The disc-electrophoretic patterns of 30*S*-15 from both sources reveal one component, and the material is homogeneous in sedimentation equilibrium experiments. Experiments in which total SmS and SmD proteins are combined in different ratios in the reconstitution mixture yield a population of ribosomes with intermediate phenotypes. The variation in degree of SmS character of these particles parallels the relative amount of SmS protein in a manner which is consistent with there being one protein per 30*S* particle that determines the Sm phenotype of the ribosomes. Preliminary experiments by Craven (*8*) which are designed to determine the nature of the chemical alteration of 30*S*-15 SmD have confirmed the conclusion that our active samples are at least 90 percent chemically homogeneous.

The altered protein of Sm resistant (SmR) ribosomes also corresponds to 30*S*-15. When a sample of our 30*S*-15 SmS was used by Ozaki *et al.* (*5*), it was found that the SmR phenotype of reconstituted ribosomes could be converted to the SmS phenotype if excess 30*S*-15 SmS is present in the reconstitution mixture. Therefore, mutational events at a single genetic locus (*9*) convert SmS ribosomes to the SmR or SmD phenotype by affecting a single protein. However, we are not certain that this genetic locus is the primary structure determinant of 30*S*-15. The only genetic locus which is known to determine the primary structure of a ribosomal protein is that determining the structure of 30*S*-8, the K character (*10*). It is likely that the alterations of 30*S*-15 which give rise to the different Sm phenotypes are alterations in the primary structure of this ribosomal protein.

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Lesions of Central Norepinephrine Terminals with 6-OH-Dopamine: Biochemistry and Fine Structure

Abstract. *Intracisternal injections of 6-hydroxydopamine produce rapid and long-lasting depletion of brain catecholamines without effects on serotonin concentrations. Depletion of norepinephrine is greatest in areas containing only nerve terminals and axons and least in areas containing monoamine cell bodies. The norepinephrine loss is accompanied by electron microscopic evidence of nerve terminal degeneration and decreased turnover. Dopamine loss is less marked and is not accompanied by degeneration or alteration of turnover rate.*

A classical approach for elucidating the function of a given neuronal population is to study the changes following their biochemical inactivation or anatomical lesioning. For the serotonin-containing nerve cells of the central nervous system this approach is typified by either electrolytic lesioning of the raphe nuclei (*1*) or inhibition of serotonin synthesis with *p*-chlorophenylalanine (*2*). Provided its accuracy and selectivity can be anatomically established, the lesioning approach has the inherent advantage that the nerve terminals undergo permanent orthograde degeneration. Both the lesioning and the biochemical approaches appear to be impractical for analysis of catecholamine nerve terminals, since the cell bodies of these monoaminergic neurons are diffusely placed in the brain stem reticular formation (*3*) and the inhibitors of norepinephrine synthesis also inhibit dopamine formation (*4*). Moreover, it is impossible to block norepinephrine biosynthesis selectively in the central nervous system without causing peripheral autonomic inhibition (*5*). The norepinephrine congener 6-hydroxydopamine (6-HDM) provides reversible chemical sympathectomy of certain portions of the peripheral sympathetic nervous system (*6, 7*) associated with ultrastructural toxic degeneration of the axons and nerve terminals (*8*). When administered intracisternally, 6-HDM will produce long-lasting depletion of brain norepinephrine levels and inhibition of uptake mechanisms (*9*). We now report that 6-HDM administered intracisternally produces selective effects on norepinephrine-containing nerve terminals accompanied by fine structural evidence of degeneration.

Sprague-Dawley male rats (NIH and Zivic Miller, Pittsburgh, strains), 150 to 300 g, were given 6-HDM by either intraventricular or intracisternal injections according to various dose schedules. In the most extensive series, rats lightly anesthetized with ether were given 200 μg of 6-HDM (in 50 μl of 0.85 percent NaCl containing 0.001 percent $Na_2S_2O_5$) followed at 72 and 96 hours by supplemental intracisternal injections of 50 μg each. Temporal assays of whole-brain monoamine levels were performed by modifying previously reported techniques (*10–15*) and were compared with control animals receiving only intracisternal injections of $Na_2S_2O_5$ in NaCl diluent.

