for humans since the conjugate is transported rapidly out of brain ($k_2 >> k_1$). The ratio of k_1 to k_2 is small so that the levels of free and conjugated MHPG in brain are dependent mainly on their formation in brain (M_c/k_2). Since monkeys form little or no conjugates of MHPG in brain, studies in these animals are valid models for humans. Ellsworth *et al.* (*5*) showed that in monkeys CSF concentrations of MHPG are proportional, but always higher, than plasma concentrations. In their study, the slope of the best-fit line for 67 matching plasma and CSF samples was close to unity; the difference between CSF and plasma MHPG concentrations was relatively constant (about 20 ng/ml). Perhaps MHPG is formed more rapidly per unit volume of CSF in monkeys than in man, or the larger difference is a result of sampling of CSF from the cisterna magna rather than from the lumbar area. Concentrations of unconjugated MHPG reflect local norepinephrine metabolism only if the equilibrium of CSF (or partition of brain tissue) with plasma MHPG is considered.

As shown above, concentrations of MHPG in the plasma and CSF are dependent on the sum of the rates of MHPG formation in both the central nervous system and the peripheral tissues and the rate of MHPG metabolism. Peripheral sources probably predominate in determining plasma MHPG levels. Neither plasma levels nor urinary excretion of MHPG are valid indices of brain norepinephrine metabolism since MHPG from brain appears to account for only about 30 percent of the total body production of MHPG (*12*). On the basis of the theoretical considerations and the supporting empirical evidence (for example, in patients with idiopathic orthostatic hypotension), it appears that concentrations of MHPG in human lumbar CSF can provide a valid index of central MHPG production, but only when appropriately corrected by subtracting 90 percent on the plasma MHPG concentrations.

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10. This may be expressed mathematically in terms of the equations that describe the two-component system: $[P] = (M_p + M_c)/k_3$, $d[P]/dM_p = 1/k_3$, and $d[P]/dM_c = 1/k_3$. For the levels in CSF: $[C] = (k_1/k_2 \cdot k_3)M_p + (k_1/k_2 \cdot k_3 + 1/k_2)M_c$, $d[C]/dM_p = (k_1/k_2 \cdot k_3)$, whereas $d[C]/dM_c = k_1/k_2 \cdot k_3 + 1/k_2$. Thus, when M_p changes, $d[C]/d[P] = k_1/k_2$, whereas when M_c changes, $d[C]/d[P] = k_1/k_2 + k_3/k_2$. If both M_p and M_c change, the slope will be intermediate (a weighted mean) between k_1/k_2 and $k_1/k_2 + k_3/k_2$, depending on the relative magnitude of the changes in M_p and M_c.
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Effects of Tyrosine Administration on Serum Biopterin in Normal Controls and Patients with Parkinson's Disease

Abstract. *After administration of tyrosine, total concentration of biopterin, the cofactor for tyrosine hydroxylase, was increased in the striatum, adrenal glands, and serum of rats, and in the serum of humans. Serum biopterin is lower in patients with Parkinson's disease than in normal controls. After oral administration of tyrosine, the increase in serum biopterin concentration was smaller in patients with Parkinson's disease (less than twofold) than in healthy controls (three- to sevenfold). These results suggest that tyrosine may have a regulatory role in biopterin biosynthesis and that patients with Parkinson's disease may have some abnormality in the regulation of biopterin biosynthesis.*

The enzymic hydroxylation of tyrosine, the first step in dopamine biosynthesis, is catalyzed by tyrosine hydroxylase, which requires L-*erythro*-tetrahydrobiopterin (BPH_4) as cofactor. Both tyrosine hydroxylase activity and total biopterin concentration are greatly decreased in the striatum of patients with Parkinson's disease (*1*). Hydroxylase cofactor content in the cerebrospinal fluid of these patients is also decreased in comparison with that of controls (*2*). Since BPH_4 is an essential cofactor for tyrosine hydroxylation, these results suggest that the reduction in dopamine in the parkinsonian striatum may be due to reductions in the concentration of striatal BPH_4 and in tyrosine hydroxylase activity. The concentration of BPH_4 in catecholaminergic neurons may be lower than the value of the Michaelis constant for the cofactor of nonphosphorylated tyrosine hydroxylase, and catecholamine biosynthesis in vivo may be regulated by both BPH_4 concentration and tyrosine hydroxylase phosphorylation (*3*). Reserpine treatment or insulin-induced hypoglycemia, which increases tyrosine hydroxylase (*4, 5*), produced a significant increase in the BPH_4 content in the adrenal medulla in rats (*6, 7*).

