

EFFECT OF 6-HYDROXYDOPAMINE ON BRAIN NOREPINEPHRINE AND DOPAMINE: EVIDENCE FOR SELECTIVE DEGENERATION OF CATECHOLAMINE NEURONS¹

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ABSTRACT

BREESE, GEORGE R. AND T. DENNIS TRAYLOR: Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmacol. Exp. Ther.* 174: 413-420, 1970. After the intracisternal administration of 6-hydroxydopamine, brain levels of norepinephrine were reduced significantly with or without pargyline pretreatment. Depletion of dopamine in the central nervous system was found to be enhanced markedly by pargyline administration at higher dose levels of 6-hydroxydopamine. Brain serotonin concentrations were not altered. The effects of 6-hydroxydopamine were long-lasting with the depletion of brain amines persisting at 78 days. After norepinephrine-³H intracisternally to animals treated with 6-hydroxydopamine, labeled norepinephrine uptake was diminished with a corresponding reduction of deaminated catechols and a marked increase in methylated amines. Tyrosine hydroxylase activity was found to be reduced in brainstem, caudate nucleus and whole brain in 6-hydroxydopamine-treated animals. Conversion of tyrosine-³H to labeled norepinephrine and dopamine was also markedly diminished. The results support the view that 6-hydroxydopamine produces a "central sympathectomy" when introduced into cerebrospinal fluid.

During the past few years, understanding of the role and mechanisms of the peripheral sympathetic nervous system has been advanced by the development of techniques of sympathectomy and through the use of an array of pharmacologic agents. Nevertheless, central noradrenergic mechanisms have been more difficult to assess. The diffuse nature of the noradrenergic system (Carlsson *et al.*, 1962) has made discrete lesioning of norepinephrine cells in brain virtually impossible. Furthermore, pharmacologic agents used to study central mechanisms have had the disadvantage of altering not only the catecholamine systems in the central nervous system but also in the periphery. A pharmacologic agent which could be introduced directly into the central nervous system and which would destroy

the central noradrenergic system without altering the peripheral system would offer obvious advantages. Recent observations suggest that 6-hydroxydopamine may prove to be such an agent (Tranzer and Thoenen, 1968; Uretsky and Iversen, 1969).

The observation by Porter *et al.* (1963) and Laverty *et al.* (1965) that 6-hydroxydopamine induced a long-lasting depletion of norepinephrine in peripheral organs with sympathetic innervation led to the hypothesis that 6-hydroxydopamine destroyed norepinephrine binding sites (Porter *et al.*, 1963). Recently, Tranzer and Thoenen (1968) have confirmed and extended these observations showing that two weeks after treatment with 6-hydroxydopamine virtually all adrenergic fibers were absent from sympathetically innervated tissues. These reports of peripheral chemical sympathectomy by 6-hydroxydopamine (Thoenen and Tranzer, 1968; Tranzer and Thoenen, 1968) led to investigation of the possibility that 6-hydroxydopamine might also produce selective destruction of brain catecholamine neurons when introduced into the central nervous system (Uretsky and Iversen, 1969).

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Bloom *et al.*, 1969; Anagnoste *et al.*, 1969; Traylor and Breese, 1970). Ungerstedt (1968) reported that 6-hydroxydopamine produced degeneration of central catecholamine neurons when injected into a localized area of brain. Utilizing electron micrographic examination of areas rich in adrenergic neurons, Bloom and associates (1969) demonstrated that 6-hydroxydopamine administered into cerebrospinal fluid caused degeneration of central noradrenergic fibers.

The present experiments were designed to investigate the depletion of brain catecholamines after intracisternally administered 6-hydroxydopamine, to determine the effect of this procedure on the retention and metabolism of intracisternally administered H³-norepinephrine and to measure the effect of this derivative on brain tyrosine hydroxylase. Experiments were performed on animals with or without pargyline pretreatment. Our data further support the view that 6-hydroxydopamine can produce selective degeneration of central catecholamine-containing neurons.

