3. C. KORNETSKY and G. F. KIPLINGER, *Psychopharmacologia* **4,** 66 (1963).
4. G. F. KIPLINGER and J. W. CLIFT, *J. Pharmac. exp. Ther.* **146,** 139 (1964).
5. E. L. WAY, H. H. LOH and F. H. SHEN, *Science, N.Y.* **162,** 1290 (1968).
6. H. H. LOH, F. H. SHEN and E. L. WAY, *Biochem. Pharmac.* **18,** 2711 (1969).
7. F. H. SHEN, H. H. LOH and E. L. WAY, *J. Pharmac. exp. Ther.* **175,** 427 (1970).
8. N. B. EDDY and D. G. LEIMBACH, *J. Pharmac. exp. Ther.* **107,** 385 (1953).
9. C. A. WINTER and L. FLATAKER, *J. Pharmac. exp. Ther.* **98,** 305 (1950).
10. Q. MCNEMAR, *Psychological Statistics*, p. 362. Wiley, New York (1962).
11. M. P. FEINBERG and J. COCHIN, *Pharmacologist* **11,** 256 (1969).
12. J. M. MILLER and J. COCHIN, *Some Aspects of Tolerance to Morphine in the Mouse.* Reported to the NAS-NRC Committee on Problems of Drug Dependence, February (1968).
13. D. B. GOLDSTEIN and A. GOLDSTEIN, *Biochem. Pharmac.* **8,** 48 (1961).
14. L. SHUSTER, *Nature, Lond.* **189,** 314 (1961).
15. A. GOLDSTEIN and D. B. GOLDSTEIN, in *The Addictive States* (Ed. A. WIKLER) p. 265. Williams & Wilkins, Baltimore (1968).
16. H. O. J. COLLIER, *Nature, Lond.* **205,** 181 (1965).

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Effect of 6-hydroxydopamine on the activity and circadian rhythmicity of hepatic tyrosine aminotransferase

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THE INDUCIBLE enzyme,[1] tyrosine aminotransferase (ECC 2.6.1.5), undergoes circadian variations in activity,[2] and it has been proposed that these variations are under noradrenergic control. According to this proposal, aminotransferase synthesis is cofactor dependent and norepinephrine, periodically released from noradrenergic terminals, disrupts synthesis by directly chelating the cofactor, pyridoxal phosphate, and removing it from the enzyme-forming site.[3] In support of this proposal it has been established that norepinephrine, together with other catechols, does complex with pyridoxal-5-phosphate *in vitro*[4,5] and that the dissociation constant for the resulting isotetraquinoline is smaller than that of the enzyme–cofactor dissociation constant. Further, administration of pyridoxine to the rat can lead to elevations in tyrosine aminotransferase activity under some conditions,[6] and norepinephrine can inhibit this elevation.[7] This inhibition is most marked if norepinephrine is administered during the nocturnal rise of enzymic activity.

In the present study we attempted to gain more information on the relationship betwen norepinephrine and enzymic activity by examining circadian changes in enzymic activity after treatment with 6-hydroxydopamine, an agent shown to be a potent tool for selective destruction of catecholaminergic terminals, particularly noradrenergic terminals.[8]

Male Long–Evans rats weighing 80–120 g were housed individually after treatment and maintained on an *ad lib.* diet of Purina Rat Chow. The animal room was maintained at 22° and lights were on from 5 a.m. to 5 p.m. Animals were decapitated at 9 a.m. or 9 p.m. which are the nadir and peak, respectively, of the diurnal rhythm of tyrosine aminotransferase under these environmental conditions. Upon decapitation, trunk blood was collected; organs were quickly removed, rinsed, weighed and placed in appropriate media for assay. Spleens and hearts from some animals were homogenized in 0·4 N perchloric acid for norepinephrine determinations. Livers were homogenized in cold neutral 0·15 M KCl and centrifuged at 105,000 *g* for 30 min at 4° and the supernatants used for liver tyrosine aminotransferase assay. Adrenal and serum corticosterone was assayed by the method of Givner and Rochefort.[9] Norepinephrine was assayed by the method of Bertler *et al.*[10] after separation by the method of Anton and Sayre.[11] Liver tyrosine aminotransferase was measured by the method of Lin *et al.*[12] and protein by the method of Lowry as described by Layne.[13] A Student two-tailed *t*-test was used for statistical comparisons.