Brains were frozen immediately after removal and stored frozen until analysis. Homogenization of tissues and their preparation for Dowex columns (*10*) was as previously described (*11*). The effluent and the eluate of a successive rinse with 4 ml of 0.1*M* Na acetate buffer, *p*H 4.5, with 0.1 percent disodium ethylenediamine tetraacetate (EDTA) were collected for tyrosine analysis (*12*). A rinse with 10 ml of 0.1*M* Na acetate buffer, *p*H 6, with 0.1 percent disodium EDTA followed. A successive wash with 5 ml of 0.4*N* HCl, containing 0.5 mg of $Na_2S_2O_5$ per milliliter, was discarded, but the following 10-ml wash with the same solution was collected for the norepinephrine analysis (sample A). The columns were rinsed with 4 ml of 4*N* HCl with 0.5 mg of $Na_2S_2O_5$ per milliliter, which was collected for dopamine analysis (sample B), and then rinsed with H_2O (2 ml). Finally, the serotonin was eluted with 4 ml of

0.5*M* Na_3PO_4 solution, which was collected (sample C) in a tube containing 15 ml of *n*-butanol, 0.5 g of Na_2HPO_4, and 2 ml of borate buffer, *p*H 10. The assay of serotonin was carried out as suggested by Snyder *et al.* (*13*). Eight milliliters of sample A and 3 ml of sample B were transferred to a 13-ml centrifuge tube containing 500 mg of Al_2O_3 prepared as suggested by Crout (*14*) and were washed with 0.1*M* disodium EDTA. After the addition of 2.5 ml of 3*M* tris (hydroxymethylamino methane solution) to sample A and 10.5 ml of the same buffer to sample B, the samples were processed as described previously for catecholamine assay (*15*). The 6-HDM did not interfere with the assay of norepinephrine and dopamine because it does not appear in the Al_2O_3 eluate used to assay norepinephrine and dopamine.

Internal standards of norepinephrine, tyrosine, dopamine, and serotonin were added to tissues of normal rats and processed simultaneously with tissues of animals treated with 6-HDM. The recoveries obtained were: tyrosine, 70 percent; norepinephrine, 85 percent; dopamine, 70 percent; and serotonin, 65 percent. Radioactivity of tyrosine, norepinephrine, dopamine, and serotonin was assayed by scintillation spectrometry in the same fractions used for fluorometric assay. The specific activity of the amines is calculated after correcting for the loss of the radioactive atoms resulting from the metabolism of the amino acid.

The three-dose schedule of 6-HDM causes a decline in whole-brain norepinephrine to a maximum of 57 percent of control concentration after 4 days. At this time the decline in dopamine concentrations is less profound, and by the seventh postinjection day the dopamine level is 70 percent of controls. There is no detectable decline in whole-brain serotonin concentration.

In the experimental analysis of 6-HDM effects upon the sympathetic nervous system, the toxic degenerative effects were restricted to the axons and nerve terminals. To pursue this possibility within the central nervous system, we repeated the previous experiments; the brains were now subdivided into regions thought to contain varying proportions of catecholamine cell bodies and terminals (*3*).

When expressed in terms of regional concentrations (Table 1), the decline in norepinephrine appears to progress to virtual completeness in the cerebellum and tele-diencephalon; these areas are presumably free of catecholamine cell bodies, with the exception of the arcuate nucleus (*3*). The tele-diencephalic dopamine, however, declines only to 50 percent of control levels. On the other hand, in the monoamine cell body areas of the brain stem, there is progressive but incomplete attenuation of norepinephrine concentration. In the subdivided brain the serotonin levels of brain stem are also transiently decreased soon after the injections of 6-HDM, but recover as the norepinephrine continues to decline.

These biochemical observations suggested that the norepinephrine-nerve terminal areas were the most likely sites of 6-HDM effects. Accordingly, ultrastructural analyses of the rat brains were undertaken in parallel experiments. Random animals from the same series of 6-HDM treatments were perfused with glutaraldehyde, exposed to osmium tetroxide, and otherwise routinely prepared for electron microscopy. In the paraventricular hypothalamus, a norepinephrine-rich area previously investigated in detail (*16*), typical ultrastructural evidence of degeneration (*17*) could be observed at 7, 14, and 21 days after the 6-HDM (Figs. 1 and 2). In this region, the reaction consisted first of increased electron opacity of the terminal axoplasm and loss of synaptic vesicle distinctness. Subsequently, axonal engulfment by glia and profuse multilamellar envelopment of degenerating processes was also found. These nerve terminals almost universally exhibited the large granular vesicles, thought to be related to catecholamine fibers in this region (*16*). Apparent degenerating fibers are also seen on neurons of the dorsal raphe nucleus and on Purkinje cell