METHODS. Male Sprague-Dawley rats (140–180 g) were given from 25 to 200 μ g of 6-hydroxydopamine hydrobromide intracisternally as described previously (Schanberg *et al.*, 1968). The compound was dissolved in Elliott's "B" solution (Baxter Laboratories, Inc., Morton Grove, Ill.) containing 1 mg/ml of ascorbic acid to prevent oxidation of the 6-hydroxydopamine; total volume administered was 25 μ l. In an attempt to decrease deamination of 6-hydroxydopamine (Breese *et al.*, 1969), some animals received 50 mg/kg of pargyline i.p. 30 minutes before the 6-hydroxydopamine injection. A second dose of 6-hydroxydopamine (200 μ g) was administered to a few animals seven days after the first injection; pargyline was administered with the first but not with the second dose. After the surgical procedure, each animal received 0.05 ml of benzathine penicillin G suspension i.m.

For the measurement of brain concentrations of norepinephrine, dopamine and serotonin, animals were killed by cervical fracture and decapitated at various times after injection. Brains were removed, rinsed in cold water, homogenized in 10 ml of ice-cold 0.4 N perchloric acid, frozen and analyzed within 24 hours. After thawing and centrifugation of the homogenate, an aliquot of the clear supernatant was adjusted to pH 8.6 with sodium hydroxide, and the catecholamines were adsorbed onto alumina (Woelm, neutral) by the method of Anton and Sayre (1962). After stirring,

the alumina was transferred to a chromatographic column and washed with 10 ml of 0.4 N sodium acetate solution (pH 8.6) and with 20 ml of glass-distilled water. The catecholamines were eluted from the alumina with 7 ml of 0.2 N acetic acid. Endogenous norepinephrine in the acetic acid eluate was assayed according to the method of Häggendal (1963) except that mercaptoethanol was substituted for British anti-lewisite. Dopamine was assayed according to the method of Anton and Sayre (1964). Values for endogenous amines were uncorrected for recovery which averaged 89% for norepinephrine and 84% for dopamine. Brain serotonin was isolated from an aliquot of the supernatant by the method of Bogdanski *et al.* (1956) and measured by the fluorescent method of Snyder *et al.* (1965).

Some animals were given norepinephrine-H³ (5 μ c, 7.1 c/mmol) intracisternally in a volume of 25 μ l. After the procedure outlined above for killing and homogenization, labeled norepinephrine and its metabolites were isolated with the method of Schanberg *et al.* (1967). When tyrosine-H³ (34.7 c/mmol) was injected either intracisternally (15 μ c) or i.v. (40 μ c), the animals were sacrificed one hour later; radioactive norepinephrine and dopamine formed from tyrosine-H³ were isolated according to the method of Sedvall *et al.* (1968). Less than 0.5% dopamine was noted in the norepinephrine fraction and *vice versa*. Radioactive amine values were uncorrected for recovery which was 80% for norepinephrine and 70% for dopamine. All radioactivity was measured by scintillation spectrometry; an internal standard of toluene-H³ was used to correct for counting efficiency.

Dissection of brain parts was performed on a moistened filter paper placed on a chilled glass plate. The term "brainstem" as used in this manuscript refers to the area of brain caudal to a cut made behind the superior colliculi after removal of the cerebellar cortices. The term "caudate" refers to that area of brain which lies adjacent to the lateral ventricle and is bounded by the radiation of the corpus callosum. Access to the caudate mass, which contained portions of the globus pallidus, was obtained through the lateral ventricle after hemisection of the whole brain. After removal of the caudate, the remainder of the brain tissue was assayed and is referred to as "rest of brain."