Since tyrosine increases catecholamine biosynthesis through the change in tyrosine hydroxylase activity (*8*), we have investigated the effect of tyrosine administration on total biopterin concentration in tissues and serums from rats and from normal human subjects and patients with Parkinson's disease.

Tyrosine (1 g per kilogram of body weight), suspended in 0.2 percent carboxymethylcellulose, was injected intraperitoneally in male Sprague-Dawley rats (200 to 250 g). The rats were decapitated at various time intervals after tyrosine administration; tissues (striatum, adrenal glands, and liver) and blood were immediately removed, and serum was separated. Total biopterin concentration was measured by a newly established, specific, sensitive radioimmunoassay for L-*erythro*-biopterin (*9*). We found that total biopterin concentration was increased 1.5- to threefold in the striatum, adrenal glands, and serum after administration of tyrosine to rats (Fig. 1), but total biopterin content in the liver was slightly decreased. These results suggest that tyrosine may regulate the biosynthesis of biopterin from guanosine triphosphate in central and peripheral catecholaminergic cells.

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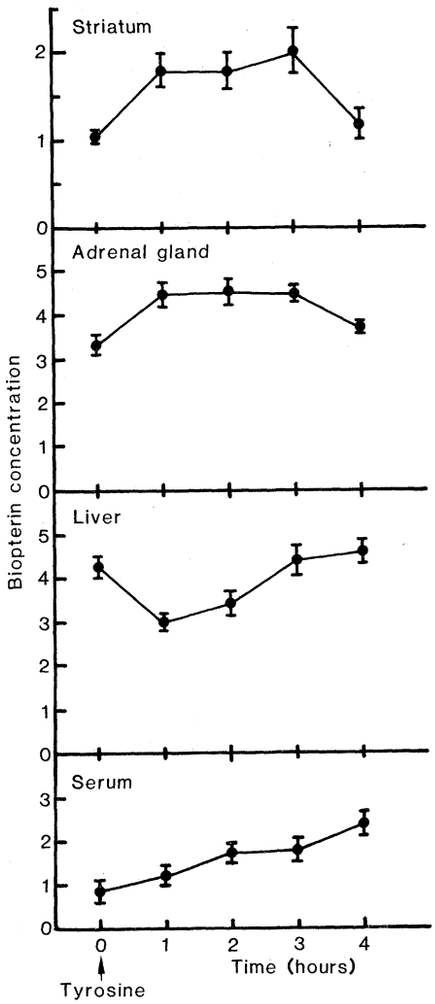
In view of these results in rats, we administered tyrosine (7 g orally) at 8 a.m. to six parkinsonian patients who had not taken L-dopa during the last 3 months and to six normal controls. The subjects were not permitted food before and during the experiments. Total biopterin in human serum varied from less than 0.5 to 25 pmole per milliliter of serum. The mean values of total biopterin in the serum of 20 normal controls and 26 parkinsonian patients were, respectively, 5.9 ± 0.8 and 3.9 ± 0.7 pmole per milliliter of serum [± standard error (S.E.M.)]. The mean value of serum biopterin in parkinsonian patients was slightly but significantly lower than that in controls ($P < .05$). After administration of tyrosine, serum biopterin in controls increased three- to sevenfold, whereas the level in parkinsonian patients was unchanged or increased less than twofold (Fig. 2). The mean (± S.E.M.) maximum increases in six controls and six parkinsonian patients were 478 ± 66 and 181 ± 23 percent, respectively ($P < .05$).

The previous finding, in rats, that tyrosine administration resulted in an increase in total biopterin concentration in the striatum and adrenal glands, but not in the liver, suggests that catecholaminergic cells are the origin of the increased biopterin. We also measured tyrosine concentration in serum in parallel with biopterin concentration. The changes in serum tyrosine did not differ significantly between parkinsonian patients and controls. The peak concentration of serum tyrosine was observed 2 to 3 hours after tyrosine administration, coinciding with that of serum biopterin. This result indicates that tyrosine absorption from the intestine in parkinsonian patients is not different from that in normal controls.

We also administered alanine instead of tyrosine to four normal controls and four parkinsonian patients, but total biopterin in the serum did not change. Two hours after alanine administration, the values were 109 ± 2 and 98 ± 4 percent, respectively, of the values before alanine administration. These results suggest that tyrosine may have a regulatory role in the biosynthesis of biopterin in catecholaminergic cells and that the regulatory system in parkinsonian patients may not be able to respond properly after tyrosine administration.

