PHARMACOLOGICAL REVIEWS
Vol. 26, No. 3

Printed in U.S.A.

# Pharmacological Actions of 6-Hydroxydopamine

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## I. Introduction

In 1967 Tranzer and Thoenen made the remarkable discovery that an isomer of norepinephrine (NE) namely, 6-hydroxydopamine (6-OHDA), produced a destruction of the terminal ground plexus of peripheral sympathetic (noradrenergic) neurons. This phenomenon has since been termed a "chemical sympathectomy" (363, 364). It is a relatively selective effect, with cholinergic neurons, Schwann cells, glia, endothelial, and other cell types remaining virtually unaffected at the ultrastructural level (47, 363, 364). When the compound was injected into the brain, dopaminergic neurons were destroyed along with noradrenergic neurons (47, 368). Since central monoaminergic neurons are implicated in numerous different types of behavioral and learning processes, such findings of relatively selective morphological and biochemical alterations were of immense importance as an aid to subsequent studies.

There has been a flood of articles that describe various aspects of the pharmacological role of 6-OHDA, and these articles increase in geometric proportions each year. Because of the rapid accumulation of a broad expanse of knowledge on this pharmacological agent it was considered appropriate to review the literature and systematize the information on 6-OHDA. A recent general review (357) and a book containing communications of a symposium on 6-OHDA (263) are available.

Because of the large variations in the degree of degeneration of the terminal adrenergic fibers within the various organs, followed by reinnervation at different time intervals, some confusion exists as to the proper use of 6-OHDA. For example, destruction of cardiac adrenergic fibers may appear to be effectively accomplished when only small amounts of NE are measured in the heart. However, since preterminal fibers remain intact, regeneration of fibers to the pacemaker takes place relatively quickly while the NE content of the four chambers of the heart remains largely reduced. At this stage of our knowledge there seems to be a need for a survey of the literature with particular emphasis on the numerous variables used which are basic to the study of all drugs, *i.e.*, dose-response, time-course of depletion and recovery of amines, species variations, routes of administration, and dose schedules. It is hoped that a clear knowledge of this information will give direction to future investigations in this field.

This review is comprehensive for most of the literature through 1972, and includes

selected 1973 articles which aid to delineate the mechanisms of 6-OHDA.

## II. Historical Review

One of the major means of studying neuronal function in a particular organ or system has been the observation of functional impairment after removal of the neuronal input (68). Traditionally, this has been accomplished most easily by making lesions of nerve tracts or removing ganglia. However, many ganglia are difficult to dissect and too often different types of nerves are present in the same trunk. In the central nervous system (CNS) this procedure is nonspecific or impossible to accomplish, primarily because of the diffuse distribution of many nuclei and their respective nerve tracts.

A major contribution to the study of the sympathetic nervous system was the development of an antiserum to a specific nerve growth factor (NGF) (84, 244, 245). This antiserum, anti-NGF, when administered to newborn animals, selectively destroyed certain pre- and paraganglia. However, the sympathectomy was never complete. Approximately 5 to 10% of the sympathetic perikarya in paraganglia and about 15 to 20% of the cell bodies in prevertebral ganglia survived, while terminal ganglia found in the male and female genitourinary tract were unaffected. Thus, although anti-NGF was of immense pharmacological value, it was far from ideal in removing sympathetic function. Also, it had no effect on central catecholamine-containing neurons because of its inability to penetrate the blood-brain barrier, and therefore, provided no further advantage in studying partial central aminergic nerve involvement in behavior.

In 1959, Senoh *et al.* (317–320), while studying the enzymatic conversions of dopamine (DA) to NE in rat tissue homogenates, discovered and isolated the metabolite 6-OHDA. Actual identification of this compound was obtained by incubating tissues with mixtures of dopamine-$\beta,\beta$-$^{3}$H and dopamine-$\gamma$-$^{14}$C. The isolated 6-OHDA had an $^{3}$H/$^{14}$C ratio of 1 while newly formed NE had a ratio of only 0.5. The product $^{14}$C-6-OHDA also could be isolated in the urine of rats pretreated with marsilid, a monoamine oxidase inhibitor, after an injection of $^{14}$C-DA; this indicates similar conversion *in vivo* of DA to 6-OHDA.

The initial biological effects of 6-OHDA were reported by Porter *et al.* (293, 294) and Stone *et al.* (338, 339). Essentially, they demonstrated that 6-OHDA produced depletion of NE in hearts of mice and dogs, but the major significance of this event was that the duration of action was longer than for any other pharmacological agent known at that time. Physiologically active NE was released, as noted by the resultant sympathomimetic responses in dogs. It was also found at this time that certain antidepressants and several other agents were capable of antagonizing the effects of 6-OHDA, which indicates that uptake into the noradrenergic neuron was a necessary prerequisite. Laverty *et al.* (240) later showed that 6-OHDA was capable of producing depletion of NE stores of numerous tissues in several species of animals and that the NE content of brain remained unchanged except when 6-OHDA was injected into immature cats.

Shortly afterward Thoenen *et al.* (350, 351, 356) and Tranzer and Thoenen (362–364), while studying the ultramorphological localization of trihydroxyphenylethylamines which act as false neurotransmitters in sympathetic neurons, discovered that 6-OHDA caused an actual destruction of the terminal endings of sympathetic neurons. This effect accounted for the long-lasting NE depletion first shown by Porter *et al.* (294) and was accompanied by a marked decrease *in vitro* of $^{3}$H-NE uptake by various end organs of the rat. Malmfors and Sachs (262) showed that 6-OHDA produced a distinct alteration in adrenergic neurons stained for visualization by fluorescence microscopy. The pattern was unlike that observed previously by any other agent and

actually resembled the morphology seen after ligation or surgical lesioning of the nerve trunk.

Ungerstedt (368) showed that 6-OHDA would also destroy central catecholamine-containing neurons after direct injection into the parenchyma of the brain. Injection into one of the brain cavities also produced degeneration of noradrenergic neurons (28, 47) and this was accompanied by marked alterations of NE and DA levels in whole brain (377, 378) and brain regions (205). Not long after this, Angeletti and Levi-Montalcini (14–16) showed that sympathetic ganglia of neonatal mice and rats were susceptible to the destructive effects of 6-OHDA, unlike the situation noted in mature animals wherein the ganglia are fairly resistant to the effects of 6-OHDA.

With these studies as a nucleus a vast literature has evolved to confirm and extend these initial findings. The function of various end organs in the absence of sympathetic innervation and the role of central catecholamine-containing neurons in numerous behavioral processes has been studied avidly. Thus, 6-OHDA has proved to be invaluable as a pharmacological tool and has become one of the most widely used agents in experimental animal research.

## III. Temporal Sequence of Neuronal Interactions with 6-OHDA

Due to the large variety of biological effects produced by 6-OHDA in both the peripheral and central nervous system, a knowledge of the manner with which 6-OHDA interacts with neurons would lead to a better understanding of subsequent processes described later in this literature review. In this section, an attempt is made to characterize the molecular complexation of 6-OHDA with neuronal elements and to describe the resultant pharmacological responses.

Immediately after 6-OHDA is administered and comes in contact with catecholamine-containing nerves, a chain of events is initiated which may be interrupted at any number of points, depending on the dose. 6-OHDA is initially accumulated by an active uptake process of the catecholamine-containing neurons and is transported to intraneuronal sites where it can displace NE and act as a false neurotransmitter (356). When critical intraneuronal concentrations of 6-OHDA or metabolites are attained, then destructive processes begin and cellular enzymes (28, 47, 356) and energy-producing cytochromes or related elements of the respiratory transport chain (384, 386) are destroyed. At about this point, the nerve terminals lose their ability to conduct action potentials, but still have a relatively intact monoamine uptake mechanism (160, 161). Because of the internal destructive processes NE is released into the synaptic cleft, from where it can act at postjunctional receptor sites to elicit a host of sympathomimetic effects (338). After a period of time, if the nerve terminals are completely destroyed, there will be a marked reduction of NE, tyrosine hydroxylase activity, and monoamine uptake capacity in the tissues under study (205). Responses to tyramine will be abolished and cocaine will not potentiate effects of NE on the system either *in vivo* or *in vitro*, although responsiveness of the gland or smooth muscle to NE will be increased markedly because of the presynaptic denervation supersensitivity. With a longer interval a postsynaptic supersensitivity to NE may develop, so that effects of phenylephrine or isoproterenol may be potentiated. However, it should be noted that after a very high dose of 6-OHDA there may be destruction or alteration of the postsynaptic receptor site (160, 161).

Thus, the series of destructive events after 6-OHDA are fairly well described and evidence for each particular point will be ascribed to in the following discussion. The destructive process is permanent only in the sense that the terminal network cannot of itself overcome the effects of high doses of 6-OHDA. However, in the periphery the event is reversible in that new sympathetic terminal plexuses are formed from the rela-

tively intact preterminal processes, beginning within a matter of days. During the regenerative stage NE levels, enzyme levels, and uptake capacity approach pretreatment conditions. The terminals conduct action potentials and the supersensitivity diminishes. The entire process may take up to three months in the iris or as little as several weeks in the vas deferens.

### *A. Selectivity of Uptake of 6-OHDA into Catecholamine-containing Neurons*

In the peripheral nervous system, 6-OHDA appears to be taken up with a high degree of selectivity by noradrenergic neurons. Numerous electron microscopic studies have indicated that cholinergic neurons, nonadrenergic neurons, myelinated axons, smooth muscle cells, Schwann cells, and endothelial cells appear normal while noradrenergic neurons are in the process of degeneration (322, 356, 363, 364).

Ljungdahl *et al.* (219, 249) demonstrated by a combination of autoradiography and fluorescence microscopy that $^3$H-6-OHDA is accumulated with a high degree of selectivity by noradrenergic neurons. In the iris where cholinergic and adrenergic neurons often lie side by side and form interdigitating plexuses, $^3$H-6-OHDA ($10^{-6}$ M, 60 minutes) was shown *in vitro* to accumulate along the course of the fluorescent adrenergic terminals. In irides previously denervated of their sympathetic supply by pretreatment of mice with 6-OHDA (50 mg/kg 2 times i.v.) or by surgical denervation, only a diffuse distribution of grains was observed.

In other experiments the total accumulation of radioactivity in mouse irides and atria after incubation *in vitro* with $^3$H-6-OHDA ($10^{-6}$ to $10^{-4}$ M, 60 minutes) was depressed markedly in tissues that had been denervated of their sympathetic supply, the total amount being identical with that found in innervated tissues incubated with $^3$H-6-OHDA at 0°C or in the presence of desmethylimipramine (desipramine, Norpramin, Pertofran) (219, 221). Since desipramine and low temperatures inactivate the monoamine active uptake mechanism (21, 170, 202), it appears that 6-OHDA is actively taken up largely by noradrenergic neurons in control tissues.

With Michaelis-Menten kinetics, 6-OHDA was shown to act as a competitive inhibitor of NE at the NE uptake sites in mouse atria (222). The affinity of 6-OHDA for the uptake sites, however, is much less than that for NE, 5-hydroxydopamine (5-OHDA), DA, and metaraminol (Aramine), as determined by tissue/media ratios of the tritium atom (219, 222).

Stone *et al.* (338) showed that various tricyclic antidepressants including amitriptyline (Elavil), nortriptyline (Aventyl), protriptyline (Vivactyl), imipramine (Tofranil), and desipramine were capable of antagonizing the NE-depleting action of 6-OHDA (7 mg/kg i.p.) on mouse heart. A number of other studies have likewise confirmed the effectiveness of desipramine and imipramine in blocking the action of 6-OHDA on noradrenergic neurons in the mouse iris (49, 99, 221, 262) and atrium (221, 262), rat heart (93, 99), chick expansor secundariorum (37), and rat brain (59). Similarly, chlorpromazine (Thorazine, Largactil, Megaphen) (37, 59, 334), metaraminol (37), and cocaine (95) have also been shown to be effective, and it is likely that the above agents are acting primarily at the monoamine uptake site in the neuronal membrane, and competing with 6-OHDA, thereby blocking its uptake and subsequent accumulation intraneuronally. Methylphenidate (Ritalin), pipradrol, and tripelennamine (Pyribenzamine) also effectively block the actions of 6-OHDA on NE depletion (338), but whether this is primarily by an action on the uptake mechanism is not known.

### *B. False Neurotransmitter Role and Nondestructive Effects of 6-OHDA*

Once inside the neuron, 6-OHDA molecules accumulate in the amine-storage granules, as can be demonstrated by increased electron opacity under the electron

microscope (37, 83, 140, 361, 364). Studies on mouse atria incubated *in vitro* with $^{3}$H-6-OHDA ($10^{-6}$ M, 60 minutes), indicate that once inside the neuron, nearly 50% of the 6-OHDA may be accumulated in the amine storage granules, a process which can be partially but not totally blocked by reserpine-nialamide pretreatment. Thus, 6-OHDA appears to be capable of entering the amine binding granules to a limited extent by a reserpine resistant mechanism (170, 171). While $^{3}$H-6-OHDA is bound to the granules, $^{3}$H-NE content of the granule fraction is reduced markedly and the proportion of $^{3}$H-NE in the cytoplasmic fraction (high-speed supernatant) is increased slightly, although total content in this fraction is also reduced. Thus, 6-OHDA decreases both granule and cytoplasmic pools of endogenous NE, thereby accounting for decreased NE content of the tissue (218, 219, 221).

In low doses 6-OHDA appears to act as a false transmitter in noradrenergic neurons. In the isolated perfused cat spleen $^{3}$H-6-OHDA (0.3 mg/kg i.v., 2 hours) was liberated as a false neurotransmitter along with NE upon sympathetic nerve stimulation. Also, when administered in low doses (1 or 3 mg/kg i.v.) and measured after short intervals (30 minutes) the amount of $^{3}$H-amines retained in rat heart and spleen after $^{3}$H-6-OHDA was inversely proportional to the dose, further indicating a displacement mechanism. However, it is also possible for 6-OHDA to be accumulated without releasing NE, as the total content of both $^{3}$H-6-OHDA and endogenous NE is greater in rat heart after low doses of $^{3}$H-6-OHDA (1 or 3 mg/kg i.v.) than pretreatment levels of NE alone (356).

Bartholini *et al.* (28, 29) have shown that 6-OHDA (200 μg ivt., 2 or 5 days) is capable of producing marked depletion of catecholamine (CA) levels in rat whole brain in the absence of ultrastructural damage in the periventricularis or caudate nucleus. Thus, displacement mechanisms may be of major importance for the depleting action of 6-OHDA, particularly since an additional dose of 6-OHDA (200 μg ivt.) causes obvious degenerative changes in the absence of further marked catecholamine depletion. However, it is unlikely that the decrease in CA observed after the first dose of 6-OHDA is solely due to displacement, since biosynthesis of CA from $^{14}$C-tyrosine is reduced markedly at this time; this suggests functional impairment of the neuronal biosynthetic apparatus probably due to tyrosine hydroxylase inhibition since dihydroxylated deaminated metabolites are decreased, especially $^{14}$C-3,4-dihydroxyphenylacetic acid. Nevertheless, impaired storage or a displacement mechanism appears to be responsible in part for the decrease in catecholamines, since the level of phenolcarboxylic acids are unaltered in the brainstem when $^{14}$C-dopa is substituted for $^{14}$C-tyrosine (28). Also, in the hypothalamus, when the NE content is reduced by more than 50% after 6-OHDA (200 μg ivt., 2 days), tyrosine hydroxylase is near normal activity (29).

It has also been found that 6-OHDA appears to be accumulated in extraneuronal sites. When atria from mice treated with 6-OHDA (50 mg/kg 2 times i.v., 16-hour interval; sacrificed 2 hours after final dose) were incubated *in vitro* with $^{3}$H-6-OHDA ($10^{-6}$ M to $10^{-4}$ M) there was a concentration-dependent accumulation of the tritium label and the amount in the atria increased with increasing incubation time (222). In previous studies fluorescence microscopy has indicated a disappearance of noradrenergic terminal endings in mouse atria after identical treatment with 6-OHDA (221, 302). The significance of these results is not yet clear but it is possible that this phenomenon is related to the so-called uptake-2 process for accumulation of NE (202). However, one must also use caution in the quantification of these findings, since the tritium content was assumed to be equal to the $^{3}$H-6-OHDA content. It is quite possible that the tritium is present because of nonspecific covalent bonding to tissue of the spontaneous auto-oxidized by-products (quinones, *etc.*) of the 6-OHDA (see section XI). In

this case the tritium may be little related to an actual uptake process. Studies of 6-OHDA measurement specifically in the presence of selective uptake-1 and uptake-2 inhibitors would better describe the process. Nevertheless, the potential role of extraneuronal uptake of $^3$H-6-OHDA must be taken into account when discussing the uptake and accumulation of 6-OHDA by tissues.

