now report that reduced pterins administered peripherally in relatively large doses do cross the blood-brain barrier and in a fashion that reflects the lipophobicity of the side-chain substitution at position 6 of the pterin ring.

Male Sprague-Dawley rats (150 to 200 g) were injected intraperitoneally with tetrahydropterins dissolved in 1 percent ascorbic acid, *p*H 7.0. Except where noted, animals received an overdose of barbiturate (500 mg/kg) 90 minutes later and were perfused through the heart with 50 ml of phosphate-buffered saline, *p*H 7.4, to flush the cerebral vasculature. Brains were removed, freed from membranes, rinsed in saline, dissected if necessary, and frozen at −70°C. Pterin content was determined by reverse-phase high-performance liquid chromatographic analysis with fluorescence detection (*8*).

Administered peripherally at a dose of 0.10 μmole per gram of body weight, BH_4 entered the brain in quantities which, although only 0.4 percent of what might have been expected on the assumption of equal body distribution, still were sufficient to increase whole-brain biopterin levels by a factor of 2 (Table 1). Analysis of the state of reduction of the accumulated pterin by differential oxidation demonstrated that more than 95 percent remained in the fully reduced, tetrahydro form (*8*).

The chemical reduction of biopterin introduces an asymmetric center at position 6 of the pterin ring, producing two diastereoisomers that can be resolved into the natural *l* and unnatural *d* isomers (*9*). Since the pterin-dependent monooxygenases exhibit different properties in the presence of the separate diastereoisomers (*9*, *10*), we investigated whether these isomers might also differ in their ability to cross the blood-brain barrier.

Following preparative isolation of the diastereoisomers of BH_4 (reduced in this laboratory and composed of a 60:40 mixture of *l* to *d*) (*9*), 0.08 μmole per gram of body weight of either isomer was injected intraperitoneally, and the BH_4 content of the brain was determined (Table 1). Although the natural *l* isomer was

Table 1. Entry of peripherally administered tetrahydrobiopterin and its diastereoisomers into rat brain. Male Sprague-Dawley rats were injected intraperitoneally with *dl*-tetrahydrobiopterin (60 percent *l* isomer) or the isolated diastereoisomers, dissolved in 1 percent ascorbic acid, *p*H 7.0. Control animals received ascorbic acid alone. Values of biopterin accumulated are expressed as means ± standard deviation (S.D.) and represent accumulations above control levels (0.486 ± 0.031 nmole/g).

Treatment	Dose (μmole/g)	*N*	Biopterin accumulated (nmole/g)
dl-Tetrahydrobiopterin	0.10	12	0.466 ± 0.028
l-Tetrahydrobiopterin	0.08	6	0.412 ± 0.064
d-Tetrahydrobiopterin	0.08	6	0.258 ± 0.043

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Table 2. Entry of peripherally administered 6-methyltetrahydropterin into rat brain. Male Sprague-Dawley rats were injected intraperitoneally with 6-methyltetrahydropterin (0.10 μmole/g) dissolved in 1 percent ascorbic acid, *p*H 7.0. In separate experiments, the distributions of biopterin and 6-methylpterin among subfractions of the crude mitochondrial fraction (P_2) of striatum were determined (*12*). Data are expressed as means ± S.D. and are presented as nanomoles per gram of starting material. The P_2B fraction contains predominantly nerve endings (synaptosomes). N.D., not detected.

Brain region	*N*	Biopterin (nmole/g)	6-Methylpterin (nmole/g)
Whole brain	4	0.509 ± 0.064	5.37 ± 0.51
Hypothalamus	6	1.84 ± 0.17	5.00 ± 0.41
Striatum	6	0.979 ± 0.131	3.27 ± 0.49
P_2 fraction		0.254	0.631
P_2A (myelin)		N.D.	N.D.
P_2B (nerve endings)		0.231	0.510
P_2C (mitochondria)		N.D.	0.082
Hippocampus	6	0.315 ± 0.029	3.41 ± 0.50
Frontal cortex	6	0.292 ± 0.038	4.02 ± 0.36
Cerebellum	6	0.163 ± 0.026	4.80 ± 1.12

found to be 60 percent more effective than the *d* isomer in entering the brain, further analysis showed that whole-blood concentrations of the natural isomer were also greater, perhaps accounting for the distinction.

Solubility in lipid is a major determining factor in a compound's capacity to cross the blood-brain barrier (*11*). We therefore investigated this capacity for 6-methyltetrahydropterin ($6MPH_4$), a model pteridine that at the 6-position has a relatively lipophilic methyl group rather than the dihydroxypropyl side chain of biopterin and that also serves quite well as a cofactor for pterin-dependent enzymes (*4*).

Administration of $6MPH_4$ (0.10 μmole/g) demonstrated that this pterin entered the brain approximately ten times more efficiently than did an equal dose of BH_4 (Table 2), even though at 90 minutes the blood levels of BH_4 (25 to 30 nmole/ml) were actually higher than the levels of $6MPH_4$ (20 to 25 nmole/ml). A regional brain analysis of endogenous BH_4 and accumulated $6MPH_4$ was performed to determine if a specific uptake mechanism exists for concentrating $6MPH_4$ in cells containing BH_4 (Table 2). The homogeneous distribution of the model pteridine, which did not correlate with the heterogeneous distribution of BH_4, suggests that no such mechanism exists. However, analysis of the subcellular distributions of endogenous BH_4 and $6MPH_4$ accumulated by the striatum (Table 2) showed the compounds to be present in the fraction containing isolated nerve endings, and therefore the pterin-dependent enzymes tyrosine and tryptophan hydroxylases (*12, 13*).