In earlier studies (*10, 11*), phenylalanine administered orally to normal subjects and to patients with classical phenylketonuria, who have abnormal phenylalanine hydroxylase but normal enzyme systems for BPH_4 biosynthesis, led to two- to fivefold increases in plasma biopterin. In contrast, patients with atypical phenylketonuria, who have an abnormality in BPH_4 biosynthesis in the liver, did not exhibit increased plasma biopterin after phenylalanine administration. Leeming *et al.* (*10*) reported that tyrosine loading in normal adults at the dose used in our study did not alter the levels of biopterin in the serum, although it did elevate these levels in our study. The reason for this discrepancy is not clear.

The response of serum biopterin to tyrosine may prove to be useful in predicting the occurrence or estimating the progress of Parkinson's disease. On the other hand, the administration of BPH_4 may be useful in increasing the biopterin cofactor in parkinsonian catecholaminergic cells.



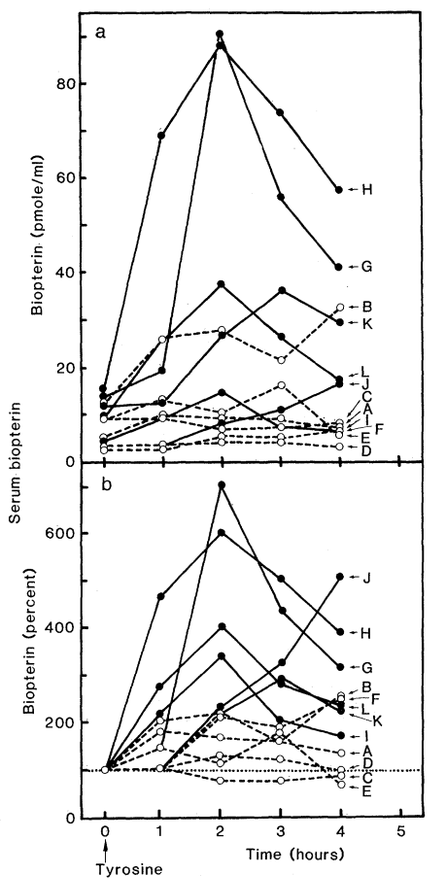


Fig. 1 (left). Time course of changes in concentration of biopterin in rat striatum, adrenal glands, and liver (expressed as nanomoles per gram of wet tissue) and in serum (picomoles per milliliter of serum) after tyrosine administration. At time zero, tyrosine (1 g per kilogram of body weight) suspended in 0.2 percent carboxymethylcellulose was administered intraperitoneally. Animals were decapitated and tissues and blood were removed immediately after tyrosine administration and at every hour thereafter for 4 hours. Values are means ± S.E.M. ($N = 5$). Fig. 2 (right). (a) Total serum biopterin concentration after tyrosine was administered orally to patients with Parkinson's disease (○) and to controls (●). Tyrosine was administered at 8 a.m., and blood samples were taken at every hour. (b) Values from (a) expressed as percentage of the serum biopterin level before tyrosine administration. The age and sex of the patients with Parkinson's disease were: A, 67 ♀; B, 77 ♂; C, 69 ♂; D, 43 ♀; E, 47 ♀; and F, 51 ♂. The age and sex of the controls were: G, 44 ♂; H, 31 ♂; I, 74 ♀; J, 74 ♂; K, 58 ♂; and L, 46 ♀.

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Role for Acetylcholine in Mediating Effects of Light on Reproduction

Abstract. *The length of day, or photoperiod, regulates the annual cycle of reproductive activity in the golden hamster. The inhibitory effects of a short-day photoperiod on testicular function were prevented by nighttime, but not daytime, intraventricular injections of carbachol, a cholinergic agonist. Short pulses of light during the night also block short-day induced testicular regression. The findings suggest that acetylcholine may play an important role in the mechanism through which information about the light-dark environment is transferred to the hypothalamic-pituitary-gonadal axis.*

In many avian and mammalian species the effects of seasonal changes in day length on neuroendocrine-gonadal activity are mediated by an internal 24-hour biological clock (*1, 2*). Although there is a clear relation between the biological clock and the hypothalamic-pituitary-gonadal axis, little is known about the neurochemical events underlying the effects of light on clock-controlled changes in reproductive function. Acetylcholine has been implicated in mediating the effects of light on the circadian system by reports that carbachol, a cholinergic agonist, mimics the phase-shifting effects of light on both the circadian rhythms of wheel-running activity in mice and pineal serotonin *N*-acetyltransferase activity in rats (*3*).