Tyrosine hydroxylase was isolated from whole brain and brain parts according to the method of Musacchio *et al.* (1969) except that the enzyme was precipitated from a 1:1 mixture of sample and ammonium sulfate solution. Enzyme activity was determined by measuring the formation of tritiated water from 3,5-ditritiotyrosine in a modified procedure of Nagatsu *et al.* (1964). The incubation mixture for whole brain, rest of brain and

brainstem consisted of tyrosine (100 μmol), 2.9×10^6 cpm of ditritietyrosine, 0.5 μmol of 2-amino-4-hydroxy-6,7 dimethyltetrahydropteridine in 0.1 ml of 1 M mercaptoethanol and 0.5 ml of

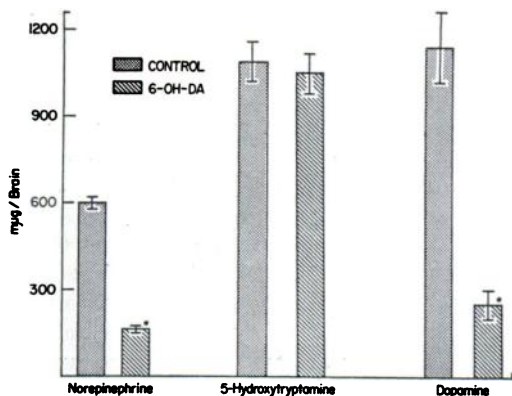


Fig. 1. Effect of 6-hydroxydopamine (6-OH-DA) on brain norepinephrine, 5-hydroxytryptamine and dopamine. Animals pretreated with pargyline received 200 μg of 6-hydroxydopamine intracisternally and were killed 21 days later. Each bar is the mean of 8 to 16 animals. Vertical bars indicate S.E.M. *, $P < .001$.

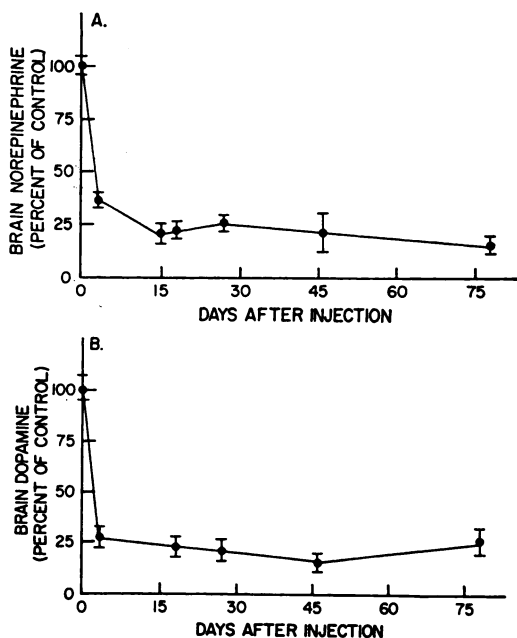


Fig. 2. Effect of 6-hydroxydopamine on brain norepinephrine (A) and dopamine (B) at various times after injection. Animals pretreated with pargyline received 200 μg of 6-hydroxydopamine and were killed at various times after this injection. Control values for norepinephrine and dopamine were 605 ± 12.2 and 1136 ± 58 μg , respectively. Vertical bars indicate S.E.M. Each value represents six to eight determinations.

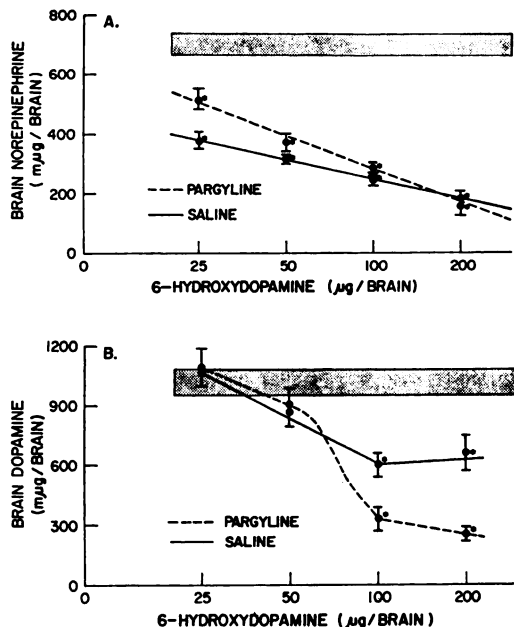


Fig. 3. Effect of pargyline on brain depletion of catecholamines (A and B) by 6-hydroxydopamine. Various doses of 6-hydroxydopamine were administered intracisternally to animals with or without pargyline pretreatment. Animals were sacrificed 16 or 21 days later. Stippled area indicates control values \pm S.E.M. of 20 animals. Each point indicates the mean of 6 to 12 animals; vertical bar indicates the S.E.M. *, $P < .01$.