TABLE 1. EFFECT OF 6-HYDROXYDOPAMINE ON LIVER TYROSINE AMINOTRANSFERASE

	Controls				Experimentals			
	N	9 a.m.	N	9 p.m.	N	9 a.m.	N	9 p.m.
Liver tyrosine aminotransferase (μmoles/min/g protein)	10	15·3 ± 1·0*	8	28·2 ± 1·7†	11	13·1 ± 1·6	9	24·9 ± 1·7*
Heart norepinephrine (μg/g)	4	0·636 ± 0·005			4	0·162 ± 0·008		
Spleen norepinephrine (μg/g)	11	1·250 ± 0·014			16	0·233 ± 0·034		

* Mean ±S.E.

† $P < 0·05$ in comparison to enzyme level at 9 a.m.

Animals were injected in the tail vein with 6-hydroxydopamine hydrobromide (Regis Chemical Co.) (50 mg/kg calculated as the free base) at 8 a.m. and again at 8 a.m. the following day. Pargyline (100 mg/kg) was injected intraperitoneally 30 min before the first injection of 6-hydroxydopamine. Animals were sacrificed 3 days after the final injection and the extent of noradrenergic depletion was estimated from the norepinephrine content of spleen and heart in representative animals.

The effects of two intravenous injections of 6-hydroxydopamine (50 mg/kg) on tissue norepinephrine concentrations and on the enzymic activity of hepatic tyrosine transaminase are shown in Table 1. As may be seen, 3 days after the final injection of 6-hydroxydopamine, heart norepinephrine concentration was reduced to 26 per cent of that for controls while splenic norepinephrine was reduced to 19 per cent. This degree of depletion is comparable to that observed by Theonen.[8] Liver depletion would be expected to closely follow splenic depletion since both are innervated by the celiac ganglia. The reduction in cardiac norepinephrine was twice that produced by a dosage of α-methyl-*p*-tyrosine sufficient to elevate tyrosine aminotransferase activity 7-fold.[14] However, as seen in Table 1, this decrease in peripheral catacholamine concentration did not affect the basal activity level or the amplitude of the circadian variation of tyrosine aminotransferase.

The model of noradrenergic modulation of pyridoxal-dependent enzymes by cofactor repression would imply that enzyme concentrations would be lowered by a decrease in available cofactor and increased by a decrease in norepinephrine. This suggestion[3] is based on observations that peripheral depletion of norepinephrine by some pharmacological agents leads to elevated activity of tyrosine aminotransferase, that repletion of norepinephrine reverses this rise[15] and that administration of norepinephrine to adrenalectomized or hypophysectomized rats initially elevates and then depresses hepatic tyrosine aminotransferase activity.[7]

In previous studies,[16] we examined the effects of pyridoxal deficiency on induction and circadian rhythmicity of several pyridoxal phosphate-dependent enzymes including tyrosine aminotransferase. The results of that study demonstrated that neither induction nor rhythmicity of enzyme activity was altered by markedly decreasing available cofactor. Although these results would argue against the cofactor repression model for sympathetic control of this enzyme, it is possible that only minimal amounts of cofactor at specific sites might be required to permit normal synthesis and that this minimal quantity might still be susceptible to chelation with norepinephrine released from sympathetic nerves. In this study we have examined the second implication of the model, namely that enzymic activity would be increased by a decrease in norepinephrine. Our results strongly suggest, however, that peripheral depletion of norepinephrine does not affect the activity or rhythmicity of this hepatic enzyme. This conclusion is in agreement with the results of Govier *et al.*[14] who observed that enzyme activity was unaltered after marked peripheral norepinephrine depletion by α-methyl-*m*-tyrosine. Wurtman *et al.*[17] have shown that a 77 per cent depletion of central norepinephrine by reserpine does not alter the hepatic transaminase rhythm in cyclically fed animals. Ancillary data on the effects of 6-hydroxydopamine are presented in Table 2. Treatment with this agent caused an initial fall in body weight and decreased food consumption. Animals evidenced diarrhea during this period. By the time of sacrifice, however, daily weight gain was normal as was daily food consumption and serum and adrenal corticoid levels.