Whole-blood levels of the pterin declined dramatically with time after intraperitoneal injection of $6MPH_4$ (0.11 μmole/g), displaying a half-life of only 0.7 hour (Fig. 1). Despite the extremely rapid decrease in blood levels, striatal $6MPH_4$ content was maximal between 0.5 and 2 hours and had a half-life of 3 hours. At no time were striatal levels of BH_4 altered, suggesting that no displacement of endogenous pteridine occurred. Enzymatic analysis demonstrated that over 85 percent of striatal $6MPH_4$ remained in the fully reduced tetrahydro form 2 hours after injection (*3*).

Administration of reduced pterins, particularly of $6MPH_4$, may be effective in preventing the neurological disorders associated with the variant forms of hyperphenylalaninemia. Although low doses of BH_4 decrease blood levels of phenylalanine in patients with variant forms of hyperphenylalaninemia (*5, 14*), there is no evidence that significant amounts of BH_4 enter the brain or that the neuropathology associated with hyperphenylalaninemia can be prevented when low doses of BH_4 are given. In contrast, our demonstration that pterins such as $6MPH_4$, and even BH_4 when given at a proper dose, do elevate pterin concentrations in the brain suggests that pterin administration might be an effective therapy for these diseases. Administration of tetrahydropterins might also provide a pharmacological technique for increasing the biosynthesis of catecholamines and serotonin in the brain and periphery and could therefore be useful in the treatment of disorders resulting from deficits in biogenic amine biosynthesis.

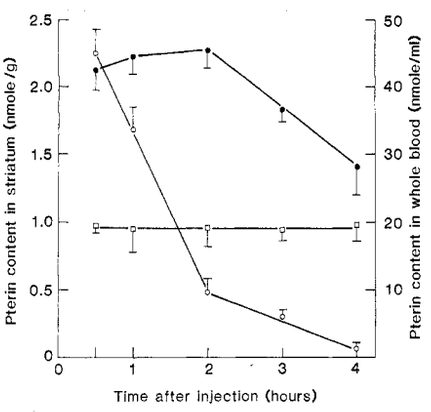

Fig. 1. Time course of entry of 6-methyltetrahydropterin into blood and striatum and clearance of 6-methyltetrahydropterin by blood and striatum. Male Sprague-Dawley rats were given intraperitoneal injections of 6-methyltetrahydropterin (0.11 μmole/g dissolved in 1 percent ascorbic acid, *p*H 7.0). At various times after injection, animals received an overdose of barbiturate, blood was collected from the heart, which was then perfused with 50 ml of phosphate-buffered saline, *p*H 7.4; the brains were removed and striata were dissected. The 6-methylpterin content in blood (○) and striatum (●) and the biopterin content of striatum (□) were determined. Values are means ± standard deviation.

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References and Notes

1. I. Smith, B. E. Clayton, O. H. Wolff, *Lancet* **1975-I**, 1108 (1975).
2. S. Kaufman, N. A. Holtzman, S. Milstien, I. J. Butler, A. Krumholz, *N. Engl. J. Med.* **293**, 785 (1975); F. Rey, J. P. Harpey, R. J. Leeming, J. A. Blair, J. Aicardi, J. Rey, *Arch. Fr. Pediatr.* **34** (Suppl.), 109 (1977).
3. S. Kaufman *et al.*, *N. Engl. J. Med.* **299**, 673 (1978).
4. S. Kaufman in *Aromatic Amino Acids in the Brain*, G. E. W. Wolstenholme and D. W. Fitzsimons, Eds. (Elsevier, New York, 1974), p. 810.
5. I. J. Butler, S. H. Koslow, A. Krumholz, N. A. Holtzman, S. Kaufman, *Ann. Neurol.* **3**, 224 (1978); R. McInnes, S. Kaufman, J. J. Warsh, S. Milstien, G. VanLoon, A. Slyper, G. Sherwood, *Pediatr. Res.* **13**, 579a (1979).
6. K. Bartholomé, D. J. Byrd, S. Kaufman, S. Milstien, *Pediatrics* **59**, 757 (1977).
7. R. Kettler, G. Bartholini, A. Pletscher, *Nature (London)* **249**, 476 (1974); E. M. Gal, G. Hanson, A. Sherman, *Neurochem. Res.* **1**, 511 (1976).
8. T. Fukushima and J. C. Nixon, *Anal. Biochem.* **102**, 176 (1980). High-performance liquid chromatography was performed on Whatman Partisil ODS-2 reverse-phase C18 columns with isocratic 5 percent methanol as the solvent system for biopterin analysis, and 20 percent methanol for 6-methylpterin determination. The eluate was monitored for fluorescence (excitation at 330 to 380 nm; emission at 460 to 600 nm), and pterins were quantified by peak area analysis. All values are corrected for recovery of an internal standard of D-erythroneopterin.
9. S. W. Bailey and J. E. Ayling, *J. Biol. Chem.* **253**, 1598 (1978).
10. H. Hasegawa *et al.*, in *Chemistry and Biology of Pteridines*, R. L. Kisliuk and G. M. Brown, Eds. (Elsevier/North-Holland, New York, 1979), p. 183.
11. A. Lajtha and D. H. Ford, *Brain Barrier Systems* (Elsevier/North-Holland, Amsterdam, 1968).
12. E. G. Gray and V. P. Whittaker, *J. Anat.* **96**, 71 (1962).
13. R. Kuczenski and A. J. Mandell, *J. Biol. Chem.* **247**, 3114 (1972); S. Knapp, A. J. Mandell, M. A. Geyer, *J. Pharmacol. Exp. Ther.* **189**, 676 (1974).
14. J. Schaub *et al.*, *Arch. Dis. Child.* **53**, 674 (1978); A. Niederwieser, H.-Ch. Curtius, M. Viscontini, J. Schaub, H. Schmidt, *Lancet* **1979-I**, 550 (1979).

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