### *C. Sympathomimetic Effects of 6-OHDA*

That 6-OHDA acutely and chronically depletes postganglionic sympathetic neurons of NE stores is a recognized fact, demonstrated by electron microscopic, histofluorescent microscopic, and autoradiographic studies of the nerve terminals as well as biochemical demonstration of reduced NE content in various peripheral organs normally innervated by these neurons. Numerous experiments, furthermore, have shown that the depleting action of 6-OHDA results in the release of intact NE molecules which elicit the typical responses of gross sympathetic chain stimulation. The intensity and duration of the effect, naturally, is related to the dose of 6-OHDA, and thereby, the degree of destruction of the peripheral sympathetic neurons.

After administration of a relatively low dose of 6-OHDA (1 to 10 mg/kg i.v.) to a rat there is a dose-related short-lived rise in the blood pressure. With higher doses ($\geq$15 mg/kg i.v.) the maximal response, while not markedly higher, is of much longer duration (381). An intravenous dose of 100 mg of 6-OHDA per kg causes a tachycardia and hypertensive response that peaks in about 5 minutes, stabilizes at an elevated level after another 15 minutes, and does not attain preinjection levels for another 30 to 90 minutes. Blood pressure continues to fall and stabilizes eventually at a level as much as 30 to 40 mm Hg below pretreatment levels (100, 101). In the dog, a similar dose-related chronotropic and hypertensive effect is noted after injection of 6-OHDA (15 $\mu$g to 1.5 mg) into the sinus node artery. After such treatment the duration of the effect varies from 12 minutes to 2.5 hours, which is indicative of the powerful sympathomimetic nature of the agent. In fact, with doses of 150 $\mu$g, blood pressure rises about 50 mm Hg and some dogs have atrial dysrhythmias in the form of premature beats that lead frequently to development of atrial fibrillation (113). In the closed-chest dog chronotropic responses are evident for 1½ to 2 hours after a single low dose of 6-OHDA (0.5 to 1.0 mg/kg i.v.), although pressor responses are short-lived (149, 338). In a preparation devoid of baroreceptor-compensatory mechanisms, as in a pithed rat, 6-OHDA (68 mg/kg i.v.) immediately brought about a rise in the blood pressure from 60 to 180 mm Hg and increased heart rate from 290 to 350 beats per minute. Both parameters did not return to preinjection values for 40 to 60 minutes, again indicating the long-lasting action of 6-OHDA on cardiovascular innervation. Study of the chronotropic action of 6-OHDA in cat hearts, isolated and perfused 5 minutes after administration of 6-OHDA (27 mg/kg i.v.), showed that the effects were immediate, with heart rate increased 110 to 195 beats per minute within 15 minutes and remaining at this level for at least 2 hours. NE was released at the rate of about 40 ng/min as determined by assay of the perfusate (160, 161). Similarly, 6-OHDA ($10^{-5}$ to $10^{-4}$ M) produced *in vitro* a transient positive inotropic and chronotropic response in isolated rat atria (236). Similar findings have been reported in cat nictitating membrane (160, 161, 338, 385, 386), mouse, rat and guinea pig vasa deferentia (139, 140), and rat palpebral aperture (252).

That the above responses are indirectly due to the release of NE was demonstrated by the ability of reserpine (100, 160, 161, 236, 381, 385), guanethidine (338), and 6-OHDA (236, 240) pretreatment to abolish or diminish pressor responses of subsequent 6-OHDA (100, 160, 381), inotropic and/or chronotropic responses in cardiac muscle (160, 161, 236, 240) and contraction of the cat nictitating membrane (160, 385). Equally effective were the *alpha*-adrenergic blocking

agents phentolamine (139, 160) and phenoxybenzamine (139), the *beta*-receptor antagonist propranolol (113, 160, 161, 236), and the agents cocaine (236) and desipramine (100, 113, 236), which inactivate the monoamine uptake mechanisms of the sympathetic neuron. In addition, it was shown that pressor and chronotropic responses to the indirectly acting sympathomimetic drugs, phenethylamine, tyramine and amphetamine, were potentiated immediately after 6-OHDA administration (338), but were greatly reduced after 16 to 48 hours (149, 338).

Actual measurements of CA in perfusates have shown that 6-OHDA releases both NE and epinephrine (E) from isolated dog adrenal glands (240), and NE from isolated cat hearts (160). Analysis of spontaneous excitatory junction potentials (MEJP) in the smooth muscle cells of the vas deferens indicates that the NE is released in discrete quanta and not simply leached out, since the MEJP's increase in frequency but not in amplitude, and the number of spontaneous contractions increases. Furthermore, were NE leached out of the nerve, there would likely be a gradual decrease in the postjunctional membrane potential associated with long slowly developing contractures of the smooth muscle. In actuality, an increased number of action potentials was recorded (138, 139). Therefore, it appears that 6-OHDA, because of its marked effect on neuronal ultrastructure and gross alterations in neuronal functional integrity, brings about a quantal release of NE which then acts at postjunctional sites to elicit the observed sympathomimetic responses.

### *D. Intraneuronal Sites Associated with the Destructive Lesioning of 6-OHDA*

Thoenen and Tranzer (356) have stressed that a critical level of 6-OHDA must be attained intraneuronally before degenerative events occur. Jonsson and Sachs (219, 222) carried out studies with mouse irides incubated *in vitro* in the presence of $^3$H-6-OHDA to estimate the actual intraneuronal content of 6-OHDA necessary to produce functional impairment. It was shown that after a 30-minute incubation with $10^{-6}$ or $10^{-5}$ M $^3$H-6-OHDA, slight impairment of uptake-storage mechanisms was produced (189, 221, 302, 303) and $^3$H-6-OHDA (plus metabolites) attained a concentration of about 10 and 30 %, respectively, of the usual endogenous content of NE in a single varicosity. However, with $10^{-4}$ M $^3$H-6-OHDA the content of 6-OHDA plus metabolites increased to approximately 10,000 $\mu$g/g, the level of NE estimated to be present normally in the varicosities of adrenergic neurons, and it was this concentration which produced not only marked alterations in the uptake-storage mechanism but actual ultrastructural lesions (189, 222). Therefore, it would appear that very high intraneuronal concentrations of 6-OHDA are necessary for bringing about destruction of catecholamine-containing neurons. In rat heart and spleen, the amount of $^3$H-amines present after injection of $^3$H-6-OHDA (1, 3 or 30 mg/kg i.v.) was proportional to the dose at one-half hour, but after 2 hours or longer the amounts of $^3$H-amines retained were inversely related to the dose, thus indicating that 6-OHDA destroyed its own binding sites (356).

The actual localization of the initial lesion produced by 6-OHDA in the neuron is unknown, and it is possible that any one of a number of regions may be the critical site, with 6-OHDA having no specificity for any one of these sites. Certainly, functional processes of all subcellular structures will eventually be deleteriously affected, but which are secondarily affected cannot be stated with certainty at this time. Tranzer (359) has discussed the possibility that the cell membrane is the site of the initial damage, since uptake capacity, conduction properties, and neurotransmission are impaired at early times. (These events will be discussed in section III E and V B.) The endoplasmic reticulum was also considered, since this structure accumulates 5-OHDA (362). One wonders whether the $^3$H-6-OHDA in the coarse particulate fraction from mouse atria

was primarily located in the outer neuronal membrane and/or associated with the endoplasmic reticulum, as this accounted for 25% of total tritium (221). Another site of action of 6-OHDA considered was the mitochondrion, since it contains the oxidative-reductive enzymes of the respiratory chain, with 6-OHDA being a strong reducing agent. 6-OHDA was shown to be as strong an uncoupler of oxidative phosphorylation as dinitrophenol, and the possibility exists that destruction of the major energy-producing subcellular component is responsible for subsequent destructive events (384, 386). This particular action is not entirely nonspecific, since 6-OHDA in similar concentrations does not inhibit monoamine oxidase (MAO) *in vitro*, which is associated with mitochondria.

Evidence to date indicates that the NE storage granules, to a certain degree, modulate the effects of 6-OHDA, but this site does not appear to be the primary target of action. Prior reserpine treatment, to prevent granule binding of the 6-OHDA, results in a definite decrease of loading in the vesicles and there appears to be less extensive degenerative lesions (37). However, with different times of pretreatment with different doses of reserpine and 6-OHDA, opposite results on NE content, monoamine uptake, and fluorescence microscopy have also been observed (220, 262, 294); others have reported reserpine to have no effect on depletion of NE by 6-OHDA (59, 93). In one study, reserpine was found to potentiate the depleting effects of 6-OHDA on DA content of rat brain (59). A dose of 6-OHDA (21 mg/kg i.v.), which alone produced no degeneration of noradrenergic terminals in the expansor secundariorum of the chick, when combined with reserpine produced the same degree of destruction as a 5-fold greater dose of 6-OHDA (37). Again, different results from studies of monoamine uptake are reported (220) that indicate the doses of both reserpine and 6-OHDA as well as the intervals between injection appear to be of primary importance in determining the effect of reserpine on 6-OHDA action. The results given above appear to indicate that, indeed, a critical concentration of 6-OHDA in the axoplasm is of major importance in determining the degenerative effects of 6-OHDA. When administration of reserpine precedes treatment with 6-OHDA the total content of 6-OHDA in the neuron will be decreased, but the cytoplasmic concentration may vary little or may change in different directions, depending on the dose and duration of the effect of reserpine. Hence, this may be one explanation for the varied results with the reserpine-6-OHDA combination. When administration of reserpine follows 6-OHDA treatment, then one would expect at least a temporary increase in the cytoplasmic concentration of 6-OHDA, since the amount formerly bound in the granules is released. Therefore, the potentiation of the destructive effects of 6-OHDA probably results from such a mechanism. In this context the emphasis has been laid on the cytoplasmic content of 6-OHDA because of associated studies with amphetamine and related agents. When amphetamine (37, 59, 220) is administered before or shortly after 6-OHDA, there is an attenuation of the effects of 6-OHDA, so that degeneration is altogether prevented or delayed (37), and NE levels (59, 220, 294) and monoamine uptake capacity (220) remain near control levels. Although amphetamine may compete at the neuronal membrane for the monoamine uptake sites, its major action on the sympathetic neuron is the release of extragranular monoamines (21, 63, 69, 153, 185, 295). Thus, when administered before 6-OHDA, amphetamine could act by both mechanisms, but when administered after 6-OHDA it appears to function primarily by releasing cytoplasmic 6-OHDA, thereby decreasing its concentration and preventing the destructive events. What later "leaks" into the cytoplasm from granule sites does not appear to be of major importance. Similar protective effects have also been noted with tyramine (294) and metaraminol (59, 294), which also affect cytoplasmic NE (71, 170, 302). It appears that

6-OHDA is a substrate for monoamine oxidase since nialamide pretreatment potentiates the effects of 6-OHDA in noradrenergic neurons (93, 220, 261, 262), and pargyline produces a similar potentiation of 6-OHDA on central dopaminergic neurons (59). Likewise, catechol-O-methyltransferase (COMT) may also be involved in the biodegradation of 6-OHDA, since inhibition of COMT with 4-tropoloneacetamide augmented the NE depleting action of 6-OHDA. Combination of 6-OHDA with both a MAO- and COMT-inhibitor produced no greater NE depletion than pargyline-6-OHDA, but toxicity was enhanced markedly (220).

These studies indicate that 6-OHDA is actively accumulated by catecholamine-containing neurons, localized in granules and produces degenerative effects in relation to the cytoplasmic content. It is a substrate for both MAO and COMT. The primary site of action of 6-OHDA is not known, although 6-OHDA does uncouple oxidative phosphorylation in concentrations calculated to occur intraneuronally. The loss of ability to conduct action potentials and destruction of intraneuronal enzymes immediately follow the destructive process of 6-OHDA (160).

### *E. Loss of Conduction Properties of Noradrenergic Terminal Endings after 6-OHDA Treatment*

Haeusler (160, 161), in studying the temporal sequence of events after 6-OHDA, showed that within 1 hour noradrenergic terminals lost their ability to conduct or generate action potentials. This occurred at a time when monoamine uptake mechanisms appeared relatively intact, so that the neurons were still anatomically present.

When rat mesenteric arteries or cat hearts and nictitating membranes were isolated *in vitro* 5 or 30 minutes after 6-OHDA treatment responses of the organs to electrical stimulation of their intact sympathetic supply were reduced markedly at 30 minutes and completely absent by 60 minutes. Assay of the perfusate of the cat Langendorff preparation showed that the amount of NE released by electrical stimulation of the stellate was greatly reduced at 30 minutes, and at 60 minutes stellate stimulation failed to release NE. That the nerves were not depleted of NE was shown by the large amounts of NE being released into the perfusate in the absence of electrical stimulation for at least 75 minutes (160, 161). Similarly, it had earlier been shown that there was a loss in sympathetic vasoconstriction at a time when NE was reduced 50% and monoamine uptake processes and postsynaptic sensitivity to NE was unchanged (268). Thus, although NE stores are still intact, electrical stimulation is ineffective in releasing the physiological transmitter, indicating a breakdown in the electrical properties of the membrane. In further support of this finding, it was shown that both acetylcholine (0.15 $\mu$M) and potassium (0.25 $\mu$M) perfusion, which normally evoke antidromic discharges in the isolated cat heart presumably *via* noradrenergic terminal endings (164, 166), failed to evoke these responses after 1 hour in preparations from 6-OHDA-treated animals (160, 161).

Numerous other investigators also have shown failure of sympathetic stimulation to produce physiological responses, although these have been studied at later time periods when nerve terminals may be depleted of NE stores, or no longer may be present morphologically. Long before it was known that 6-OHDA destroyed catecholamine-containing neurons, Stone *et al.* (339) demonstrated the deleterious effect of 6-OHDA on transmission of nerve impulses. From 16 to 24 hours after injection of 6-OHDA into dogs it was found that reflex pressor responses to stimulation of the central ends of the vagi and to carotid artery occlusion were greatly reduced. Identical results were shown even after direct stimulation of sympathetic neurons in both dogs and cats between the first and eighth days after 6-OHDA (149, 175, 240, 339) and after stimulation of the entire sympathetic outflow in pithed rats (151, 160).

With atropine-perfused isolated cat hearts

sympathetic stimulation after administration of 6-OHDA (96 mg/kg total, 1 week) produced only a slight chronotropy, and no frequency of stimulation produced inotropy (164). Loss of vasoconstrictor responses of isolated rat mesenteric arteries to sympathetic stimulation was also found to occur 4 to 10 days after administration of 6-OHDA (133, 268). In the frog retrolingual membrane the same loss of neurogenic vasoconstrictor tone was observed after 6-OHDA was painted on the membrane over a two-hour period (320).

It is apparent that after adequate doses of 6-OHDA, the regeneration of sympathetic terminal fibers is relatively slow in most peripheral tissues, as shown by the prolonged reduction in end-organ responses to direct or indirect sympathetic stimulation. Furthermore, this loss in functional activity first occurs while the terminals are still present, so that there appears to be a true breakdown in the membrane properties before the terminal fibers completely disappear.

### *F. Development of Presynaptic Supersensitivity after 6-OHDA Treatment*

Trendelenburg and Weiner (366) described two mechanisms for development of supersensitivity. The first, presynaptic supersensitivity is seen after cocaine or desipramine or 24 hours after surgical denervation, and is attributable to a decreased uptake of NE by presynaptic nerve terminals. The other mechanism is postsynaptic supersensitivity and is described as a proliferation of the postsynaptic receptors 12 to 16 days after surgical denervation. These adrenergic receptors are capable of being activated by either NE or isoproterenol (isopropylarterenol, isopropylnorepinephrine, isoprenaline, Isuprel).

Haeusler *et al.* (160, 161, 165, 175) first noted a nearly 100-fold increase in sensitivity to NE in the intact nictitating membrane of the spinal cat and isolated mesenteric artery of the rat when studied 4 to 16 days after the last of a series of injections of 6-OHDA. Time-course studies revealed that the supersensitivity developed from as early as three hours after a single large dose of 6-OHDA (27 mg/kg i.v. or 30 mg/kg s.c.) to a maximum at 14 hours (160, 161) to 25 hours (384, 385). Supersensitivity to NE was also noted in isolated mouse vas deferens (201), pig trachea (85), rat palpebral aperture (252), perfused rat mesenteric arteries (132, 161), and chick vena cava (36), and to isoproterenol in rat duodenal preparations (152) after administration of 6-OHDA.

With isolated aortae from rats (234) a slight increase in sensitivity to NE was originally reported, although this result was not consistent (258). Because of the reported absence of adrenergic nerve endings in rat aortae (290) one would not expect to see supersensitivity after 6-OHDA treatment. In rabbit aorta strips supersensitivity to NE was reported one hour to one week after 6-OHDA treatment (258, 321) but no change was found in guinea pig aorta strips after one to two weeks (258) which is somewhat surprising because of the dense adrenergic innervation normally present in the vessel (290). In this case, it appears that the terminal endings regenerated rapidly so that supersensitivity either did not develop or had regressed by the time studies were made (see section VIII C).

In demedullated rats treatment with 6-OHDA increased pressor responses to NE for at least 10 days (234) and in pithed rats it was shown that both the magnitude and duration of NE pressor responses were potentiated after 6-OHDA treatment (131). Similar results on pressor responses to NE were also found in intact rats (81) and cats (165) after administration of 6-OHDA.