TABLE 2. EFFECT OF 6-HYDROXYDOPAMINE ON NUTRITIONAL AND ENDOCRINE PARAMETERS*

	Controls (mean ± S.E.)	Experimentals (mean ± S.E.)
Body wt (g)		
Initial	84·5 ± 2·6	84·9 ± 1·3
Final	97·5 ± 3·8	89·0 ± 4·5
ΔBody wt (overall)	13·0 ± 2·3	3·9 ± 2·8†
ΔBody wt (last day)	4·0 ± 0·3	4·5 ± 1·2
Liver wt (g)	4·03 ± 0·26	3·84 ± 0·21
Adrenal wt (mg)	24·4 ± 0·9	22·4 ± 1·4
Adrenal corticoids (μg/g)	18·6 ± 6·2	18·5 ± 7·5
Serum corticoids (μg/100 ml)	15·4 ± 5·8	23·9 ± 10·5
Food consumption‡ (g)		
Day	4 ± 1	5 ± 1
Night	17 ± 2	18 ± 1

* Each value represents the mean for eleven animals except those for adrenal and serum corticoids which are the means for six animals.

† Differs from controls by $P < 0{\cdot}05$.

‡ Food consumption during the day and night preceding sacrifice. Day consumption was from 9 a.m. to 4:30 p.m. Night consumption from 4:30 p.m. to 9 a.m.

In summary, then, the present results argue against noradrenergic control of the circadian rhythmicity of tyrosine aminotransferase by stoichiometric inhibition of cofactor induction. They do not rule out neural regulation of this enzyme.

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REFERENCES

1. E. C. C. LIN and W. E. KNOX, *Biochim. biophys. Acta* **26,** 85 (1957).
2. M. CIVEN, R. ULRICH, B. M. TRIMMER and C. B. BROWN, *Science, N.Y.* **157,** 1563 (1967).
3. I. B. BLACK and J. AXELROD, in *Biochemical Action of Hormones* (Ed. G. LITWACK), Vol. 1, p. 135. Academic Press, New York (1970).
4. H. F. SCHOTT and W. G. CLARK, *J. biol. Chem.* **196,** 449 (1952).
5. I. B. BLACK and J. AXELROD, *J. biol. Chem.* **244,** 6124 (1969).
6. O. GREENGARD and M. GORDON, *J. biol. Chem.* **238,** 3708 (1963).
7. I. B. BLACK and J. AXELROD, *Archs. Biochem. Biophys.* **138,** 614 (1970).
8. H. THEONEN, in *Six-hydroxydopamine and catecholamine neurones* (Eds. T. MALMFORS and H. THEONEN), p. 75. North Holland Pbl., Amsterdam (1971).
9. M. L. GIVNER and J. G. ROCHEFORT, *Steroids* **6,** 485 (1965).
10. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* **44,** 273 (1958).
11. A. H. ANTON and D. F. SAYRE, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
12. E. C. C. LIN, C. M. PITT, M. CIVEN and W. E. KNOX, *J. biol. Chem.* **233,** 668 (1958).
13. E. LAYNE, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN), Vol 3, p. 447. Academic Press, New York (1957).
14. W. C. GOVIER, W. LOVENBERG and A. SJOERDSMA, *Biochem. Pharmac.* **18,** 2661 (1969).
15. I. B. BLACK, *J. Pharmac. exp. Ther.* **174,** 283 (1970).
16. A. YUWILER, E. GELLER and S. EIDUSON, *Biochim. biophys. Acta* **244,** 557 (1971).
17. R. J. WURTMAN, W. L. SHOEMAKER, F. LARIN and M. ZIGMOND, *Nature, Lond.* **219,** 1049 (1968).