To identify the neurotransmitters involved in the circadian-based photoperiodic control of reproduction, we focused on a possible role for acetylcholine. We used male golden hamsters (*Mesocricetus auratus*) because maintenance of gonadal function in these animals does not require daily prolonged periods of photostimulation (*4, 5*). Testicular regression occurs in hamsters exposed to nonstimulatory short days, but can be prevented by exposing them to as little as 1 second of light near the middle of the night either every day or every other day (*6*). We sought to determine whether the administration of carbachol during the night could mimic the effects of short pulses of light on gonadal function in hamsters transferred from a stimulatory light-dark (LD) cycle of 14 hours of light and 10 of darkness to a nonstimulatory LD 6:18.

Twenty-eight adult male golden hamsters (*7*) that had been housed five to six

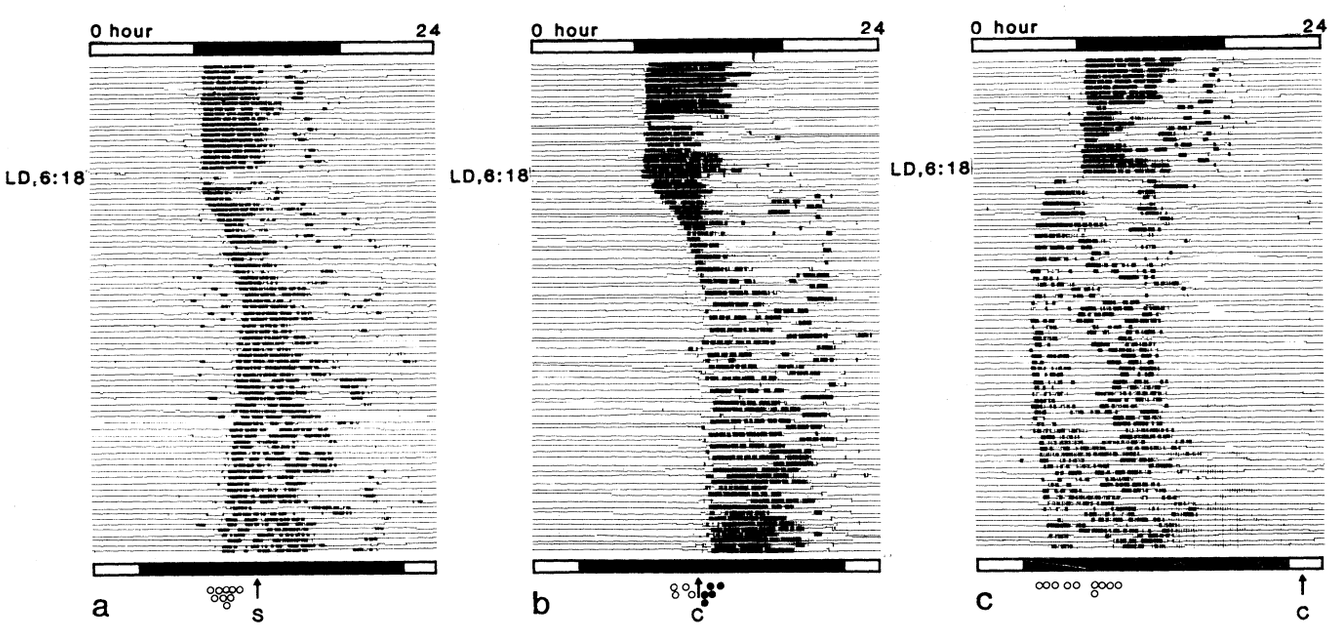

Fig. 1. The activity records of three individual hamsters that were first exposed to LD 14:10 and then transferred to LD 6:18 on the day indicated. LD 14:10 is illustrated at the top and LD 6:18 at the bottom of the activity charts, with the time of darkness represented by the black bars. (a) The activity of an animal injected every other day with saline (*S*) 8 to 8.5 hours after lights-off (time of injections represented by arrow). (b) The activity of an animal injected with carbachol (*C*) 8 to 8.5 hours after lights-off. (c) The activity of an animal injected with carbachol 1 to 1.5 hours after lights-on. The mean (± S.E.M.) activity onsets for animals in groups 1, 2, and 3 occurred 5.9 ± 0.2, 8.1 ± 0.4, and 3.9 ± 0.6 hours after lights-off, respectively. The circles below the activity records depict the time of the onset of activity relative to LD 6:18 for individual animals in the three groups of animals. Open circles represent animals with small testes (paired testis weight < 1000 mg); closed circles represent animals with large testes (paired testis weight > 1000 mg) at the end of the experiment.

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