Potentiation of chronotropic responses to NE have been noted in the atropine-perfused cat heart (165), the isolated-perfused rat heart (65, 277), and isolated rat (100, 277) and rabbit atria (321) after treatment with 6-OHDA; potentiation of chronotropic responses to isoproterenol in isolated rat atria was also observed (152). Supersensitivity of inotropic effects of NE were demonstrated

in the atropine-perfused cat heart (165), perfused rat heart (277), and rabbit atria (321).

In conjunction with the above findings, there were other reports, namely: markedly reduced responses to or ineffectiveness of tyramine on initiating vasoconstriction in isolated rabbit aortae (258); pressor responses in intact rats (81, 131) and cats (165, 175); and chronotropic and inotropic responses in isolated rabbit atria (321), rat atria (100, 258), whole rat hearts (277), and perfused cat hearts (165). Ineffectiveness of methamphetamine and dihydroxyamphetamine on the rat aorta (234) and dimethylphenylpiperazinium (DMPP) on changes in heart rate (165, 321) and force of contraction (321) were also noted. Cocaine and desipramine had little or no effect in potentiating responses to NE in the guinea pig trachea and rat mesenteric arteries (131), while chronotropic responses to isoproterenol were unaltered (277) after treatment with 6-OHDA.

These studies demonstrate that the noradrenergic terminals are depleted of their NE stores after treatment with 6-OHDA, as indicated by the ineffectiveness of the indirectly acting sympathomimetic amines (tyramine, methamphetamine, and dihydroxyamphetamine) and by lack of effects of DMPP in bringing about sympathomimetic effects through ganglionic stimulation. Furthermore, since cocaine and desmethylimipramine, which act primarily at the noradrenergic terminal neurons to inhibit NE uptake, fail markedly to potentiate responses to NE, it appears that the nerve terminals are virtually absent because of degeneration so that uptake processes are no longer functional. Thus, because of its destructive action on noradrenergic neurons and the eventual loss of uptake mechanisms for monoamines, administration of 6-OHDA brings about the development of presynaptic supersensitivity of various end organs to catecholamines or direct acting sympathomimetic amines which are normally taken up by the sympathetic nerve endings.

### *G. Development of Postsynaptic Supersensitivity after 6-OHDA Treatment*

Haeusler *et al.* (164) first demonstrated the development of postsynaptic supersensitivity of the medial smooth muscle of cat nictitating membrane two weeks after initial treatment with 6-OHDA (14 mg/kg i.v., 2 times on day 1, plus 34 mg/kg i.v., 2 times on day 7). It was shown that the NE dose-response curve of the isolated muscle from 6-OHDA-treated cats was shifted to the left by a factor of 100, while cocaine ($10^{-5}$ M), in the presence of an innervated preparation, shifted the curve to the left by a factor of only 15. Similarly, both NE and isoproterenol constrictor responses were shown to be potentiated after 1 to 3 days in aorta strips from 6-OHDA-treated (50 mg/kg i.v.) rabbits and in trachea of 6-OHDA-treated guinea pigs (85, 321). Therefore, the same basic physiological processes occurring after surgical denervation are shown to occur after chemical sympathectomy.

### *H. Effects of 6-OHDA on Adrenergic Receptor Sites*

Haeusler (160, 161) showed that 30 minutes after a toxic dose of 6-OHDA (139 mg/kg i.v.) the constrictor responses of isolated perfused rat mesenteric arteries to NE were reduced markedly. The entire dose-response curve was shifted to the right and the maximal response was depressed. Responses of arteries to $CaCl_2$ (in a depolarizing solution) and KCl were normal, indicating that 6-OHDA did not exert a nonspecific depressant action on the smooth muscle. In rats pretreated with phentolamine (20 mg/kg i.p. 30 minutes) responses of arteries to NE were similar to those in the control group after 20 hours, thereby indicating that 6-OHDA is capable of producing a blockade or destruction of *alpha*-adrenergic receptor sites. Since recovery of sensitivity did not begin for 20 to 48 hours, and was not complete until five days, it appears that 6-OHDA either forms a covalent bond in the region of the receptor, with resultant blockade or produces an

alteration in the protein configuration at the receptor site.

It was suggested that the adrenergic receptors normally compete with the nerve terminals for 6-OHDA, but because of their active uptake mechanisms the adrenergic neurons take up much more 6-OHDA. In addition, the NE released by 6-OHDA may compete for the receptor and thereby provide additional protection for this site (160, 161). Therefore, although 6-OHDA has the potential to produce adrenergic receptor blockade or destruction, the basic physiological processes and sympathomimetic actions of 6-OHDA work against this effect, so that they may appear only after the sympathetic neurons have been destroyed previously, or after toxic doses of 6-OHDA have been administered.

## IV. Differential Sensitivity of Catecholamine-containing Neurons to 6-OHDA

Thoenen and Tranzer (356) noted that tissues in which the NE content was reduced as much as 95% were able to recover from the effects of 6-OHDA, so that after a period of weeks the adrenergic terminal plexus had regenerated. Thus, although 6-OHDA treatment brought about marked destruction of noradrenergic terminals, the perikarya appeared to be intact. Malmfors and Sachs (262), with fluorescence microscopy, confirmed and extended these findings by showing that the fluorescent terminals decreased in number and surviving terminals displayed diminished fluorescence. Perikarya remained intact, but the axons acquired a marked degree of fluorescence, usually accompanied by swelling and irregular beading or bulging along their course. Thus, a pattern was seen similar to that observed after surgical sympathectomy of an end organ (see section VI).

When ganglia of 6-OHDA-treated animals were assayed, there appeared to be no marked alteration of NE content (240), nor tyrosine hydroxylase (T-OH) (62). These findings were extended to noradrenergic neurons in the CNS by Bartholini *et al.* (28) and Bloom *et al.* (47) who demonstrated the same differential sensitivity of noradrenergic neurons to 6-OHDA (i.c. or ivt.). Iversen and Uretsky (205, 206) and Uretsky *et al.* (380) also showed that uptake of $^3$H-NE into synaptosomes from the pons medulla of rats treated intraventricularly with 6-OHDA was decreased to a greater degree than uptake of $^3$H-NE in slices from this region; this reflected a lesser alteration of function in the preparation containing the perikarya and axons, in addition to the damaged terminal endings studied solely in the former preparation. Also, the pons medulla, a region containing a high proportion of monoaminergic cell bodies, had less biochemical alterations than those regions containing primarily noradrenergic terminal processes, such as the cerebral cortex, hippocampus, cerebellum, and hypothalamus (see section V).

From these studies, it was apparent that different parts of the noradrenergic neuron were not affected to the same degree by 6-OHDA. However, the monoaminergic perikaryon was not totally insensitive to 6-OHDA, since injection of 6-OHDA directly into the substantia nigra, which contains dopaminergic cell bodies (5, 6, 8, 96), brought about destruction of the perikaryon (368, 375). Also, intracisternal or intraventricular administration of 6-OHDA resulted in the destruction of the perikaryon although this was often associated with retrograde degenerative changes (105, 292). However, in newborn animals there was a distinct sensitivity of the perikaryon to 6-OHDA so that a large percentage of the cell bodies in sympathetic ganglia were completely and permanently destroyed (11, 15, 16).

The reason for decreased sensitivity of the noradrenergic perikaryon to 6-OHDA in adult animals is not yet understood. The ganglion cells take up and retain less $^3$H-NE than nonterminal axons and varicose nerve terminals and it is possible that the perikarya accumulate less 6-OHDA than the terminal endings, so that the critical threshold level for degenerative events is not attained (360).

It was also considered that surface geometry could account for the differential sensitivity of the neuron since the surface to volume ratio of the cell body is much less than that of the neuronal varicosity and the cell body would need to accumulate a much greater amount of 6-OHDA to reach the same critical intracellular concentration of 6-OHDA (204). Angeletti (12) suggested that there is a true shift of NE uptake efficiency from the cell body of nondifferentiated tissue to the peripheral parts of the neuron with development. It was also noted that 6-OHDA produces a greater destructive effect on mouse adrenergic neurons than on rat neurons (260). Therefore, it appears that geometry may be of some importance in determining the differential neuronal susceptibility to 6-OHDA, but other factors appear to be involved.

Differences in blood supply of 6-OHDA to the affected region may also account for the lesser susceptibility of the noradrenergic perikarya to 6-OHDA (356), although terminal endings in ganglia appear to be affected to the same degree as in end organs (360), which indicates that the arterial circulation carries a reasonable amount of 6-OHDA to all parts of the neuron. The importance of blood supply was illustrated by the finding that the amount of $^3$H-NE retained in rat heart one hour after intravenous injection is 10 times the amount in the spleen (356). Although both tissues have nearly the same NE content ($\mu$g/g), the heart receives a much greater blood supply and therefore, more $^3$H-NE. Nevertheless, even when a large dose of 6-OHDA is injected directly into the ganglion or *via* the arteries, degeneration of the cell bodies does not occur, thereby indicating a relative insensitivity of the noradrenergic perikarya to 6-OHDA (349, 360).

Size differences between perikarya of young and adult animals are not great and therefore should not account for the different sensitivities to 6-OHDA (138). However the extent to which satellite cells encompass the ganglion cells in young animals may be important since they may protect perikarya. If, in the neonate, the cell bodies are incompletely covered by a layer of satellite cells, then this too could be of major consequence in explaining differences in susceptibility to 6-OHDA (45). In addition, the size of the dendritic tree may be important in sequestering significant amounts of 6-OHDA. In the neonate, there is far less arborization of terminal plexuses, so that much more 6-OHDA is available for uptake by perikarya.

The effects of 6-OHDA on sympathetic chain ganglia, stellate, and superior cervical ganglia of two-week-old lambs have been studied in greater detail (76), and the findings have been confirmed and extended in rat stellate and superior cervical ganglia (61, 62). There are distinct biochemical and morphological alterations, and the effects of 6-OHDA appear to be due to both a direct and indirect action, the latter as a consequence of nerve terminal destruction (62, 76). When the sympathetic nerve terminals are destroyed either by surgical or chemical (6-OHDA) means, the ganglia appear to be unresponsive to increased preganglionic activity insofar that T-OH is not compensatorily increased as it is after reserpine (62). Such an effect has been noted at three and seven days after treatment with 6-OHDA (136 mg/kg, times 2, i.v.) in the stellate ganglion of rats. Protein synthesis in the ganglion appears to occur at a normal rate in the first few hours after treatment with 6-OHDA as indicated by $^3$H-leucine incorporation. However, there is a simultaneous 50% decrease in soluble dopamine-$\beta$-hydroxylase (DBH) activity of the ganglion and a slight fall in particulate DBH, which indicates that protein synthesis may be directed to repair of the damaged cell (61). One day after treatment with 6-OHDA the Nissl substance was dispersed in the cytoplasm and the nucleus displaced toward one side of the cell. Neurotubules were disrupted and became swollen and electron-lucid. Large numbers of granules accumulated in the axon profiles and even within the perikaryon itself and axons became highly fluorescent (76).

It is likely that the 100% rise in NE content of the ganglion is related at least partially to the disruption in axoplasmic flow. It has also been suggested that the rise in NE may be coupled with decreased MAO activity, although measurements indicated a fall in activity only at a later time (76). Measurements of both T-OH and DBH show that both are only slightly altered at this time (62, 76). It has been reported that DBH synthesis is identical to that of controls, when measured 20 to 40 hours after 6-OHDA. However, between 40 and 55 hours postinjection, there is a marked decrease in synthesis of DBH and levels fall about 50% of control for at least the next five days (61). T-OH and MAO synthesis also decreases and levels are far below control at seven days. NE content is elevated at this time, coupled with a reduced MAO activity (76). It was suggested that axonal transport is unaltered, since the ratio of DBH activity in axonal segments from control and treated rats is unchanged (61). Studies *in vitro* on fluorescent-stained adrenergic nerves likewise support the view that axoplasmic transport is unaltered (38). By 21 days after 6-OHDA, T-OH activity of ganglia is at or above control levels, while MAO is still reduced, although the content of NE is about the same as at seven days. Therefore, it would appear that the ganglia recover to a large degree by three weeks and possibly part of the elevation in T-OH is due to transsynaptic induction. Neurotubular transport still appears to be somewhat altered, although only a small percentage of the axons are fluorescent (76). It would be of interest to correlate the effect of 6-OHDA on the intraganglionic system of varicose terminals, axons, and cell bodies with changes in enzyme and amine levels. A recent study (172) has demonstrated that the intraganglionic adrenergic terminals are capable of massive sprouting of new fibers four days after initial destruction of these ganglionic fibers by vinblastine treatment. Unless these parameters are known, interpretation of changes in the ganglion remain confusing.

In summary, both morphological and biochemical data indicate alterations in noradrenergic perikarya after administration of 6-OHDA. The effects appear to be short-lived, with recovery of rat superior cervical ganglion and lamb stellate ganglion being nearly complete three weeks after administration of 6-OHDA. The exact nature of the sequential changes still need to be determined and it is important to know the temporal relationship between intraganglionic varicose terminal destruction and loss of cell body responsiveness to preganglionic activity.

## V. Effects of 6-OHDA on Catecholamine-containing Neurons in the CNS

### *A. Monoamine Content*

Destruction of catecholamine-containing neurons in the CNS was first observed by Ungerstedt (368), who used fluorescence microscopy to study changes in neuronal structure. Injection of 6-OHDA (2 to 8 μg) into the area dorsolateral to the interpeduncular nucleus resulted in the degeneration of noradrenergic axons coursing through the region, in addition to dopaminergic cell bodies of the substantia nigra. A similar amount of 6-OHDA injected directly into the caudate-putamen caused a marked degeneration of dopaminergic terminal processes. Thus, 6-OHDA destroyed not only noradrenergic terminals but dopaminergic terminals as well; this has been confirmed under the electron microscope (EM) (see section VI). Furthermore, when 6-OHDA is injected into the zona compacta of the substantia nigra, the cell bodies of dopaminergic neurons are "chemically lesioned," as evidenced by the anterograde degeneration of the nigro-neostriatal tract. From these findings, it became evident that neurons which actively accumulate 6-OHDA, *via* NE-, DA-, or related amine-uptake mechanisms, undergo degenerative phenomena when the 6-OHDA is present in sufficient amounts. Also, while peripheral adrenergic perikarya usually are affected to only a minor degree

by 6-OHDA, central and peripheral cell bodies are also susceptible to damage when the agent is present in high enough local concentrations.

Shortly after this initial report, it was found that 6-OHDA (1 to 500 μg), injected intraventricularly produced a dose-dependent decrease in whole brain NE levels. A dose of 5 μg was capable of reducing whole brain NE by 30% within two days while 25 to 50 μg 6-OHDA decreased NE by 50% (377). DA content remained unaltered up to this point (205), indicating a relatively selective action of 6-OHDA on noradrenergic neurons (see table 1). With higher doses of 6-OHDA, both NE and DA decreased simultaneously, with the decline of DA being steeper than the drop in NE content, until both were reduced by about 80% after a total dose of 500 μg (121, 205). Up to this point toxicity of 6-OHDA was mild, but with doses near to or greater than 500 μg toxicity became marked. It was found that divided doses were much less lethal and produced the same degree of catecholamine depletion as a single large dose of 6-OHDA (205). Intracisternal (i.c.) administration of 6-OHDA produced essentially the same effect as 6-OHDA *via* the intraventricular route at all time periods studied (58; see table 1).

When discrete regions of the cerebrospinal axis were taken for CA analysis it was shown that all components were affected by 6-OHDA, albeit not to the same degree. It was also found that each region was depleted of CA in a dose-dependent manner, as noted in whole brain (table 1). The neocortex showed a 40% reduction in NE content one day after intraventricular administration of 41 μg of 6-OHDA. Increasing the dose to 136 μg reduced NE 60% at the same period (241), while 500 μg (250 μg 2 times, 48-hour interval), reduced NE by 95% after 16 to 25 days. The latter dose of 6-OHDA produced a similar reduction in NE in the hippocampus and reduced both NE and DA in the striatum to a similar degree (205). NE content of the hypothalamus was reduced approximately 50% after intraventricular administration of 200 μg of 6-OHDA (29, 275), while NE content of the thalamus was somewhat less altered (184). When the dose of 6-OHDA was increased to 500 μg (250 μg 2 times ivt.), NE was reduced by approximately 80% in both the hypothalamus and the midbrain (205). In the pons medulla 6-OHDA (250 μg 2 times) depleted NE to only 35% of control (378). It appears that the major portion of this change was due to an effect of 6-OHDA in the pontine regions, inasmuch as when the medulla alone was studied, NE content was reduced less than 35% after intraventicular administration of 200 μg of 6-OHDA (29). When the dose of 6-OHDA was increased to 750 μg (250 μg 3 times), NE in the medulla was only decreased 60%. Thus, it would appear that NE in the pons is reduced to a level similar to that observed in the midbrain after treatment with 6-OHDA. When 140 μg of 6-OHDA is administered intraventricularly, NE in the cerebellum is reduced approximately 70% and becomes undetectable after administration of 500 μg of 6-OHDA (378). The spinal cord, despite its distance from the lateral ventricles, appears to be affected markedly by 6-OHDA, since NE levels are reduced 90% after administration of 500 μg (250 μg 2 times, ivt.) (205). These studies indicate that 6-OHDA affects noradrenergic neurons throughout the CNS, with prepontine fibers being somewhat more susceptible to damage or depletion than lower brainstem neurons (207). By far, noradrenergic neurons in the medulla appear to be the least susceptible to NE depletion by 6-OHDA.