enzyme preparation. For measurement of activity in caudate, half quantities of each reactant were used with the final volume measuring 0.5 ml. Incubation was at 37°C for 30 minutes. The reaction was terminated by the addition of 0.25 ml of 6% trichloroacetic acid. The mixture was centrifuged and the supernatant placed on a column of Dowex-50 (NH_4^+) (3 by 0.8 cm). The effluent and one wash were combined and mixed with 10 ml of phosphor-Triton (1:1) and counted. Monoamine oxidase activity was measured according to the method of Wurtman and Axelrod (1964).

The 6-hydroxydopamine HBr was purchased from Regis Chemical Company (Chicago, Ill.) and used without further purification. Pargyline was kindly supplied by Abbott Laboratories (North Chicago, Ill.) H^3 -tyrosine (34.7 c/mmol), H^3 -norepinephrine (7.1 c/mmol) and C^{14} -tryptamine (5 mc/mmol) were purchased from New England Nuclear Corporation (Boston, Mass.). All doses of the compounds used in this study are expressed as their free base.

RESULTS. *Effect of 6-hydroxydopamine on brain norepinephrine, dopamine and 5-hydroxytryptamine.* After the intracisternal injection of

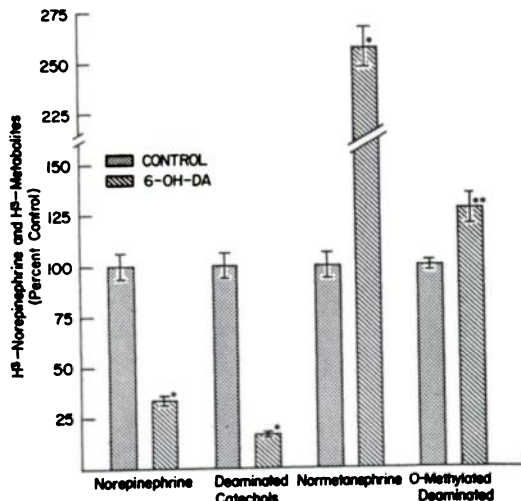


FIG. 4. Effect of 6-hydroxydopamine (6-OH-DA) on the metabolism of intracisternally administered norepinephrine- H^3 . Two doses of 6-hydroxydopamine (200 μ g) were administered: the first was injected to pargyline-treated animals 21 days before sacrifice and the second dose was administered 7 days after the first dose. Animals were sacrificed 2 hours after the intracisternal injection of H^3 -norepinephrine (5 μ c). Results are expressed as percentage of control \pm S.E.M. Each value represents the mean of at least 10 determinations. Control mean norepinephrine- H^3 = 1000 m μ c/brain; normetanephrine- H^3 = 136 m μ c/brain; H^3 -deaminated catechols = 3 m μ c/brain; labeled O-methylated deaminated metabolites = 700 m μ c/brain. *, $P < .001$; **, $P < .05$.

200 μ g of 6-hydroxydopamine in animals pretreated with pargyline, brain norepinephrine and dopamine were reduced without effecting brain serotonin (fig. 1). The depleting effect on brain norepinephrine and dopamine of a single 200 μ g injection of 6-hydroxydopamine after monoamine oxidase inhibition was noted as early as 3 days and persisted for as long as 78 days (fig. 2).

Effect of pargyline pretreatment on the reduction of brain catecholamines by 6-hydroxydopamine. The administration of 6-hydroxydopamine intracisternally was found to lower whole brain norepinephrine significantly at all doses administered irrespective of the presence or absence of pargyline pretreatment (fig. 3). However, at the lowest dose of 6-hydroxydopamine the administration of pargyline was found to inhibit significantly ($P < .01$) the depleting effect of 6-hydroxydopamine upon brain norepinephrine. The effect of 200 μ g of 6-hydroxydopamine in pargyline-treated animals was not significantly

different from that in 6-hydroxydopamine-treated animals which did not receive pargyline. Thus, pargyline pretreatment does not appear to offer any advantage in the 6-hydroxydopamine-induced depletion of brain norepinephrine. With or without pargyline, heart norepinephrine was not affected by intracisternally administered 6-hydroxydopamine.