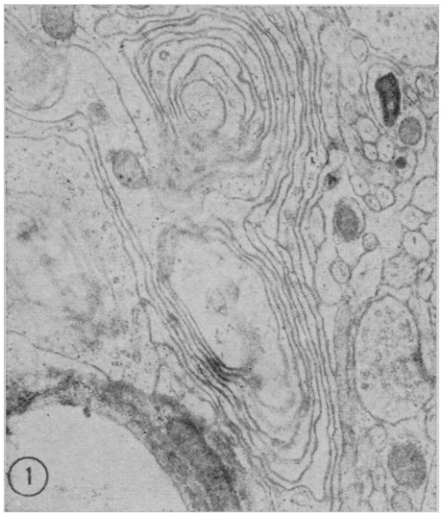
Table 1. Mean monoamine concentrations (μg/g $\pm$ S.E.) in tele-die ncephalon, brain stem, and cerebellum of rats injected intracisternally with 6-OH-dopamine (6-OHDM). Two hundred micrograms of 6-OHDM were injected intracisternally and two additional doses of 50 μg each followed after 72 and 96 hours respectively. Each concentration value shown is the mean of at least four assays. Monoamines: 5-HT, serotonin; NE, norepinephrine; DM, dopamine.

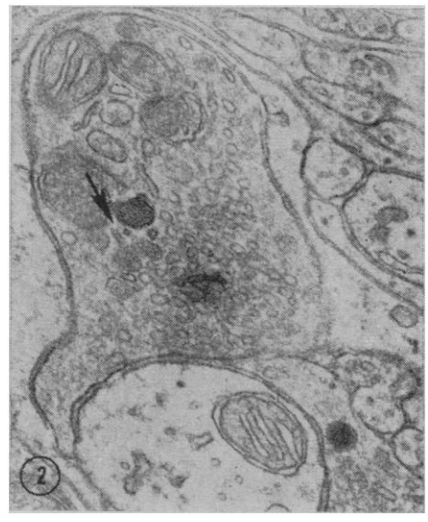
Days after last 6-OHDM injection	In tele-diencephalon			In brainstem		In cerebellum	
	5-HT	NE	DM	5-HT	NE	5-HT	NE
0	0.57 $\pm$ 0.015	0.42 $\pm$ 0.015	1.11 $\pm$ 0.12	0.95 $\pm$ 0.16	0.59 $\pm$ 0.035	0.46 $\pm$ 0.013	0.15 $\pm$ 0.013
5	0.57 $\pm$ 0.025	0.12 $\pm$ 0.0043	0.76 $\pm$ 0.17	0.49 $\pm$ 0.093	0.42 $\pm$ 0.037		0.067 $\pm$ 0.0044
7	0.58 $\pm$ 0.027	0.18 $\pm$ 0.015	0.96 $\pm$ 0.11	0.58 $\pm$ 0.039	0.41 $\pm$ 0.025	0.46 $\pm$ 0.062	0.052 $\pm$ 0.0019
21	0.49 $\pm$ 0.014	0.13 $\pm$ 0.0056		0.73 $\pm$ 0.034	0.35 $\pm$ 0.040	0.54 $\pm$ 0.053	0.072 $\pm$ 0.0081
28	0.58 $\pm$ 0.047	0.10 $\pm$ 0.0083	0.81 $\pm$ 0.17	0.84 $\pm$ 0.079	0.28 $\pm$ 0.047	0.64 $\pm$ 0.16	Undetectable

Table 2. Conversion of ^{3}H-tyrosine into ^{3}H-NE and ^{3}H-DM in rats injected with 6-HDM and infused with ^{3}H-tyrosine. The 6-HDM was injected according to the schedule shown in Table 1, 28 days before infusion. L-Tyrosine-3,5-^{3}H (specific activity 3.5 c/mmole) was infused intravenously for 45 minutes at the constant rate of 1.7 mc kg^{-1} hr^{-1}. Values are means $\pm$ S.E. Numbers of animals are shown in parentheses.