In the CNS, the damaged CA-containing neurons do not appear to regenerate to any substantial degree. No structural recovery of noradrenergic neurons was observed for as long as 8 to 10 months after a single injection of 6-OHDA (46). With CA levels as an index, no tendency towards recovery of central CA-containing neurons was observed in the CNS at intervals of 32 days (241, 377), 75 days (378), or 142 days (205). However, Vetulani *et al.* (382), found that NE content of rat whole brain recovered

TABLE 1

*Effects of 6-OHDA on catecholamine content (μg/g) in rat whole brain and brain regions*

| Brain Region | Total Dose of 6-OHDA (Free Base) | Route | Time of Sacrifice after Final 6-OHDA Dose | Control | | Ref. |
|---|---|---|---|---|---|---|
| | | | | NE | DA | |
| | μg | | | % | % | |
| Whole brain | 5 | ivt. | 2 days | 70 | | 377 |
| | 25 | | 2 days | 55 | N.S.[a] | 377, 378 |
| | | i.c. | 2 weeks | 65 | N.S. | 59 |
| | | | 16–21 days | 55 | N.S. | 58 |
| | 25[b] | | 16–21 days | 70 | N.S. | 58 |
| | 28 | | 1 day | 35 | 70 | 136 |
| | 50 | | 1 day | 65 | 75 | 176 |
| | | ivt. | 2 days | 50 | N.S. | 377, 378 |
| | | i.c. | 2 weeks | 60 | N.S. | 59 |
| | 50[c] | | 2 weeks | 45 | N.S. | 59 |
| | 50 | | 14 days | 50 | 70 | 176 |
| | | | 16–21 days | 45 | N.S. | 58 |
| | 50[b] | | 16–21 days | 55 | N.S. | 58 |
| | 50[c] | | 3 weeks | 45 | N.S. | 59 |
| | 75[d] | ivt. | 7 days | 25 | N.S. | 315 |
| | 100 | | 2 hours | 45 | 55 | 120 |
| | | | 1 day | 40 | | 377 |
| | | | 2 days | 40 | 65 | 121 |
| | | | 2 days | 40 | 70 | 377, 378 |
| | | | 8 days | 30 | | 377 |
| | | i.c. | 2 weeks | 45 | 70 | 59 |
| | 100[c] | | 2 weeks | 40 | N.S. | 59 |
| | | ivt. | 16 days | 40 | | 377 |
| | | i.c. | 16–21 days | 35 | 60 | 58 |
| | 100[b] | | 16–21 days | 45 | 35 | 58 |
| | 100[d] | | 4 weeks | 35 | 75 | 59 |
| | 100 | ivt. | 32 days | 45 | (65)[e] | 377, 378 |
| | | | 75 days | 50 | | 378 |
| | | | 1 hour | 45 | N.S. | 241 |
| | 136 | | 2 hours | 35 | N.S. | 241 |
| | | | 4 hours | 30 | N.S. | 241 |
| | | | 6 hours | 40 | 115 | 241 |
| | | | 1 day | 45 | 85 | 241 |
| | | | 2 days | 45 | 60 | 241 |
| | | i.c. | 5 days | 20 | 30 | 111 |
| | | ivt. | 8 days | 35 | 50 | 241 |
| | | | 16 days | 35 | 50 | 241 |
| | | | 32 days | 35 | 50 | 241 |
| | 150 | i.c. | 1 hour | 15 | N.S. | 55 |
| | | ivt. | 4 hours | 25 | | 315 |
| | | i.c. | 5 days | 40 | | 55 |
| | 170[f] | ivt. | 2 hours | 90 | 160 | 382 |
| | | | 9 days | 40 | 70 | 382 |
| | 170 | | 2–3 months | 45 | 80 | 382 |
| | 170[f] | | 2–3 months | 35 | 65 | 382 |
| | | | 4 months | 65 | 85 | 382 |
| | 200 | i.c. | 1 hour | 30 | | 316 |
| | | | 1 day | 50 | 50 | 176 |
| | | | 1 day | 35 | 70 | 28 |

TABLE 1—*Continued*

| Brain Region | Total Dose of 6-OHDA (Free Base) | Route | Time of Sacrifice after Fina 6-OHDA Dose | Control | | Ref. |
|---|---|---|---|---|---|---|
| | | | | NE | DA | |
| | μg | | | % | % | |
| Whole brain | 200[b] | i.c. | 3 days | 35 | 25 | 58 |
| | 200 | ivt. | 5 days | 30 | | 334 |
| | | i.c. | 14 days | 30 | 45 | 176 |
| | | | 14 days | 35 | 50 | 59 |
| | 200[c] | ivt. | 2 weeks | 30 | | 59 |
| | 200[b] | i.c. | 15 days | 20 | | 58 |
| | | | 17 days | 20 | 25 | 58 |
| | 200 | ivt. | 16–21 days | 25 | 65 | 58 |
| | 200[b] | i.c. | 16–21 days | 20 | 25 | 58 |
| | | | 21 days | 25 | 25 | 58 |
| | | | 28 days | 25 | 20 | 58 |
| | | | 40 days | 35 | 20 | 89 |
| | | | 46 days | 25 | 20 | 58 |
| | 200[b] | | 78 days | 15 | 25 | 58 |
| | | | 94 days | 30 | 25 | 89 |
| | 205[g] | ivt. | 5 days | 30 | 65 | 28 |
| | 250 | | 2 hours | 25 | 25 | 120 |
| | | | 2 days | 35 | 45 | 120 |
| | | | 2 days | 30–35 | 45–50 | 378 |
| | | | 10 days | 45 | 55 | 67 |
| | 272 | i.c. | 5 days | 10 | 25 | 111 |
| | 400[h] | | 7 days | 20 | 20 | 58 |
| | 400[c] | ivt. | 7 days | 10 | | 316 |
| | | | 2 weeks | 20 | 40 | 59 |
| | 400[h] | i.c. | 2 weeks | 35 | 20 | 58 |
| | 400[c] | | 57 days | 20 | 25 | 89 |
| | 400[i] | | 21 weeks | 40 | | 337 |
| | 500 | ivt. | 2 days | 15 | | 377 |
| | 500[j] | ivt. | 2 days | 20 | 35 | 378 |
| | 500[j] | | 3 days | 20 | 35 | 378 |
| | 500[c] | | 4 days | 20 | 25 | 121 |
| | 500[j] | | 10 days | 15 | 45 | 309 |
| | 500[c] | | 15 days | 20 | 35 | 315 |
| | 500[j] | | 32 days | 15 | 25 | 378 |
| | 500[c] | | 4–6 weeks | 10 | 20 | 315 |
| | 600[k] | i.c. | 1 day | 15 | 40 | 176 |
| | 600[k] | | 14 days | 5 | 15 | 176 |
| | 750[l] | | 2 days | 10 | | 378 |
| Cortex | 7 | ivt. | 1 day | N.S. | | 241 |
| | 41 | | 1 day | 60 | | 241 |
| | 136 | | 1 day | 40 | | 241 |
| | | | 32 days | 30 | | 241 |
| | 250 | | 7 hours | 55 | 125 | 184 |
| | | | 3 days | 65 | N.S. | 184 |
| | 500[c] | | 16–25 days | 3 | | 205 |
| Hippocampus | 500[c] | ivt. | 16–25 days | 10 | | 205 |
| Telediencephalon | 25 | | 2 weeks | 55 | N.S. | 100 |
| | 50 | | 2 weeks | 15 | 40 | 100 |
| | 300[d] | i.c. | 9 days | 30 | 70 | 47 |
| | | | 11 days | 45 | 85 | 47 |

TABLE 1—*Continued*

| Brain Region | Total Dose of 6-OHDA (Free Base) | Route | Time of Sacrifice after Final 6-OHDA Dose | Control | | Ref. |
|---|---|---|---|---|---|---|
| | | | | NE | DA | |
| | μg | | | % | % | |
| Telediencephalon | 300[d] | i.c. | 25 days | 30 | | 47 |
| | | | 32 days | 25 | 75 | 47 |
| Striatum | 7 | ivt. | 1 day | N.S. | N.S. | 241 |
| | 25 | | 30 minutes | | 185 | 35 |
| | 41 | | 1 day | 40 | N.S. | 241 |
| | 136 | | 2 days | 60 | 85 | 241 |
| | | | 32 days | 50 | 30 | 241 |
| | 250 | | 30 minutes | | 170 | 35 |
| | | | 2 hours | | 120 | 35 |
| | | | 7 hours | 135 | 120 | 184 |
| | | | 24 hours | | 135 | 35 |
| | | | 48 hours | | 15 | 35 |
| | | | 3 days | N.S. | N.S. | 184 |
| | 400[h] | i.c. | 7 days | 25 | 20 | 58 |
| | 500[c] | ivt. | 16–25 days | | 5 | 205 |
| | | | 23 days | | 5 | 377 |
| Hypothalamus | 25 | ivt. | 30 minutes | 60 | | 35 |
| | 200 | | 2 days | 40 | | 29 |
| | | | 10 days | N.S. | | 29 |
| | 205[g] | | 5 days | 65 | | 275 |
| | 250 | | 30 minutes | 70 | | 35 |
| | | | 2 hours | 45 | | 35 |
| | | | 7 hours | 40 | 115 | 184 |
| | | | 24 hours | 20 | | 35 |
| | | | 48 hours | 25 | | 35 |
| | | | 3 days | 50 | 85 | 184 |
| | 500[c] | | 16–25 days | 15 | | 205 |
| | 600[d] | | 15 days | 20 | | 163 |
| Thalamus | 250 | ivt. | 7 hours | 80 | N.S. | 184 |
| | | | 3 days | 65 | N.S. | 184 |
| Midbrain | 7 | ivt. | 1 day | 75 | | 241 |
| | 41 | | 1 day | 45 | | 241 |
| | 136 | | 1 day | 25 | | 241 |
| | | | 2 days | 40 | | 241 |
| | | | 32 days | 40 | | 241 |
| | 250 | | 7 hours | 45 | N.S. | 184 |
| | | | 3 days | (75) | N.S. | 184 |
| | 500[c] | | 16–25 days | 10 | | 205 |
| Brainstem | 25 | ivt. | 2 weeks | 75 | | 100 |
| | 50 | | 2 weeks | 25 | | 100 |
| | 100 | | 18–20 days | 60 | | 390 |
| | 200 | | 18–20 days | 40 | | 390 |
| | 300[d] | i.c. | 9 days | 70 | | 47 |
| | | | 11 days | 70 | | 47 |
| | | | 25 days | 60 | | 47 |
| | | | 32 days | 50 | | 47 |
| | 400[h] | | 7 days | 20 | | 50 |
| Lower brainstem | 206 | ivt. | 7 days | 85 | | 207 |
| | 411 | | 7 days | 30 | | 207 |

TABLE 1—*Continued*

| Brain Region | Total Dose of 6-OHDA (Free Base) | Route | Time of Sacrifice after Final 6-OHDA Dose | Control | | Ref. |
|---|---|---|---|---|---|---|
| | | | | NE | DA | |
| | μg | | | % | % | |
| Prepontine region | 206 | ivt. | 7 days | 60 | N.S. | 207 |
| | 411 | | 7 days | 15 | 40 | 207 |
| | 660[m] | | 8 days | 15 | 45 | 207 |
| | | | 30 days/70 days | 15/15 | 35/25 | 207/207 |
| Pons medulla | 7 | ivt. | 1 day | N.S. | | 241 |
| | 41 | | 1 day | 50 | | 241 |
| | 140 | | 1 day | 30 | | 241 |
| | | | 2 days | 45 | | 241 |
| | | | 32 days | 55 | | 241 |
| | 250 | | 7 hours | N.S. | N.S. | 184 |
| | | | 3 days | N.S. | N.S. | 184 |
| | 500[c] | | 16–25 days | 35 | | 205 |
| Medulla | 200 | ivt. | 2 days | 80 | | 29 |
| | | | 10 days | 65 | | 29 |
| | 750[d] | | | 40 | | 241 |
| Cerebellum | 7 | ivt. | 1 day | 90 | | 241 |
| | 41 | | 1 day | 60 | | 241 |
| | 140 | | 1 day | 5 | | 241 |
| | | | 2 days | 25 | | 241 |
| | | | 32 days | 35 | | 241 |
| Spinal cord | 500[c] | ivt. | 16–25 days | 10 | | 205 |

All values for catecholamines are to the nearest 5%.

[a] N.S., no significant difference between 6-OHDA- and control groups.

[b] A single dose of 6-OHDA preceded 30 minutes by pargyline (50 mg/kg i.p.).

[c] Two equimolar doses of 6-OHDA, 48-hour interval.

[d] Three equimolar doses of 6-OHDA, 48-hour intervals.

[e] Numbers in parentheses are indicated literature value with undetermined degree of significance from control group.

[f] A single dose of 6-OHDA preceded 16–18 hours by nialamide (140 mg/kg i.p.).

[g] 136 μg plus 68 μg 6-OHDA, weekly interval.

[h] Two equimolar doses of 6-OHDA, with second dose preceded by pargyline (50 mg/kg i.p.) one week after first dose.

[i] Two equimolar doses of 6-OHDA, weekly interval.

[j] Two equimolar doses of 6-OHDA, 24-hour interval.

[k] 200 μg plus 400 μg 6-OHDA (i.c.), 72-hour interval.

[l] Equimolar doses of 6-OHDA on days 1 (i.c.), 2 (ivt)., and 5 (ivt.).

[m] Two equimolar doses of 6-OHDA, 72-hour interval.

from 65 to only 35% depletion between 2 and 4 months after treatment with nialamide-6-OHDA (250 μg). A greater recovery was found for DA which was reduced 35% at 2 to 3 months and only 15% 4 months after treatment. Recovery of NE from 75 to 60% depletion in rat whole brain between 1 and 21 weeks after administration of 6-OHDA (200 μg and 300 μg i.c., 72-hour interval) was also found by Hartman *et al.* (174). Further work is necessary to clarify the conditions under which regeneration of new CA-containing nerves will occur. A dose range of 6-OHDA may be found that could result in degeneration followed by axonal sprouting. This has been observed with the use of 6-hydroxydopa (299) (see section XIV).

As can be seen in table 1, the acute changes in NE or DA do not always correlate well with the long-term effects of 6-OHDA on CA

levels. Iversen and Uretsky (205, 206) studied the initial time-course of alteration of various parameters of monoamine function in rat brain in an attempt to determine whether 6-OHDA could acutely deplete catecholamines before occurrence of chronic neuronal degenerative changes. In the hypothalamus, a region rich in noradrenergic terminals, tyrosine hydroxylase activity, *in vitro* uptake capacity for NE, and NE content were simultaneously decreased 30 to 40%, 30 minutes after a large dose of 6-OHDA (250 μg ivt.). At various intervals up to 48 hours, further parallel decreases in enzyme activity and NE content occurred so that alteration in NE content appeared to be related to the impairment of neuronal function, *i.e.*, the severity of degeneration. In the striatum, the same measurement revealed that both the tyrosine hydroxylase activity and NE uptake capacity were reduced far in advance of the decline in DA content. In fact, DA content increased by 25 to 70%, 30 minutes to 24 hours postinjection, so that at 24 hours DA was 135% of control while tyrosine hydroxylase and NE uptake were only 50% of control values. Even after a low dose of 6-OHDA (25 μg, ivt.) DA was markedly elevated (90%) while the other two parameters were unaffected. The reason for the rise in DA levels is unexplained, but could be related to an inhibition of MAO in this region, although studies both *in vivo* and *in vitro* indicate that 6-OHDA has little effect on activity of this enzyme (refer to section VC). Whether the "dopamine-monoamine oxidase" isozyme is selectively inhibited remains to be determined. Only after a low dose of 6-OHDA (25 μg, ivt.) does there appear to be an indication that NE depletion precedes ultrastructural damage (27, 35).

In an effort to produce a more selective depletion and/or destruction of only one division of the central catecholamine neuronal systems, various procedures have been employed. Evetts and Iversen (120) first took advantage of the finding that several of the tricyclic antidepressants such as desipramine and protriptyline, exert a more selective inhibition of catecholamine-uptake on noradrenergic neurons as compared with dopaminergic neurons (148, 167, 332). It was shown that when administration of protriptyline (15 mg/kg i.p.) preceded 6-OHDA treatment (100 or 150 μg ivt.) by 2 hours, there was a selective depletion of brain DA after four days. DA levels were reduced 50 to 60% while NE content of whole brain was unchanged. Similarly, when administration of desipramine (25 mg/kg i.p.) preceded 6-OHDA treatment (200 μg i.c.), a preferential depletion of DA occurred in the CNS, with NE content remaining virtually unchanged (59). Thus, uptake of 6-OHDA into noradrenergic neurons was partially inhibited, so that more 6-OHDA molecules were allowed to come into contact with the monoamine uptake sites of dopaminergic neurons.