In contrast to the pargyline effect on norepinephrine depletion by 6-hydroxydopamine, a quite different depleting pattern was noted on dopamine (fig. 3). Without pargyline, 6-hydroxydopamine caused a dose-dependent reduction of dopamine from 25 to 100 μ g. The lower doses of 6-hydroxydopamine did not cause a significant depletion of dopamine in either the pargyline or nontreated animals. However, 6-hydroxydopamine administered at the 100 μ g and 200 μ g doses to pargyline-treated animals had marked effects on brain dopamine, depleting the amine 66 and 76%, respectively. Animals which did not receive pargyline before intracisternally administered 6-hydroxydopamine had their brain dopamine reduced approximately 40%.

Metabolism of intracisternally administered H^3 -norepinephrine in 6-hydroxydopamine-treated rats. Two hours after the intracisternal administration of H^3 -norepinephrine, labeled norepinephrine in brain tissue was found to be reduced by 76% (fig. 4). At the same time, H^3 -normetanephrine was increased 264% and deaminated catechols were reduced by 83%. The O-methylated deaminated products of labeled norepinephrine were only slightly elevated in 6-hydroxydopamine-treated animals.

Formation of labeled norepinephrine and dopamine from H^3 -tyrosine. After the administration of radioactive tyrosine, labeled dopamine and norepinephrine are formed (Sedvall et al., 1968). Whether the label tyrosine was administered i.v. or intracisternally, animals treated with 6-hydroxydopamine were found to have reduced amounts of labeled dopamine as well as norepinephrine when compared to controls (table 1). In an attempt to determine if 6-hydroxydopamine might be interfering with the storage of amines but not synthesis, animals given H^3 -tyrosine i.v. were pretreated with pargyline in an attempt to spare catecholamines which might be synthesized. Nevertheless, results by the two routes of administration were

TABLE 1

Effect of 6-hydroxydopamine on the synthesis of H³-catecholamines from H³-tyrosine^a

Tyrosine Administration	Treatment	H ³ -Tyrosine		H ³ -Norepinephrine	H ³ -Dopamine
Intravenous ^b	Saline	243,000 ± 13,000		710 ± 70	565 ± 40
	6-Hydroxydopamine	266,000 ± 16,000		130 ± 25 ^c	140 ± 25 ^c
Intracisternal	Saline	168,000 ± 9,500		940 ± 100	555 ± 51
	6-Hydroxydopamine	174,000 ± 17,000		218 ± 34 ^c	192 ± 13 ^c

^a Results expressed as counts per minute per brain and are the mean ± S.E.M. of 6 to 12 determinations. Rats received 40 μg of H³-tyrosine i.v. or 15 μg intracisternally 21 days after the intracisternal injection of 6-hydroxydopamine (200 μg, 2 doses). Animals were sacrificed 1 hour after the labeled tyrosine.

^b Animals received pargyline (50 mg/kg) 30 minutes before the administration of H³-tyrosine.

^c P < .001.

comparable. After i.v. injection of H³-tyrosine, tritium-labeled norepinephrine formed was reduced 82% while H³-dopamine formed was reduced 75%. The amount of radioactive tyrosine found in brain was not significantly different from its corresponding control.

Effect of 6-hydroxydopamine on brain tyrosine hydroxylase and monoamine oxidase activity. In animals treated with 6-hydroxydopamine in combination with pargyline, whole brain tyrosine hydroxylase activity *in vitro* was found to be decreased by approximately 80% (table 2). This was in close agreement with the depletion of whole brain catecholamines (fig. 1; table 2). A comparable loss of tyrosine hydrox-

ylase activity and norepinephrine depletion was also observed in brainstem. Tyrosine hydroxylase activity in the caudate, normally an active region (McGeer *et al.*, 1967), was markedly reduced in 6-hydroxydopamine-treated rats. Dopamine concentration in the caudate of animals similarly treated was reduced by some 81% (table 2). Tyrosine hydroxylase activity in brain portions remaining after removal of the caudate was approximately 20% of control with catecholamine content being altered proportionally. Brain monoamine oxidase showed no decrease in whole brain or in brain parts examined (table 2).