Treatment group	Plasma tyrosine (mμmole/ml)	Plasma ^{3}H-tyrosine (dpm/mμmole)	Brain NE (mμmole/g)	Brain ^{3}H-NE (dpm/mμmole)	R*	Brain DM (mμmole/g)	Brain ^{3}H-DM (dpm/mμmole)	R*
6-HDM (6)	40.58 $\pm$ 7.89	7679 $\pm$ 783	1.36 $\pm$ 0.14†	2325 $\pm$ 329	0.82	4.07 $\pm$ 0.36†	938 $\pm$ 90†	0.99
Controls (3)	47.68 $\pm$ 6.40	6659 $\pm$ 1518	2.61 $\pm$ 0.14	1967 $\pm$ 368	1.52	6.21 $\pm$ 0.32	595 $\pm$ 71	1.10

* $R = \frac{\text{dpm/m}\mu\text{mole of amine} \times 2}{\text{dpm/m}\mu\text{mole of } ^3\text{H-plasma tyrosine}} \times$ mμmole of amine/g. This value indicates turnover rate and is valid only for comparative purposes. † $P < .05$.





Figs. 1 and 2. Electron micrographs of the paraventricular hypothalamic area, illustrating the major manifestations of the degenerative process following intracisternal injection of 6-hydroxydopamine. Tissues were fixed on day 5 of the three-dose schedule (see text) by perfusion with glutaraldehyde and exposure to osmium tetroxide; after embedment in Maraglas, thin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM 9A. In Fig. 1, parts of four degenerating axons are enveloped in multiple lamellae of glia membranes at the periphery of a small capillary. The small axons and nerve endings at right appear normal (× 20,200). Fig. 2. Two axodendritic junctions in the same region, illustrating the increased electron-opacity of the terminal axoplasm, and the loss of structural integrity of both the small vesicles and the large granular vesicles (arrow) (× 60,700).

dendrites in the cerebellar cortex where norepinephrine afferents may be present.

However, despite the decrease in tele-diencephalic dopamine, we were unable to find any apparent changes in caudate nucleus nerve terminals or axons, which suggests that the depletion of this catecholamine occurs by a different mechanism than that of norepinephrine. The data listed in Table 2 indicate further substantive differences between the effects of 6-HDM on the two catecholamines: the turnover rate of brain norepinephrine is reduced in rats treated with 6-HDM, while dopamine turnover is unchanged. Possibly 6-HDM in these doses simply acts to deplete dopamine storage without influencing overall synthesis, while in norepinephrine-containing neurons, after the presumed degeneration of their terminals, the residual turnover of amine is much slower. In the experiment reported in Table 2, L-tryptophan 3-^{14}C (specific activity 22.7 mc/mmole) was infused at a constant rate (120 μc kg^{-1} hr^{-1}) simultaneously with ^{3}H-tyrosine for 45 minutes. The turnover rate calculated from the conversion of plasma ^{14}C-tryptophan into brain serotonin was equal in control and 6-HDM–treated animals.

From these results, 6-HDM would indeed appear to produce elimination of central norepinephrine-containing nerve terminals. Without specific fine structural data on catecholamine perikarya [only the locus coeruleus is easily located (*3*)] the apparently predominant biochemical and morphological damage to nerve terminals could be attributed to the unequal concentrations of drug entering the brain after intracisternal injection. The "terminal" areas analyzed here also happen to be close to ependymal or pial surfaces. Although the primary effects are on norepinephrine, dopamine levels are depressed initially, and regional serotonin levels are also influenced. If central catecholamine perikarya are relatively undamaged by 6-HDM, their failure to regenerate axons may reveal further differences between central and peripheral neurons. The dosage schedule and route of administration are important variables for the selectiveness of the 6-HDM effects. For example, we have found that one dose of 300 μg is less depletive than the split dosage schedule and that intraventricular injections have different relative effects on dopamine levels. Furthermore, direct interstitial injection of 6-HDM into the dopamine-containing neurons of the substantia nigra (*18*) clearly produces loss of nigral and caudate dopamine, indicating the dose-dependency of this effect.

A method for selective elimination of catecholamine-containing neurons would significantly contribute to an understanding of their role in normal and pharmacologically modified behavior. In this regard we find that rats treated with 6-HDM have normal ability to control temperature in hot or cold environments, have normal intake of food and water, and exhibit a normal hyperthermic response to small doses of *d*-amphetamine (1 mg/kg, intraperitoneally). Their main distinguishing features in our observations are an obvious lack of self-grooming, slightly increased aggressiveness when provoked, and a lack of hyperthermia (*19*) after implantation of morphine pellets. Moreover, when rats given 6-HDM and morphine were challenged with norallylmorphine (50 mg/kg, intraperitoneally) they failed to exhibit hypothermia. Chemical adrenolysis with 6-HDM appears to be a most promising tool for neuropharmacology, although sufficient data to use it effectively are still needed.

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