Administration of a series of low doses of 6-OHDA (25 μg, 3 times ivt., 48-hour intervals; or 25 μg 2 times plus 50 μg i.c., weekly intervals) has been found to selectively deplete the CNS of NE, with DA stores remaining essentially unaltered (59, 324). With the lower dose of 6-OHDA, the NE and DA content of rat brain was 25 and 100% of control, respectively, seven days after treatment (324). The higher dose reduced NE by 60% while DA was unaltered (59). Breese and Traylor (58) also showed that when administration of pargyline (50 mg/kg i.p.) preceded 6-OHDA treatment (25 to 200 μg i.c.) by 30 minutes, there was a partial inhibition of the usual NE depleting action with lower doses of 6-OHDA (25 to 50 μg), and essentially no modification of NE depletion with higher doses of the neurotoxic agent. Conversely, pargyline pretreatment had no effect on DA depletion produced by lower doses of 6-OHDA (25 to 50 μg), but greatly potentiated the DA depleting action with higher doses of 6-OHDA (100 to 200 μg). Before pargyline administration, 6-OHDA (100 to 200 μg) reduced brain DA by 40%, but after pargyline administration the same doses of 6-OHDA reduced DA content

by 65 to 75%. Thus, administration of a high dose of 6-OHDA (200 μg i.c.) preceded by pargyline (50 mg/kg i.p.) would produce a high degree of depletion of both NE and DA stores in the brain, with each system now being about equally sensitive to 6-OHDA. When a second injection of 6-OHDA is administered a week after the first one, in the absence of pargyline, an even greater depletion of catecholamines is produced in the CNS.

Maximal depletion of both NE and DA in the CNS is accomplished most easily with this regimen or with two or more intraventricular injections of 6-OHDA. Two of the most commonly employed dosage regimens are those of Iversen and Uretsky (205) and Breese and Traylor (59). Because an intraventricular injection is given unilaterally, one might wonder whether the damage to catecholamine-containing neurons is greater on the injected side. There are indications that the intraventricular administration of 6-OHDA may produce asymmetrical effects, at least initially, with the injected side being most altered. Such appeared to be the case in studies by Laverty and Taylor (241), and Vetulani *et al.* (382), who noted asymmetrical motor activity in rats during the first few hours after treatment. Analysis of hemisections of the brain during this period showed that NE content was reduced 15% on the side in which 6-OHDA was injected (169 μg, after nialamide), and only 5% on the section which received no injection. This asymmetry was found to persist for at least four months. It was also found that DA rose to a higher level on the side in which 6-OHDA was injected (175%) than on the side of the brain which received no injection (150%), although this difference was nonexistent within nine days. No marked asymmetrical differences of 5-HT content were found after administration of nialamide and 6-OHDA (382). Whether asymmetrical biochemical alterations are produced after intraventricular injections of 6-OHDA alone remains to be determined, although none was found from several hours to 32 days after treatment. No analyses were performed during the period when asymmetrical motor activity was observed, however, so that acute effects of this type are possible (241). In the rats treated with nialamide and 6-OHDA, needle placement was not verified histologically (382) so that a possible injection into the striatum cannot be ruled out. Such an event probably would produce the observed motor effect.

Because various anesthetic agents often mask or potentiate certain physiological responses due to their effects at the cellular level, it is particularly noteworthy that pentobarbital was found to reduce the NE depleting action of 6-OHDA. Anesthetization of rats with pentobarbital during intraventricular injections of 6-OHDA (411 μg) was found to result in a 25% reduction of NE levels in prepontine regions while ether anesthesia, during a similar procedure, resulted in a 70% reduction of NE in that region. 6-OHDA, administered during pentobarbital anesthesia, did not alter NE content in the lower brainstem, while similar treatment during ether anesthesia decreased NE by 25% (207). The effect of other anesthetic agents on 6-OHDA still remains to be studied, but it is noteworthy that in almost all studies to date ether anesthesia is used during intraventricular injections of 6-OHDA.

Only a few studies relating to intracerebral injections of 6-OHDA in species other than the rat have been reported (see table 2). In general, it appears that the regions of the CNS which exhibit the greatest degree of NE depletion after administration of 6-OHDA are the same as those found in the rat, namely, the spinal cord, cerebellum, and telencephalon. Comparisons between species of sensitivity to 6-OHDA are difficult to make because of the limited number of studies and also because of the differences in the relative doses used. Thus, as the brain increases in size the total dose of 6-OHDA needs to be increased to maintain cerebrospinal fluid concentrations of the drug identical with those in smaller animals after lower

TABLE 2

*Effects of 6-OHDA on catecholamine content (μg/g) in whole brain and brain regions of various species*

| Species | Brain Region | Total Dose 6-OHDA (Free Base) | Route | Time of Sacrifice after Final 6-OHDA Dose | Control | | | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | | | NE | DA | E | |
| | | μg | | | % | % | % | |
| Frog | Forebrain | (100 mg/kg) | Dorsal | 4 days | N.S.[a] | | N.S. | 198 |
| | Hypothalamus | | lymph | | 45 | | 55 | 198 |
| | Pituitary | | sac | | 20 | | 15 | 198 |
| Rabbit | Whole brain | 600 | i.c. | 1 week | 55 | | | 74 |
| | | | | 3 weeks | 30 | | | 74 |
| | Telencephalon | 200 | i.c. | | 45 | | | 74 |
| | | 600 | | | 15 | | 45 | 74 |
| | Hypothalamus | 200 | | | 55 | | | 74 |
| | | 600 | | | 30 | | | 74 |
| | Midbrain | 200 | | | 40 | | | 74 |
| | | 600 | | | 35 | | | 74 |
| | Pons medulla | 200 | | | 60 | | | 74 |
| | | 600 | | | 50 | | | 74 |
| | Cerebellum | 200 | | | 20 | | | 74 |
| | | 600 | | | 3 | | | 74 |
| | Cervical cord | 200 | | | 20 | | | 74 |
| | | 600 | | | 10 | | | 74 |
| | Thoracolumbar cord | 200 | | 3 weeks | 30 | | | 74 |
| | | 600 | | | 5 | | | 74 |
| | Lumbrosacral cord | 200 | | | 35 | | | 74 |
| | | 600 | | | 3 | | | 74 |
| | | | | 1 week | 40 | | | 74 |
| | Spinal cord | 600 | | 3 weeks | 10 | | | 74 |
| Monkey | Cortex | 21,000[b] | ivt. | 16 days | 30 | | | 254 |
| | Hypothalamus | | | | 10 | | | 254 |
| Cat | Cortex | 300 | ivt. | 14 days | 40 | | | 291 |
| | | 600 | | | 40 | | | 291 |
| | | 1200 | | | 45 | | | 291 |
| | | 2500 | | | 35 | | | 291 |
| | | 5000[c] | | | 35 | | | 291 |
| | Striatum | 300 | ivt. | | 85 | N.S. | | 291 |
| | | 600 | | | 75 | 80 | | 291 |
| | | 1200 | | | 70 | 85 | | 291 |
| | | 2500 | | | 55 | 60 | | 291 |
| | | 5000[c] | | | 60 | 55 | | 291 |
| Cat | Hypothalamus | 300 | ivt. | | 40 | | | 291 |
| | | 600 | | | 40 | | | 291 |
| | | 1200 | | | 45 | | | 291 |
| | | 2500 | | | 35 | | | 291 |
| | | 5000[c] | | | 40 | | | 291 |
| | Thalamus | 300 | ivt. | | 45 | | | 291 |
| | | 600 | | | 45 | | | 291 |
| | | 1200 | | | 45 | | | 291 |
| | | 2500 | | | 40 | | | 291 |
| | | 5000[c] | | | 30 | | | 291 |
| | Midbrain | 300 | ivt. | | 55 | | | 291 |
| | | 600 | | | 60 | | | 291 |
| | | 1200 | | | 65 | | | 291 |
| | | 2500 | | | 50 | | | 291 |
| | | 5000[c] | | | 50 | | | 291 |

TABLE 2—*Continued*

| Species | Brain Region | Total Dose 6-OHDA (Free Base) | Route | Time of Sacrifice afer Final 6-ODDA Dose | Control | | | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | | | NE | DA | E | |
| | | μg | | | % | % | % | |
| Cat | Pons medulla | 300 | ivt. | | 50 | | | 291 |
| | | 600 | | | 40 | | | 291 |
| | | 1200 | | | 75 | | | 291 |
| | | 2500 | | | 50 | | | 291 |
| | | 5000[c] | | | 40 | | | 291 |
| | Cerebellum | 300 | ivt. | | 40 | | | 291 |
| | | 600 | | | 30 | | | 291 |
| | | 1200 | | | 45 | | | 291 |
| | | 2500 | | | 30 | | | 291 |
| | | 5000[c] | | | 30 | | | 291 |
| | Cervical cord | 300 | ivt. | | 65 | | | 291 |
| | | 600 | | | 75 | | | 291 |
| | | 1200 | | | 85 | | | 291 |
| | | 2500 | | | 45 | | | 291 |
| | | 5000[c] | | | 35 | | | 291 |

[a] N.S., no significant difference between 6-OHDA- and control groups.
[b] 0.68, 1.40, 2.70, 5.40 mg of 6-OHDA at 12-hour intervals plus 10.7 mg of 6-OHDA after a seven-day interval.
[c] Two equimolar doses of 6-OHDA, 24-hour interval.

doses. Specific doses employed in different species of animals are presented in table 2.

### *B. Uptake of Catecholamines*

In the CNS, 6-OHDA inhibited uptake *in vivo* of both $^3$H-NE and $^3$H-DA by rat whole brain within one hour, and effects were still notable three weeks later (58, 241). Subsequent studies demonstrated that 6-OHDA reduced uptake *in vitro* of $^3$H-NE in homogenates of rat hypothalamus and striatum by 30 and 15%, respectively, after 30 minutes. By two hours, uptake was near maximal reduction, about 60%, in both homogenates and slices of hypothalamus. In the striatum there was a progressive reduction of $^3$H-NE uptake through 48 hours, at which time uptake by homogenates and slices was reduced approximately 60% (35). Uptake of NE is reduced more rapidly or effectively in the hypothalamus and striatum than in the pons medulla. Alterations in uptake are seen during the first hour in the former two regions but are not apparent in the pons medulla until six hours have passed (380).

Since the hypothalamus and striatum are areas rich in CA nerve endings, they would be most susceptible to effects of 6-OHDA, while the pons medulla contains a large proportion of CA cell bodies which are least affected by the drug. Therefore, the reduced $^3$H-NE uptake in the striatum and hypothalamus appears to be due to a loss of uptake sites through degeneration, whereas $^3$H-NE accumulation by the pons medulla, to a larger degree, is probably into cell bodies and/or nonterminal axons (neuronal components which are least sensitive to 6-OHDA). It is noteworthy that the small effect of 6-OHDA in the pons medulla may not be just a simple matter of a lack of diffusion of the agent to this region since uptake from the ventricle of $^3$H-NE by the spinal cord *in vitro* was markedly reduced after treatment with 6-OHDA (205, 380).

It appears that 6-OHDA alters uptake of NE by noradrenergic neurons more selectively than by dopaminergic neurons since $^3$H-NE uptake is reduced 40% in the hypothalamus 2 or 16 days after treatment with 6-OHDA (100 μg ivt.), while striatal uptake

of NE is not significantly impaired (377). Also, uptake of $^3$H-GABA and $^3$H-5-HT into slices of rat hypothalamus is unaffected by 6-OHDA (378), although octopamine uptake into rat brain homogenates is reduced. Octopamine uptake was also inhibited by drugs known to block CA uptake, which indicates that this substance is taken up by adrenergic neurons (24).

When the brains of rats treated with 6-OHDA were dissected into discrete regions, uptake of $^3$H-NE was reduced 85 to 90% in homogenates from the hippocampus, neocortex, hypothalamus, and midbrain. In the striatum, pons medulla, cerebellum, and spinal cord the same dose of 6-OHDA (250 $\mu$g, ivt. 2 times, 48-hour interval) reduced uptake of $^3$H-NE by 70 to 80% after 16 to 25 days. Thus, uptake of $^3$H-NE was altered markedly in all regions of the brain (205). When these results were compared with those obtained under similar conditions on slices of striatum, hypothalamus and pons medulla (380) it could be seen that the findings were identical in the former two regions, but less uptake-inhibition was observed on slices of the pons medulla. As noted above, this region contains the majority of the noradrenergic perikarya found in the brain and this component of the neuron is most resistant to alteration by 6-OHDA, whereas the noradrenergic terminals are least resistant. The study on slices would include uptake of $^3$H-NE by perikarya, axons, and terminals, while the synaptosomal preparation would only include study on the NE uptake into terminals. Therefore, uptake of NE would be expected to be reduced to a significantly greater degree in synaptosomes (75% reduction) from the pons medulla than in slices (55% reduction) (205, 380).

Iversen (203) studied the kinetics of $^3$H-NE uptake-inhibition by 6-OHDA in homogenates of rat hypothalamus and striatum, with Michaelis-Menten kinetics. Such analysis showed that 6-OHDA (20 $\mu$M) inhibited NE uptake competitively, but more effectively in the hypothalamus ($K_i$ = 21.9) than in the striatum ($K_i$ = 36.7). It was suggested that this difference accounted, at least in part, for the greater effectiveness of 6-OHDA in depleting brain NE rather than DA.

### *C. Enzyme Alterations*

Destruction of the entire catecholamine-containing neuron leads to a simultaneous decrease in the intracellular enzymes associated with these neurons in various tissues. T-OH, commonly used as a marker for noradrenergic or dopaminergic neurons, is found to decrease in activity whenever morphological damage is observed. It has also been suggested that decrease in T-OH may be the first observable index of destruction in noradrenergic neurons after 6-OHDA (200 $\mu$g i.c.), since conversion of $^{14}$C-tyrosine to $^{14}$C-catecholamine is reduced 75% in rat brainstem when no signs of destruction were observed under the electron microscope (29).

In CNS of the rat after treatment with 6-OHDA (250 $\mu$g ivt. 2 times, 48-hour interval), T-OH was found to be most reduced in the spinal cord, where activity remained only 10% of control 16 to 25 days after injection (205). In the neocortex, hippocampus, hypothalamus, and striatum T-OH was reduced by 70 to 80%, while in the midbrain and pons medulla the enzyme activity was reduced only about 50%. Thus, with T-OH as an index of neuronal integrity, it would appear that catecholamine-containing neurons in the brainstem are least damaged by 6-OHDA, while in all other regions of the brain these neurons are damaged to a nearly equal degree. On the other hand, axonal sprouting of fibers in the brainstem could result in higher levels of T-OH activity, and similarly, retrograde accumulation of the enzyme in the axons could also biochemically mask the severity of damage to terminal endings in this region.

Because of the reduction in the number of catecholamine-containing neurons and the decrease in T-OH activity, turnover of catecholamines in the brain decreases. Several investigators have found that the conversion *in vivo* of $^3$H-tyrosine to $^3$H-DA (58) or $^3$H-NE (28, 29, 47, 58, 291) is reduced in rat

brain after 6-OHDA, although formation of $^{3}$H-DA from $^{3}$H-dopa and conversion of $^{14}$C-tryptophan to $^{14}$C-5-HT is unaffected (47). The decreased rate of synthesis of DA from tyrosine has also been confirmed *in vitro* (281).

Despite the decreased turnover of NE in rat brain after 6-OHDA, Uretsky *et al.* (206, 380) have shown that the rate of disappearance of $^{3}$H-NE in the striatum and pons medulla of treated rats is greatly accelerated, so that surviving neurons apparently have a higher rate of turnover of NE (380). Total turnover of NE as stated previously, is still reduced in brains of rats treated with 6-OHDA because of the greatly reduced size of NE stores and lesser amounts of amine that can be released. Interestingly, turnover of brain NE was found to increase during the initial 48-hour interval after peripheral administration of 6-OHDA (20 mg/kg i.v.), at a dose which effectively destroyed a major portion of sympathetic terminals in numerous end organs (80).

Another marker of noradrenergic neurons, DBH, has also been found to decrease in rat cortex, hypothalamus (297), and sympathetic ganglia after administration of 6-OHDA (62, 76). Because DBH is presumably released along with NE upon nerve stimulation (103, 104, 150, 328, 329), it would be expected that serum DBH levels might decrease after destruction of a major portion of sympathetic terminals (20). Weinshilboum and Axelrod (388) found that such was indeed the case, although the reduction was minimal (20%) 72 hours after a high dose of 6-OHDA. It was postulated that the survival of sympathetic terminals in numerous structures such as blood vessels and gastrointestinal tract accounted for this fact. Demedullation did not affect the results and the possible elevation of serum levels of DBH inhibitors was ruled out. It would be of interest to know what contribution brain noradrenergic neurons contribute to the serum DBH levels.