DISCUSSION. The reports (Tranzer and

TABLE 2

Effect of 6-hydroxydopamine (6-OH-DA) on brain catecholamines and tyrosine hydroxylase activity^a

Brain Part ^b	Norepinephrine		Dopamine		Tyrosine Hydroxylase		Monoamine Oxidase	
	Control	6-OH-DA	Control	6-OH-DA	Control	6-OH-DA	Control	6-OH-DA
	μg/g		μg/g		μmol/mg/hr		μmol/mg/hr	
Whole Brain	330 ± 9.5 (25)	74 ± 4 ^c (25)	304 ± 13 (25)	55 ± 4 ^c (25)	4.33 ± 0.26 (8)	0.96 ± 0.16 ^c (8)	1.11 ± 0.10 (8)	1.09 ± 0.10 (8)
Brainstem	450 ± 18 (3)	91 ± 6 ^c (3)			6.26 ± 0.55 (8)	1.30 ± 0.45 ^c (8)	1.46 ± 0.12 (8)	1.41 ± 0.15 (8)
Caudate	174 ± 20 (4)	46 ± 15 ^c (4)	5880 ± 203 (9)	1093 ± 33 ^c (8)	20.7 ± 2.10 (14)	2.53 ± 0.20 ^c (10)	1.40 ± 0.15 (8)	1.41 ± 0.14 (8)
Rest of brain	319 ± 18 (5)	79 ± 10 ^c (5)	296 ± 23 (5)	140 ± 12 ^c (5)	4.33 ± 0.24 (8)	0.88 ± 0.18 ^c (8)	1.14 ± 0.11 (8)	1.12 ± 0.10 (8)

^a Each value represents the mean ± S.E.M.; numbers in parentheses indicate the number of animals per group. Brain dissections described in METHODS.

^b Animals in which whole brain and brainstem were assayed were given 2 doses of 6-hydroxydopamine (200 μg) as described in METHODS. Animals in which the caudate and rest of brain were assayed received 200 μg of 6-hydroxydopamine with pargyline. All animals were sacrificed 14 days after the initial treatment.

^c P < .001.

Thoenen, 1968; Malmfors and Sachs, 1968) that 6-hydroxydopamine caused a chemical sympathectomy peripherally prompted several laboratories to investigate whether this compound would display the same neurotoxicity toward central catecholamine neurons (Uretsky and Iversen, 1969; Groppetti *et al.*, 1969; Traylor and Breese, 1970). Uretsky and Iversen (1969) have found brain norepinephrine to be lowered for as long as 32 days, thus lending strong support to the view that noradrenergic degeneration could be induced by 6-hydroxydopamine. The present study shows that depletion of brain catecholamines persists at 78 days. With the use of electron microscopy, Bloom and co-workers (1969) have confirmed the presence of degenerating norepinephrine neurons within the central nervous system after administering 6-hydroxydopamine intracisternally. Several reports have indicated that sympathetic denervation decreases tyrosine hydroxylase activity in peripheral organs with adrenergic innervation (Sedvall *et al.*, 1967; Mueller *et al.*, 1969). Therefore, the marked reduction of tyrosine hydroxylase activity in brain (table 2) and the decreased norepinephrine synthesis from tyrosine (table 1) after 6-hydroxydopamine treatment not only add support to the view that noradrenergic neuronal degeneration occurs but also suggests that destruction is quite widespread.

Bloom and associates (1969) recently reported that norepinephrine neurons were more sensitive to depletion by 6-hydroxydopamine than dopaminergic nerve cells. The results of this present study are in full agreement with these earlier results (fig. 3). This observation might have been expected because of the report that dopaminergic neurons are resistant to depletion by intracisternally administered amphetamine derivatives which lower brain norepinephrine levels significantly (Breese *et al.*, 1970). Preliminary evidence indicates that a multiple injection sequence of lower doses of 6-hydroxydopamine may deplete norepinephrine to an even greater extent than a single injection with minimal influence on brain dopamine levels (unpublished data).