Studies have indicated that treatment with 6-OHDA (250–500 μg ivt.) decreases dopa decarboxylase activity in rat frontal cortex by 65 to 70% after 3 days (126) and in rat striatum and hypothalamus by 60 and 25% respectively, after 16 days (378), although conversion of $^{14}$C-dopa to $^{14}$C-catecholamines is unaltered by a lower dose of 6-OHDA (28). Recently two different isozymes of dopa decarboxylase have been characterized by using different assay conditions, and were found to have different affinities for dopa and 5-hydroxytryptophan as substrates (326). In rats treated with 6-OHDA (169 μg i.c., pargyline pretreatment), the 5-hydroxytryptophan decarboxylase activity was unaltered in every brain region studied up to one week, while dopa decarboxylase activity was reduced throughout the brain. Two days after injection, activity was decreased only in the medial pontine medullary region, but by 7 days, the enzyme activity was lowered throughout the entire brainstem. By the 15th day dopa decarboxylase activity was also reduced in cortical regions of the brain, and 5-hydroxytryptophan decarboxylase activity was elevated by 35 to 40% in the cerebellum, hypothalamus, and lateral pons medulla. It was proposed that the changes in dopa decarboxylase activity were due secondarily to destruction of noradrenergic neurons. However, after 2 days, when marked destruction was noted, only slight changes in dopa decarboxylase activity were found. Thus, the enzyme appears to be localized to a large degree in nonadrenergic structures. The rise in tryptophan decarboxylase at 2 weeks appears to be due to a compensatory change that reflects a possible formation of tryptaminergic heterotypical synapses at noradrenergic or dopaminergic receptor sites (325). In this regard, sprouting has been observed in the CNS after treatment with 6-OHDA (285).

The actual effect of 6-OHDA on MAO and COMT, enzymes involved in the destruction of catecholamines, has not been well defined. Several reports have indicated that the levels of MAO (58, 378) and COMT (378) in hypothalamus or rat whole brain are little altered after administration of 6-OHDA. Lack of an

effect of 6-OHDA (20 mg/kg i.v.) on MAO in mouse atria, observed at intervals of one hour to one week, have been reported (221) while rat heart and salivary gland MAO activity is reduced 20 to 40%, respectively, three days after treatment with a higher dose of 6-OHDA (100 mg/kg i.v.) (100). Also, 6-OHDA *in vitro* does not alter MAO activity of rat liver at a concentration which effectively uncouples oxidative phosphorylation (384, 385). However, because of the many different isozymes of MAO (156, 169, 217, 235, 310, 391) and the importance of substrate specificity for different types, it is imperative that these factors be taken into account when studying the effect of an agent on activity. Agid *et al.* (2) have shown that injections of 6-OHDA (8 or 20 μg) directly into the substantia nigra bring about a decrease in activity of "dopamine monoamine oxidase," an isozyme showing a high degree of affinity for dopamine as a substrate. This particular isozyme, which has an activity in the striatum 70 times greater than in the cerebral cortex (86), is reduced approximately 30% while DA in the striatum is decreased 90% after treatment with 6-OHDA. Thus, degeneration of the nigroneostriatal tract after treatment with 6-OHDA, results in a slight to moderate decrease in the "dopamine monoamine oxidase," presumably because of intracellular localization in the dopaminergic neurons comprising this tract. The importance of selecting dopamine as substrate for observance of an effect, is illustrated by the finding that MAO activity in the same region is unaltered when kynuramine is substituted as the substrate. In the same study it was also found that aldehyde dehydrogenase activity of the striatum was reduced 55 to 60%, and previous studies (110) also have suggested localization of this enzyme in the dopaminergic nerves. These studies indicate that the effects of 6-OHDA on MAO in various organs or structures in different species will have to be determined on the isolated isozymes with different substrates in order to determine the actual degree of alteration in activity. It is also noteworthy that the amount of a particular enzyme in a specific type of nerve fiber may be small compared to that in the end organ or brain tissue under study (214–216) so that any alteration may be greatly masked.

Because of a lack of any overt action of 6-OHDA on MAO or COMT activity, the catabolic products of catecholamines more directly reflect their metabolism after the sympathetic discharge. After administration of 6-OHDA there is a proportionate increase in O-methylated and deaminated products, as has been found in isolated mouse atria (302) and rat heart (49, 183, 186, 356).

After injection of 6-OHDA into the CSF, the same basic type alterations in catecholamine metabolism are produced as after peripheral injection. There is a proportionate increase in both the O-methylated and deaminated metabolites, with the former elevated to a greater extent (58, 380). Urinary levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) presumably are increased during the sympathetic discharge (241) but decreased at later time periods (57, 254). Since MHPG has been suggested as the principle metabolite of NE in the brain of several species, (53, 255, 266, 313) the levels of MHPG may reflect turnover of brain NE. Because the decrease of MHPG is much less than the decrease seen in brain NE after treatment with 6-OHDA, a small decrease in urinary MHPG may reflect a much greater depletion of NE in the CNS (57). Vanillylmandelic acid (VMA), which is derived mainly from metabolism of NE outside the CNS (154, 255–257), is unaltered after injection of 6-OHDA into the CNS, since peripheral NE stores are unchanged (254).

It is interesting that increased production of cyclic adenosine monophosphate (cAMP) was stimulated by NE after incubation *in vitro* of the hypothalamus, brainstem, and cerebral cortex of rats treated with 6-OHDA (250 μg 2 times, ivt.). These results suggest a denervation supersensitivity in the CNS, and would reflect increased production of

adenyl cyclase in the postsynaptic cell (288). Supersensitivity after treatment with 6-OHDA has also been noted in the caudate (125, 368, 371, 372, 376) but not in the cerebellum (288).

## VI. Morphological Alterations of Catecholamine-containing Neurons after 6-OHDA Treatment

As stated in the INTRODUCTION, the discovery that administration of 6-OHDA brought about the destruction of sympathetic nerve terminals in various organs of the rat and cat (356, 363, 364) has been confirmed by numerous investigators in different tissues from various species (19, 48, 106, 109, 135, 194, 198, 229, 238, 243, 298, 322). The time-course of ultramorphological damage of the nerve terminals has been studied in great detail. It can be seen after a low dose of 6-OHDA (0.8 to 1.6 mg/kg i.v.) that the large vesicles of adrenergic neurons become filled with a dense electron opaque granulation after one hour, while higher doses of 6-OHDA (4.1 to 8.2 mg/kg i.v.) are required for loading of the small vesicles of the same neurons. The changes in morphology seen with these smaller amounts of 6-OHDA appear to be reversible since the adrenergic neurons no longer are filled with dark-staining vesicles and appear completely normal after 24 hours (37). Still larger doses of 6-OHDA (21 to 83 mg/kg i.v.) shorten the time required for loading, increase the proportion of vesicles affected, and increase the density and diameter of the cores of the filled vesicles. The granulation stage was followed shortly by a degranulation of the vesicles. Most axon profiles became swollen and some axonal membranes ruptured. Axon inclusions disappeared and abnormal necrotic mitochondria and broken tissue elements could be observed (37, 140). Because most of the axon profiles showed only temporary changes after low doses of 6-OHDA it was apparent that terminals were able to recover from the initial damage. However, with a higher dose of 6-OHDA most nerve terminals were damaged progressively, and the changes in this phase were classified as a "primary degeneration," the direct results of toxic intraneuronal concentrations of 6-OHDA. A further deterioration of nerves with membrane breakdown, classified as "secondary degeneration," occurred within several days after treatment with 6-OHDA.

These secondary changes appear to be the result of retrograde degenerative events after axonal damage, and have been studied in rat locus coeruleus (105). With the dose employed, 20% of the total population of noradrenergic perikarya disappeared within 48 hours as observed with fluorescent staining. At the end of one week, the noradrenergic perikarya were still capable of active amine uptake processes, active synthesis of NE from L-dopa, and appeared to have the capability of synthesizing protein. However, by the end of one month, only 35% of the original number of perikarya remained, with the remainder apparently destroyed by retrograde degenerative events, "secondary" to terminal-ending or axonal lesioning.

Despite the marked effect of 6-OHDA on noradrenergic terminals, it was found that cholinergic nerve endings (243, 322, 356), myelinated axons (243), Schwann cells (106, 243, 356), smooth muscle fibers (243, 356), and endothelial cells (243) were intact in the peripheral nervous system. Because axon sheaths are enveloped by Schwann cell processes or glial cells during the degenerative phase after treatment with 6-OHDA and because of the large number of inclusions in the Schwann cells or glial cells during this period, it appears that these cells are closely associated with the breakdown and elimination of nerve debris (19, 47).

The ultrastructural changes in the neuron could be correlated with disappearance of the number of nerves as seen with fluorescence staining (109, 198, 229), a loss of function (268), decreased tyrosine hydroxylase activity (see sections V and VIII), and reduced amine active uptake processes (see sections V B and VIII).

In the CNS, Bloom *et al.* (47), first described electron microscopic evidence of

neuronal damage after administration of 6-OHDA (200 μg plus 50 μg 2 times i.c.). Damage was found in the paraventricular nucleus of the hypothalamus, dorsal raphe nucleus, and cerebellar cortex, but not in the caudate nucleus. Later studies showed that higher doses of 6-OHDA (250 μg 2 times ivt.) would cause damage to terminals in this region (28, 29).

Therefore, microscopical observations confirm the biochemical data indicating that noradrenergic neurons are more sensitive to the deleterious effects of 6-OHDA than are dopaminergic terminals. Specific data of such studies are described in section V A.

In conjunction with electron microscopic observations of degenerating neurons the technique of Falck *et al.* (122–124) for fluorescent-staining of adrenergic nerves has also been used to study the neurotoxic actions of 6-OHDA. The specific microscopic alterations seen in adrenergic nerves in the CNS and in various end organs with this method are described in other sections along with related findings (see sections V and VIII). Rather than duplicate the details of such studies it was decided that an overview of the present status of central injections of 6-OHDA would be appropriate. Particularly because of the recent report of nonspecific damage after injection of 6-OHDA into the cerebrospinal fluid or into the parenchyma of the brain (292), there appears to be some confusion concerning the selectivity of 6-OHDA for CA-containing neurons in the CNS.

Currently, most studies that utilize 6-OHDA in the CNS are based on administration by the intraventricular or the intracisternal route. In these studies, diffusion from the ventricles is concentration-dependent, ranging from 0.5 mm penetration from the periventricular zone after 25 μg/20 μl to 1.5 to 2 mm after 200 μg/20 μl (371). These studies further have the disadvantage that, particularly with the larger doses, both noradrenergic and dopaminergic neurons are affected. Intracerebral injections, on the other hand, have the distinct advantage of destroying individual discrete systems of aminergic nerves and enables the study of neuronal pathways by fluorescence microscopic observation of the accumulated transmitter in axons caudal to the injection of 6-OHDA. The drug can be injected into cell body regions, axon bundles, or terminal areas (368, 371, 373). Such well defined, regional elimination of aminergic systems in the brain, correlated with behavioral changes, would provide functional evidence for the role of CA in the CNS. The initial studies on intracerebral injections of 6-OHDA were reported by Ungerstedt (368, 371, 373). The drug has been injected into CA cell groups (A1, A6, A9, A10, A12), axon bundles, or terminal areas. It appears that the cell groups have different levels of sensitivity to the drug. A dose of 8 to 20 μg in 4 to 5 μl has no effect on the NE-containing cell bodies of groups A1, A6 (locus coeruleus), or the dopamine-containing cell bodies of group A12 (arcuate nucleus). However, the 8-μg dose is capable of destroying the dopamine-containing substantia nigra cell bodies (A9, A10).

Injection of 6-OHDA into NE or DA axon bundles causes a retrograde accumulation of amine in the axons in six hours as shown by fluorescence microscopy (371). The maximum accumulation is observed at about one week after drug injection and then starts to decline. The fluorescence build-up of CA has been followed caudally in axons for up to 5 to 6 mm. The DA nerve terminals in the caudate nucleus lose their fluorescence 30 to 48 hours after injection of 6-OHDA into the axon bundles. The NE nerve terminals show a more gradual decrease in fluorescence over a period of four to seven days. No reappearance of nerve terminals was observed after two years in the cortex (NE terminals) or the caudate nucleus (DA terminals), which is convincing evidence for an actual degeneration of the axonal nerve trunks. Application of 6-OHDA to DA axons also causes retrograde destruction of substantia nigra cell bodies. This is also reminiscent of the slow retrograde degeneration of the locus coeru-

leus (A6) cell bodies caused by intraventricular injection of a large dose of 6-OHDA (105).

Injection of 8 μg of 6-OHDA in 4 μl of solution into the caudate nucleus resulted in a 1.5- to 2-mm area without amine fluorescence (371). It is pertinent to note that when the same amount of drug is injected in varying amounts of fluid the same area is essentially affected regardless of the volume of diluent. In addition to the specific destruction of CA neurons, intracerebral injection of 6-OHDA causes a nonspecific toxic damage at the tip of the cannulae (191, 292, 371). The extent of the damage increases with increasing doses of 6-OHDA. Poirier *et al.* (292) stated that the nonspecific damage caused by 6-OHDA makes interpretations of CA involvement unfeasible. However, Hökfelt and Ungerstedt (191) note that under proper dose conditions 6-OHDA is extremely useful for intracerebral administration. They point out that Poirier *et al.* (292) used a concentration of 6-OHDA five times higher. There is no question, however, that histological examination of the site of injection should be performed. The influence of unspecific damage should be studied by varying the site of injection in proximity to the target aminergic area.

## VII. Effects of 6-OHDA on Other Central Putative Transmitters

The effects of 6-OHDA on nonadrenergic neuronal systems have not been defined to nearly the same degree as with CA-containing neurons. Initial EM, autoradiographic, and histofluorescent studies indicated the relatively specific effect of 6-OHDA on noradrenergic and dopaminergic neurons (27, 28, 47, 87, 221, 249, 262, 322, 364, 368, 375), thereby discouraging detailed investigation of other neuronal systems. Also, 5-HT determinations in rat whole brain (44, 47, 58, 181, 205, 207, 331, 378, 379, 382) indicated for the most part that serotonergic neurons were little affected by 6-OHDA (169 to 600 μg i.c. or ivt., 2 hours to 4 months), if at all. Only scattered reports of 5-HT alterations appeared in the literature, and for the most part, they indicated that alterations in central levels of 5-HT were merely transient. Burkard *et al.* (67) first reported a 15% reduction of 5-HT in rat whole brain after treatment with 6-OHDA (250 μg ivt.) and this was substantiated by Bartholini *et al.* (27). Regional analysis of rat brain indicated that 5-HT in the telediencephalon (47), cerebral cortex (184), cerebellum (47), thalamus, and hypothalamus (184) were unaffected, but a 15% fall was noted in the mesencephalon accompanied by a 75% increase in the pons medulla, 7 hours after treatment with 6-OHDA (250 μg ivt.) (184). As much as a 50% reduction of 5-HT was observed in the brainstem 5 days after treatment with 6-OHDA (300 μg total, i.c.), and complete recovery occurred by four weeks (47). Similarly, a 55% reduction in 5-HT was observed in rat whole brain five days after treatment with 6-OHDA (272 μg 2 times, i.c.), although, as was noted by the authors, such a remarkable decline could have been more related to experimental stresses, as the animals were part of a shock-induced aggression study (111).

Other studies in the rat indicated that there was a normal complement of serotonergic neurons present after administration of 6-OHDA, as demonstrated by unimpaired ability of various brain regions to accumulate $^{3}$H-5-HT (46, 181, 223, 281, 303), and to spontaneously release an amount of 5-HT identical to that from brain slices of control rats (281). Also, formation *in vivo* of 5-HT from infused $^{14}$C-L-tryptophan indicated that tryptophan hydroxylase activity and turnover rate of 5-HT was unaltered in rat brain after injection of 6-OHDA (47) and measurement of tryptophan hydroxylase 40 to 60 days after treatment of neonatal rats with 6-OHDA (100 μg i.c.) directly demonstrated that enzyme activity was unaltered (331). Studies *in vitro* with raphe cultures further suggest that 6-OHDA (50 μg/ml) does not alter 5-HIAA production, *i.e.*, turnover of 5-HT (168).

Studies performed recently, however, suggest that 6-OHDA may have some direct effect on 5-HT-containing neurons, or at least result in compensatory increased activity. When rat brains are observed 43 hours to 8 days after administration of 6-OHDA, it can be seen that 5-HIAA levels are increased while 5-HT content is unaltered. Estimation of turnover of 5-HT by administration of the monoamine oxidase inhibitor, phenelzine (20 mg/kg), indicates that there is a 60% increased rate of 5-HT synthesis in the telediencephalon. Also, increased formation of both $^{3}$H-5-HT and $^{3}$H-5-HIAA from $^{3}$H-tryptophan substantiates the increased synthesis and utilization of 5-HT in various regions of the brain, including the cortex, cerebellum, pons medulla, and mesencephalon (44). Such findings are in direct contrast to earlier findings, and it is possible that the differences are more related to the interval between treatment and sacrifice. Also, turnover studies by Blondaux *et al.* (44) were performed after a single injection of labeled tryptophan, while Bloom *et al.* (47) infused tryptophan over a 45-minute period, so that differences in findings may be more related to the experimental procedure.