The study of the effects of 6-hydroxydopamine peripherally indicated that selective degeneration of adrenergic nerves occurred only in the distal part of the neuron, but not in the perikaryon (Tranzer and Thoenen, 1968). In brain,

Bloom and associates (1969) found depletion of norepinephrine by 6-hydroxydopamine to be greatest in areas containing axons and nerve terminals and least in those areas which contained biogenic amine cell bodies. Whereas electron microscopic evaluation of fiber endings would strongly suggest that 6-hydroxydopamine eliminates norepinephrine-containing terminals, the morphologic evidence concerning the fate of the cell bodies is not yet available (Bloom *et al.*, 1969). In the present study, all brain areas seem to be influenced uniformly by the neuronal toxicity of 6-hydroxydopamine. Whether this increased effect may be due to adrenergic cell body destruction or to an increased effectiveness on fiber terminals was not established. Whatever may be the case, it is clear that the effects are quite prolonged (fig. 2), suggesting that axonal regeneration does not occur within the central nervous system as previously reported for the periphery (Thoenen and Tranzer, 1968).

After the monoamine oxidase inhibitor pargyline, 6-hydroxydopamine (200 μg) was found to decrease not only brain norepinephrine but also dopamine (fig. 1). Early observations in our laboratory had indicated that brain dopamine was only moderately altered by 6-hydroxydopamine. With this in mind, it was reasoned that taking advantage of the fact that amines pass slowly from the central nervous system after monoamine oxidase inhibition (Schanberg, *et al.*, 1968; Breese *et al.*, 1969) this minor effect of 6-hydroxydopamine could be amplified. As shown in figure 1, this procedure has permitted consistent depletion of brain dopamine as well as norepinephrine (Breese *et al.*, 1970). Furthermore, the results of the present study and the recent work of Uretsky and Iversen (1970) emphasize that dopaminergic neurons are not only depleted but are probably affected by the neurotoxicity of 6-hydroxydopamine. This is exemplified by the prolonged depletion of dopamine (fig. 2), by lack of *in vivo* synthesis of labeled dopamine from H^3 -tyrosine (table 1) and by the reduction of tyrosine hydroxylase activity in the basal ganglia, (table 2) well known for its high dopamine content (Carlsson, 1959). In further support of this view, F. E. Bloom (personal communication) has recently found degenerating fibers in the caudate of pargyline-treated animals which have received 6-hydroxydopamine intracisternally.

After the administration of H^3 -norepinephrine the amount of labeled normetanephrine isolated from brains of 6-hydroxydopamine-treated animals was increased considerably over levels in control brains. This suggests that the enzyme catechol-O-methyltransferase is not altered by 6-hydroxydopamine treatment. The reduction of deaminated catechols and the increased amounts of normetanephrine observed after 6-hydroxydopamine treatment is similar to but greater than that reported to occur after desipramine (Schanberg *et al.*, 1967; Schildkraut *et al.*, 1967). These results are probably linked to the decreased neuronal uptake observed to occur after both desipramine and 6-hydroxydopamine (Schanberg *et al.*, 1967, fig. 2).

Selective elimination of central norepinephrine or norepinephrine and dopamine neurons should aid significantly in the effort to define the central role of these amines. However, the usefulness of 6-hydroxydopamine as a pharmacologic agent for evaluating the central role of catecholamines has yet to be established. In view of the central function attributed to these amines, it has been somewhat surprising to find that the general appearance of rats "centrally sympathectomized" with 6-hydroxydopamine has thus far been grossly indistinguishable from that of untreated controls except for a slight decrease in body weight and a lack of self-grooming. Certainly these animals do not display a classical "reserpine syndrome" (Bein, 1953). Experiments are presently underway to evaluate further the role of 6-hydroxydopamine as a specific tool for the study of central adrenergic mechanisms.

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