Sims and Bloom (325), in an attempt to define further the specificity of 6-OHDA, showed that specific dopa decarboxylase activity was decreased in all 10 of the different brain areas studied 2 and 15 days after treatment, but at no time was there a decrease in 5-hydroxytryptophan decarboxylase. Rather, there was an increase in 5-hydroxytryptophan decarboxylase activity in the cerebellum, hypothalamus, and lateral pons medulla, thus indicating increased tryptaminergic neuronal activity. It was suggested by the authors that such changes may be a reflection of formation of new functional, heterotypical synapses at sites deprived of their usual adrenergic innervation. Such sprouting phenomena have been observed by Nygren *et al.* (285) after administration of 6-OHDA. Neuronal plasticity (271) may be of importance in recovery from behavioral deficits observed after administration of 6-OHDA.

In the cat brain, 6-OHDA appears to have more of a direct action on 5-HT-containing neurons than in the rat (291). Fourteen days after graded doses of 6-OHDA (300 μg to 5 mg ivt., total dose of dual injections) there is a decrease in endogenous levels of 5-HT in cervical spinal cord and seven different regions of the brain, with a dose-related effect evident in most areas. Associated with these alterations is a decreased 5-HIAA content, suggestive of decreased activity of tryptaminergic neurons. Surprisingly, chlorimipramine, an inhibitor of monoamine uptake in serotonergic neurons (70), prevented the 5-HT depletion in all regions studied, even after the highest dose of 6-OHDA (291). Thus, there appears to be a species difference of the effects of 6-OHDA on 5-HT-containing neurons, further suggesting that 6-OHDA may not be as selective as originally suggested. Whether the species variation is related to a difference in neuronal uptake-capacity or intraneuronal degradative enzyme differences or to some other factors associated with the tryptaminergic and/or catecholaminergic neurons remains to be defined. Follow-up studies should shed more light on the functional, integrative, and associative role of the monoaminergic neurons in various species.

Aside from the studies on 5-HT, only a sparse number of reports are available on the effects of 6-OHDA on other putative CNS neurotransmitters. Studies to date indicate that glutamic acid, glutamine, glycine, aspartic acid, and other amino acids as taurine, alanine, threonine, and serine are unaltered in rat whole brain after treatment with 6-OHDA (330 μg 2 times, ivt.) (207). Likewise, *gamma*-aminobutyric acid (GABA) levels appear to be unaltered by 6-OHDA (207, 378). Acetylcholine levels reportedly are unaltered 15 days after treatment with 6-OHDA (330 μg 2 times, ivt.), which indicates that central cholinergic neurons also are not affected (207), although results on choline acetyltransferase (CAT)

indicate some ambiguity on this point. One study reports that CAT activity was unaltered in rat whole brain, striatum, hypothalamus, brainstem, and "rest of brain" 40 to 60 days after neonatal treatment with 6-OHDA (100 μg/g) (331). However, Ho and Loh (188) report that 6-OHDA (200–250 μg, i.c.) increases CAT by 25% in rat cerebral cortex after four days, although no alterations in activity of the enzyme are seen in the "rest of brain." Also, a 10 to 15% increase in CAT was found in the optic lobe of chicken brain 24 hours after treatment with 6-OHDA (200 mg/kg 2 times, i.p.) (265). It appears that such an effect is compensatory in nature and not part of a direct effect of 6-OHDA, since 5-OHDA and DA produce similar alteration in brain CAT (188). In addition, a 40% increase in CAT is observed in rat submaxillary glands after 6-OHDA, as with surgical sympathetic denervation (112). It should also be noted that 6-OHDA and DA ($5 \times 10^{-3}$ M) *in vitro* reduce CAT activity of rat brain homogenates, while 5-OHDA ($5 \times 10^{-3}$ M) slightly increases activity (188).

Naturally, selective small regional effects of 6-OHDA on the above substances may be masked by whole brain analysis, so that any negative results can only be used as an index of the generalized effect of 6-OHDA on the putative transmitter. Conversely, a change in content of any of the substances could be more a reflection of a secondary change, accompanying destruction of CA-containing neurons. Such phenomena still remain to be more fully investigated.

## VIII. Effects of 6-OHDA on Peripheral Autonomic Structures

When 6-OHDA is administered to animals by an intravenous, intraperitoneal, or subcutaneous route, the monoamine is accumulated selectively by postganglionic sympathetic (noradrenergic) neurons (see section III A). The selectivity of action appears to be due to the active, energy-dependent, accumulation of 6-OHDA *via* the monoamine-uptake pump of the noradrenergic neurons. Numerous agents known to inhibit this process are capable of antagonizing the biochemical and morphological alterations brought about by 6-OHDA (see section V). To date no studies of 6-OHDA have been made on postganglionic cholinergic sympathetic neurons.

From observation of the data in table 3, several features are apparent. 6-OHDA is capable of decreasing the NE content of all sympathetically-innervated end organs, as well as the NE content of sympathetic pre- and paraganglia. The temporal sequence for production of the chemical lesions is different among organs, as is the sensitivity of various organs between species. Recovery times of various organs have been shown to be somewhat different, a factor of major importance when studying a particular system that has been chemically denervated. Numerous ramifications of the effects of 6-OHDA on peripheral organs will be discussed in this section.

### *A. Route of Administration of 6-OHDA*

An intraperitoneal and intravenous route of administration of 6-OHDA appears to be equally effective in producing depletion of NE in various end organs innervated by the peripheral autonomic nervous system (see table 3). Depletion of NE in the mouse heart was measured at intervals from 15 minutes to about two weeks after a 10 mg/kg dose by either an intravenous or intraperitoneal route (240). At all time periods the percent of depletion of NE was nearly equal. In the rat whole heart, a 20 mg/kg i.v. dose appeared to be nearly equivalent to a 30 mg/kg i.p. dose when measured after 48 hours, although recovery appeared to be occurring more rapidly in animals injected intraperitoneally (80, 268). With high doses of 6-OHDA, the intraperitoneal route appeared to be less effective in reducing the NE content of the whole heart, since a 170 mg/kg intraperitoneal dose produced only a 75% depletion of NE after 24 hours (65), while a 100 mg/kg i.v. dose produced 95% depletion (277). However, in

TABLE 3

*Effect of 6-OHDA on NE content of various tissues in several species of animals*

| Species | Tissue | Total Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Mouse | Whole heart | 1 | i.v. | 1 hour | 80 | 221 |
| | | | | 20 hours | 85 | 221 |
| | | | | 3 days | N.S.[a] | 221 |
| | | 2.5 | i.v. | 24 hours | 65 | 294 |
| | | 3.0 | | 24 hours | 35 | 294 |
| | | 3.4 | | 2 hours | 40 | 95 |
| | | 5.0 | i.p. | 16 hours | 20 | 338 |
| | | 5.0[b] | i.v. | 16–24 hours | 60 | 294 |
| | | 5.0 | i.v. | 24 hours | 35 | 294 |
| | | | | 48 hours | 30 | 294 |
| | | 6.0[b] | | 16–24 hours | 35 | 294 |
| | | 6.0 | | 24 hours | 15 | 294 |
| | | 6.7 | i.p. | 16 hours | 40 | 240 |
| | | | | 17 hours | 70 | 240 |
| | | 9.0[b] | i.v. | 16–24 hours | 15 | 294 |
| | | 10 | i.v. | 15 minutes | 60 | 240 |
| | | | i.p. | | 85 | 240 |
| | | | i.v. | 30 minutes | 70 | 240 |
| | | | i.p. | | 50 | 240 |
| | | | i.v. | 1 hour | 30 | 240 |
| | | | i.p. | | 10 | 240 |
| | | | i.v. | 17 hours | 15 | 240 |
| | | | i.p. | | 5 | 240 |
| | | | i.p. | 24 hours | 10 | 293 |
| | | | | 48 hours | 15 | 293 |
| | | | i.v. | 48 hours | 15 | 240 |
| | | | i.p. | | 15 | 240 |
| | | | | 4 days | 20 | 293 |
| | | | i.v. | 7 days | 25 | 240 |
| | | | i.p. | 8 days | 30 | 293 |
| | | | i.v. | 14 days | 20 | 240 |
| | | | i.p. | 14 days | 85 | 240 |
| | | | i.p. | 16 days | 50 | 293 |
| | | | i.p. | 24 days | 55 | 293 |
| | | | | 44 days | 70 | 293 |
| | | 20 | i.v. | 15 minutes | 75 | 221 |
| | | | | 1 hour | 20 | 221 |
| | | | | 8 hours | 5 | 221 |
| | | 34 | s.c. | 2 hours | 40 | 354 |
| | | 100[c] | i.v. | 2 hours | 5 | 221 |
| | | | | 1 week | 25 | 221 |
| | | | | 2 weeks | 35 | 221 |
| | | | | 4 weeks | 50 | 221 |
| | | | | 8 weeks | 90 | 221 |
| Rat | Whole heart | 1.0 | i.v. | 2 hours | 65 | 356 |
| | | | | 24 hours | 90 | 356 |
| | | | | 8 days | 90 | 356 |
| | | 3.0 | | 2 hours | 65 | 356 |
| | | | | 24 hours | 55 | 356 |
| | | | | 8 days | 55 | 356 |

TABLE 3—*Continued*

| Species | Tissue | Total Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Rat | Whole heart | 20 | i.v. | 30 minutes | (90)[d] | 80 |
| | | | | 3 hours | (90) | 80 |
| | | | | 16 hours | 10 | 80 |
| | | | | 24 hours | 15 | 80 |
| | | | | 52 hours | 10 | 80 |
| | | 30 | | 2 hours | 20 | 356 |
| | | | | 24 hours | 15 | 356 |
| | | | i.p. | 2 days | 30 | 239 |
| | | 30 | | 2 days | 15 | 268 |
| | | | | 3 days | 20 | 268 |
| | | | | 4 days | 40 | 268 |
| | | | | 8 days | 40 | 268 |
| | | | i.v. | 8 days | 15 | 356 |
| | | 80 | i.v. | 5 days | 10 | 390 |
| | | | | 5 days | 15 | 390 |
| | | 80[e] | i.v. | 8 days | 30 | 264 |
| | | 83 | i.v. | 72 hours | 10 | 207 |
| | | 100 | i.v. | 1 hour | 30 | 100 |
| | | | | 2 hours | 5 | 100,277 |
| | | | | 7 hours | 5 | 100 |
| | | | | 24 hours | 5 | 277 |
| | | | | 72 hours | 10 | 277 |
| | | | | 1 week | 15 | 100 |
| | | | | 2 weeks | 15 | 277 |
| | | | | 3 weeks | 30 | 100 |
| | | 170 | i.p. | 24 hours | 25 | 65 |
| | | 200[f] | i.v. | 8 days | 15 | 390 |
| | | | | 18 days | 20 | 390 |
| | | 204[g] | i.v. | 1 week | 10 | 356 |
| | | | | 8 days | 15 | 40 |
| | | | | 2 weeks | 10 | 356 |
| | | | | 1–2 weeks | 20 | 356 |
| | | | | 4 weeks | 30 | 356 |
| | | 204[b] | | 3 days | 25 | 183 |
| | | 219[h] | | 72–78 days | 0 | 158 |
| | | 272[b] | | 1 day | 10 | 62 |
| | | 1300[i] | | 1 week | 2 | 277 |
| Guinea pig | Whole heart | 10 | i.v. | 2 hours | 20 | 240 |
| | | | i.p. | 16 hours | 65 | 240 |
| | | 15 | i.v. | 2.5 hours | 20 | 240 |
| | | 34 | i.p. | 24 hours | 5 | 40 |
| | | 204[g] | i.v. | 1–2 weeks | 25 | 9 |
| Rabbit | Whole heart | 50 | i.v. | 20 hours | 5 | 258 |
| Cat | Whole heart | 96[j] | i.v. | 7 days | 3 | 356 |
| | | | | 2 weeks | 3 | 165 |
| | | | | 4 weeks | 15 | 165 |
| | | | | 8 weeks | 30 | 165 |
| | | | | 14 weeks | 80 | 165 |
| Mouse | Cardiac ventricles | 1 | i.v. | 24 hours | 90 | 221 |
| | | 2 | | | 55 | 221 |
| | | 5 | | 1 hour | 50 | 221 |

TABLE 3—*Continued*

| Species | Tissue | Total Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Mouse | Cardiac ventricles | 5 | i.v. | 4 hours | 35 | 221 |
| | | | | 24 hours | 40 | 221 |
| | | | | 96 hours | 60 | 221 |
| | | 10 | | 24 hours | 30 | 221 |
| Cat | Auricle | 70[b] | i.v. | 1 week | 10 | 383 |
| Dog | R. auricular appendage | 0.62[b] | i.v. | 16–24 hours | 35, 50 | 339 |
| | | 1.25[b] | i.v. | 16–24 hours | 10, 25 | 339 |
| | | 2.5[b] | i.v. | 16–24 hours | 10, 15 | 339 |
| | | 5.0[b] | i.v. | 16–24 hours | 20 | 339 |
| Rat | Cardiac ventricles | 16.5 | i.p. | 1 day | 5 | 155 |
| | | | | 1 week | 35 | 155 |
| | | | | 2 weeks | 45 | 155 |
| | | | | 4 weeks | 50 | 155 |
| | | | | 8 weeks | 60 | 155 |
| | | | | 12 weeks | 70 | 155 |
| | | | | 16 weeks | 70 | 155 |
| | | 100 | i.v. | 1 hour | 30 | 99 |
| | | | | 2 hours | 5 | 99 |
| | | | | 7 hours | 5 | 99 |
| | | | | 1 week | 10 | 99 |
| | | | | 2 weeks | 20 | 99 |
| | | | | 3 weeks | 30 | 99 |
| | | | | 4 weeks | 30 | 99 |
| | | | | 6 weeks | 50 | 99 |
| Rat | Right ventricle | 0.8 | i.v. | 2.5 hours | 85 | 155 |
| | | | | 24 hours | 75 | 155 |
| | | 4.1 | | 2.5 hours | 45 | 155 |
| | | | | 24 hours | 30 | 155 |
| | | 8.2 | | 2.5 hours | 10 | 155 |
| | | | | 24 hours | 10 | 155 |
| | | 16.5 | | 2.5 hours | 5 | 155 |
| | | | | 24 hours | 5 | 155 |
| Cat | Right ventricle | 70[b] | | 1 week | 15 | 383 |
| Rat | Left ventricle | 0.8 | i.v. | 2.5 hours | 65 | 155 |
| | | | | 24 hours | 80 | 155 |
| | | 4.1 | | 2.5 hours | 20 | 155 |
| | | | | 24 hours | 25 | 155 |
| | | 8.2 | | 2.5 hours | 10 | 155 |
| | | | | 24 hours | 20 | 155 |
| | | 16.5 | | 2.5 hours | 10 | 155 |
| | | | | 24 hours | 10 | 155 |
| Cat | Left ventricle | 70[b] | | 1 week | 20 | 383 |
| Rat | Aorta | 204[g] | i.v. | 8 days | 40 | 40 |
| Guinea pig | Thoracic aorta | 34 | i.p. | 24 hours | 45 | 40 |
| | | 204[g] | i.v. | 1–2 weeks | 15 | 258 |
| Rat | Renal artery | 204[g] | i.v. | 1 day | 35 | 128 |
| | | | | 7 days | 75 | 128 |
| | | | | 14 days | N.S. | 128 |
| | | | | 21 days | (115) | 128 |
| Guinea pig | Mesenteric artery | 34 | i.p. | 24 hours | 50 | 40 |
| Rat | Mesenteric artery | 204[g] | i.v. | 1 day | 40 | 128 |
| | | | | 7 days | 50 | 128 |

TABLE 3—*Continued*

| Species | Tissue | Total Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Rat | Mesenteric artery | 204[g] | i.v. | 8 days | 70 | 40 |
| | | | | 14 days | 70 | 128 |
| | | | | 21 days | 85 | 128 |
| Rat | Mesenteric vein | 34 | i.p. | 24 hours | 15 | 40 |
| | | 204[g] | i.v. | 8 days | 25 | 40 |
| Mouse | Spleen | 6.7 | i.p. | 16 hours | N.S. | 240 |
| Rat | Spleen | 1 | i.v. | 2 hours | 75 | 356 |
| | | | | 24 hours | N.S. | 356 |
| | | | | 8 days | N.S. | 356 |
| | | 3 | i.v. | 2 hours | 70 | 356 |
| | | | | 24 hours | N.S. | 356 |
| | | | | 8 days | N.S. | 356 |
| | | 30 | i.v. | 2 hours | 30 | 356 |
| | | | | 24 hours | 20 | 356 |
| | | | i.p. | 2 days | 20 | 268 |
| | | | i.v. | 2 days | 45 | 239 |
| | | | i.p. | 3 days | 40 | 268 |
| | | | | 4 days | 65 | 268 |
| | | | | 6 days | 25 | 268 |
| | | | | 8 days | 55 | 239 |
| | | | i.v. | 8 days | 25 | 356 |
| | | 80 | | 5 days | 15 | 390 |
| | | 96[i] | i.v. | 8 weeks | 75 | 165 |
| | | | | 14 weeks | N.S. | 165 |
| | | 100 | | 2 weeks | 25 | 99 |
| | | 200[j] | | 8 days | 15 | 390 |
| | | | | 18 days | 25 | 390 |
| | | 204[g] | i.v. | 1 week | 5 | 356 |
| | | | i.v. | 8 days | 15 | 40 |
| | | 204 | i.v. | 2 weeks | 3 | 356 |
| | | | | 4 weeks | 10 | 356 |
| Guinea pig | Spleen | 10 | i.v. | 2 hours | 25 | 240 |
| | | | i.p. | 16 hours | 50 | 240 |
| | | 15 | i.v. | 2.5 hours | 30 | 240 |
| Cat | Spleen | 70[k] | i.v. | 1 week | 35 | 383 |
| | | 96[i] | i.v. | 1 week | 30 | 356 |
| | | | | 2 weeks | 5 | 165 |
| | | | | 4 weeks | 15 | 165 |
| Rat | Salivary gland | 0.8 | i.v. | 2.5 hours | N.S. | 155 |
| | | | | 24 hours | N.S. | 155 |
| | | 4.1 | | 2.5 hours | 20 | 155 |
| | | | | 24 hours | 25 | 155 |
| | | 8.2 | | 2.5 hours | 10 | 155 |
| | | | | 24 hours | 20 | 155 |
| | | 16.5 | | 2.5 hours | 10 | 155 |
| | | | i.p. | 24 hours | 2 | 155 |
| | | | i.v. | 24 hours | 5 | 155 |
| | | | i.p. | 1 week | 40 | 155 |
| | | | | 2 weeks | 40 | 155 |
| | | | | 4 weeks | 70 | 155 |
| | | | | 8 weeks | N.S. | 155 |
| | | | | 12 weeks | N.S. | 155 |
| | | | | 16 weeks | N.S. | 155 |

TABLE 3—*Continued*

| Species | Tissue | Tota Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Rat | Salivary gland | 70[k] | i.v. | 2 weeks | 15 | 383 |
| | | 100 | | 2 weeks | 70 | 99 |
| Mouse | Iris | 20 | i.v. | 30 minutes | 55 | 189 |
| | | | | 1 hour | 5 | 189 |
| | | | | 16 hours | 10 | 189 |
| | | | | 1 week | 30 | 189 |
| | | | | 6 weeks | 75 | 189 |
| Cat | Iris | 96[i] | i.v. | 1 week | 3 | 356 |
| | | | | 2 weeks | 5 | 165 |
| | | | | 4 weeks | 20 | 165 |
| | | | | 8 weeks | 80 | 165 |
| | | | | 14 weeks | N.S. | 165 |
| Cat | Nictitating membrane | 30 | s.c. | 3 hours | 40 | 385 |
| | | 70 | i.v. | 3 weeks | 30 | 383 |
| | | 96[i] | | 7 days | 10 | 356 |
| | | | | 6–10 days | 10 | 165 |
| | | | | 2–3 weeks | 20 | 165 |
| | | | | 8–9 weeks | 70 | 165 |
| Dog | | 0.62[b] | i.v. | 16–24 hours | (90) | 339 |
| | | 1.25[b] | | 16–24 hours | 65 | 339 |
| | | 2.5 | | 16–24 hours | (70) | 339 |
| | | 5 | | 16–24 hours | (85) | 339 |
| Rat | Kidney | 80 | i.v. | 5 days | 35 | 390 |
| | | 80 | i.v. | 5 days | 40 | 390 |
| | | 100[f] | | 8 days | 55 | 390 |
| | | | | 18 days | 55 | 390 |
| Mouse | Lung | 6.7 | i.p. | 16 hours | N.S. | 240 |
| Cat | Lung | 70[k] | i.v. | 1 week | (75) | 383 |
| Rat | Intestine | 100 | i.v. | 2 weeks | 30 | 99 |
| Cat | Stomach | 70[k] | | 3 weeks | 25 | 383 |
| | Duodenum | 70[k] | | 3 weeks | 10 | 383 |
| | Colon | 70[k] | | 3 weeks | 10 | 383 |
| Rat | Vas deferens | 16.5 | i.p. | 24 hours | N.S. | 155 |
| | | | | 1 week | N.S. | 155 |
| | | | | 2 weeks | N.S. | 155 |
| | | | | 4 weeks | N.S. | 155 |
| | | | | 8 weeks | N.S. | 155 |
| | | | | 12 weeks | N.S. | 155 |
| | | | | 16 weeks | N.S. | 155 |
| | | 30 | i.p. | 2 days | 45 | 239 |
| | | | | 8 days | N.S. | 239 |
| | | 204[g] | i.v. | 1 week | 20 | 356 |
| | | | | 2 weeks | 20 | 356 |
| | | | | 4 weeks | 50 | 356 |
| Lamb | Vas deferens | 82.5 | i.v. | 1 day | 20 | 76 |
| | Superior cervical ganglion | 272[b] | i.v. | 1 day | 210 | 76 |
| | | | | 7 days | N.S. | 76 |
| | | | | 21 days | N.S. | 76 |
| | Stellate ganglion | | | 1 day | 205 | 76 |
| | | | | 7 days | (155) | 76 |
| | | | | 21 days | 155 | 76 |

TABLE 3—*Continued*

| Species | Tissue | Total Dose of 6-OHDA | Route | Time of Sacrifice after Last Dose of 6-OHDA | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|
| | | *mg/kg* | | | *μg/g* | |
| Lamb | Sympathetic chain | 272[i] | | 1 day | 230 | 76 |
| | | | | 7 days | (220) | 76 |
| | | | | 21 days | (190) | 76 |
| Rat | Adrenal | 136[i] | i.v. | 1 day | (95) | 354 |
| | | 204[f] | | 1 week | (95) | 356 |
| | | 204[f] | | 8 days | (110) | 40 |
| | | 219[h] | | 72–78 days | 200 | 158 |
| | | | | | 65 E | 158 |
| | | 272 | | 1 day | (110) E | 62 |
| | | 600[i] | | 1 day | (90) | 354 |
| Lamb | Adrenal | 82.5 | | 1 day | (90) | 76 |
| | | | | 7 days | (95) | 76 |
| | | | | 21 days | (75) | 76 |

[a] N.S., no significant difference between 6-OHDA- and control groups.
[b] Two equimolar doses of 6-OHDA, 24-hour interval.
[c] Two equimolar doses of 6-OHDA, 16-hour interval.
[d] Numbers in parentheses are indicated literature value with undetermined degree of significance from control group.
[e] Four equimolar doses of 6-OHDA during 48-hour period.
[f] Two equimolar doses, 12-hour interval.
[g] 34 mg/kg 6-OHDA on days 1 and 2, plus 68 mg/kg 6-OHDA on days 7 and 8.
[h] Same as treatment [g], plus 15 mg/kg 6-OHDA on day 14.
[i] Thirteen equimolar doses of 6-OHDA, weekly intervals.
[j] 14 mg/kg 6-OHDA on days 1 and 2, plus 34 mg/kg 6-OHDA on days 7 and 8.
[k] 20 mg/kg 6-OHDA plus 50 mg/kg 6-OHDA, 14-day interval.
[l] A single dose of 6-OHDA preceded 16–18 hours by nialamide (140 mg/kg i.p.).

the rat spleen little difference of NE depletion was noted after either an intravenous or intraperitoneal route (239, 268, 356). Thus, although there may be slight differences in specific instances as to the effectiveness of different routes of administration of 6-OHDA, the differences are generally only marginal in the mouse and rat. In other species, insufficient data are available to draw conclusions as to the relative effectiveness of 6-OHDA *via* different routes of administration.

### *B. Differences in Organ Susceptibility to 6-OHDA*

It was originally shown by Thoenen and Tranzer (356) that the rat heart was more readily depleted of NE than the spleen, and the vas deferens was least affected. Subsequent studies by numerous investigators have shown similar differences of susceptibility among other end organs to 6-OHDA. By comparing the effectiveness of 6-OHDA on NE depletion in various organs of the rat, it appears that the decreasing order of tissue susceptibility is cardiac ventricles = salivary gland > whole heart > spleen > vasa deferentia > major vascular arteries. The change in NE content correlates reasonably well with other measures such as changes in uptake of $^3$H-NE by tissue slices, and observations made on the number or integrity of the nerve terminals in the organ by histofluorescence or electron microscopy. In other species such generalities concerning differential organ sensitivity cannot be made because of insufficient data, although it is expected that a similar order of tissue sensitivity to 6-OHDA would be obtained as in the rat.

Various hypotheses can be invoked to

explain marked differences in organ susceptibility to 6-OHDA. Malmfors and Sachs (262) suggested that the length of the sympathetic postganglionic axon influenced the sensitivity of the neuron to 6-OHDA so that the short axonal processes of neurons whose perikarya are located in terminal ganglia of the genitourinary tract are least affected by 6-OHDA. Thus, noradrenergic terminals of the vasa deferentia are only slightly affected by even high doses of 6-OHDA, but another possibility is that neurons with shorter axons regenerate more rapidly. Differences in blood supply and therefore, differences in local concentrations of 6-OHDA may also account for the variations in organ sensitivity (262, 356). In regard to this last proposal it should be realized that blood supply could easily be a limiting factor in the effect of 6-OHDA, particularly in tissues such as the vas deferens which contain a high density of terminal endings, so that any individual ending will accumulate only a small amount of 6-OHDA as compared to a tissue with the same perfusion rate but with a lower density of terminals. A third possible determining factor accounting for differential organ susceptibility to 6-OHDA is the effect of the agent on the vascular tone, which would also tend to alter perfusion rate to various organs. The anatomical presence of certain diffusional barriers, such as connective tissue sheaths or actual differences in uptake capacity of terminals in different end organs could also alter susceptibility of neurons to impairment by 6-OHDA (303). Differences in the surface/volume relationship could likewise be a determining factor (see section IV). Such possible factors still remain to be investigated.

### *C. Studies of Sympathetic Neurons in Individual End Organs after Treatment with 6-OHDA*

Since most studies dealing with the effects of 6-OHDA on peripheral sympathetic neurons have been carried out in the rat it is difficult to make interspecies comparisons of the relative susceptibility of nerve terminals in different end organs. Also, since the intravenous route is frequently employed, it is often difficult to obtain a simple comparison of different routes of administration on various end organ effects in the same species. With other studies, generalizations cannot be drawn because of patchy overall data, as can be observed from table 3. Nevertheless, with these limitations in mind, generalizations will be made when it is felt that the data warrant such statements.

*1. Cardiac tissue.* Numerous studies on the effects of 6-OHDA on cardiac tissue have been made. In general, it appears that adrenergic terminals in mouse heart are more susceptible to damage by 6-OHDA than those in rat heart, as shown in table 3. Similar doses of 6-OHDA (*e.g.*, 20 mg/kg i.v.) decrease the NE content of the mouse heart to a somewhat greater extent than in the rat heart at all time periods studied. The initial change in NE in the heart has been found to correlate well with changes in $^3$H-NE uptake (218, 223, 303, 307) and changes in the numbers of nerves seen with fluorescence microscopy (99, 100, 155, 223).

In the mouse a 3 mg/kg intravenous dose of 6-OHDA produces a 65% decrease in whole heart NE content (294) which indicates the high degree of potency of 6-OHDA. After a high dose of 6-OHDA (100 mg/kg) NE content of mouse whole heart is nearly depleted within two hours, and recovery is not complete before two months (221, 240, 303). During the process of regeneration it appears that the nerves regain the ability to take up NE more rapidly than their ability to store, retain, or synthesize NE (221, 224), since endogenous NE content of mouse heart is reduced 35% as compared to only a 10% reduction in $^3$H-NE uptake capacity six weeks after treatment with 6-OHDA (50 mg/kg 2 times, i.v.) (221).

In rat heart a moderately high dose of 6-OHDA (20 mg/kg i.v.) is capable of reducing the NE content by 90% at 24 hours, with recovery to control values occurring in four weeks (80). However, even with such a

marked reduction, it appears that some of this effect is partially due to displacement of NE by 6-OHDA and possibly metabolites, or due to only partial damage of the nerve endings. Recovery takes a much longer time after a higher dose of 6-OHDA (100 mg/kg i.v.), with NE content of cardiac ventricles being only 50% of control after six weeks (100, 277). However, further damage of preterminals, the components from which regenerating terminals are derived, cannot be ruled out. In detailed studies with rat atria and ventricle, with NE assays and histofluorescent observation of adrenergic nerves, it has been shown that the cardiac ventricles are far more susceptible to damage by 6-OHDA, than are the atria (155). With a dose of 6-OHDA (16.5 mg/kg i.p.) that reduced NE content of the cardiac ventricles by 95% after 24 hours, it was demonstrated that there was a marked decrease in the number of nerve fibers seen in both atria and ventricles. In the ventricles, only sparsely distributed terminal endings could be seen, and even the fibers coursing along blood vessels were rarely seen. In atria these vascular fibers were generally spared, as were those found in the septum. In this region, through which the large nerve trunks course, preterminal axons were usually swollen and intensely fluorescent. Perfusion of isolated rat hearts with 1 or 20 $\mu$g/ml of NE failed to unmask any fibers in atria and ventricles 24 hours after treatment with 6-OHDA by the histofluorescence method. At this concentration of NE, a great deal of extraneuronal uptake (uptake 2) occurs (248) and any nerves with functional uptake processes would accumulate NE. Thus, by 24 hours, marked destruction of cardiac sympathetic neurons could be observed, and the neurons were not merely depleted of NE stores. After one week, nerve endings were still absent in the auricular appendages and were not noted until after two weeks, when a few fibers in the region were observed along blood vessels and in the musculature. In the ventricles, NE was reduced 65% and the fibers reappeared for the most part only along the vasculature. By two weeks, there was still only a small number of varicose nerves in ventricle muscle and in the auricular appendages, although the preterminal nerve trunks appeared far less distorted and swollen. At four weeks, the blood vessels in the heart had abundant innervation, the preterminal trunks lost their fluorescent appearance, and a few fibers were observable in the auricular appendages. The NE content of ventricles was 50% of control and the number of nerves seen varied from few to numerous. At eight weeks, the number of nerve endings in atria and ventricles was about normal, although NE was still reduced 40%. By 12 weeks, the atria had a completely normal pattern of innervation and the same was observed in the ventricles at 16 weeks. Thus, in the heart, a moderate dose of 6-OHDA produces alterations in cardiac sympathetic neurons lasting for a period of three to four months as determined by morphological observations, and even longer when using NE alterations as an index.

Similar alterations in neuronal integrity have been observed in cat and dog hearts and are accompanied by a notable loss in sympathetic function, as noted by the mild chronotropic responses measured after stellate stimulation or carotid artery occlusion (149, 165). Both a chronotropic (165, 277) and inotropic (277) supersensitivity to NE are observed, with the effect lasting for a period of weeks (165, 277). As would be expected, there is also a reduction in $^3$H-NE uptake velocity *in vivo* (99, 113, 356). Also, when $^3$H-NE is administered prior to 6-OHDA *in vivo*, there is a marked decrease in residual cardiac $^3$H-NE depots (95, 113). The proportion of deaminated and *O*-methylated metabolites likewise increases (49 113). Changes in levels of DBH (270) and T-OH (274) have accompanied the above alterations, while MAO was found to be unchanged from one hour to 14 days after moderate doses of 6-OHDA (221, 251) but somewhat reduced after higher doses (100).

*2. Irides.* Malmfors and Sachs (262) first

described the histofluorescent microscopic alterations produced by 6-OHDA on peripheral noradrenergic neurons, using stretch preparations of mouse irides and staining the neurons by the Falck-Hillarp method (122–124). Fifteen minutes after a relatively low dose of 6-OHDA (3.5 mg/kg i.v.) there was a marked decrease in the number of noradrenergic nerve terminals and by one hour, when the NE content was reduced 95% (189, 221), no nerves were discernible. By eight hours large intensely fluorescent preterminal axonal swellings were seen, most probably due to the accumulation of storage granules transported to this site by axoplasmic flow. At 16 hours, regeneration was detectable, as noted by the appearance of a few fluorescent terminal endings in the iris. Complete recovery occurred between 2.5 weeks (262) and two months (189, 218, 221), after which time the intensity of fluorescence and number of nerve terminals was nearly at pretreatment levels. With lower doses of 6-OHDA disappearance of fluorescence is less complete (155, 221, 262) and with higher doses the morphological changes occur more rapidly and for a longer duration (221). At least at the lower doses of 6-OHDA (1 to 10 mg/kg, i.v.) the morphological changes paralleled changes in $^3$H-NE uptake capacity, and such a relationship has been followed for at least four days after administration of 6-OHDA (5 mg/kg i.v.) (223).

Goldman and Jacobowitz (155) used a dose of 6-OHDA (16.5 mg/kg i.p.) in rats that was of the same magnitude as that used by Malmfors and Sachs in the mouse. It was found that the anatomical changes were slightly less severe, although the temporal sequence of events was basically unaltered. DeChamplain (99) studied the time-course of reinnervation after a high dose of 6-OHDA (100 mg/kg i.v.), which produced a virtually complete destruction of the noradrenergic terminal endings in the rat iris. After this dose of 6-OHDA the noradrenergic terminal endings in the rat iris were markedly diminished in number within 30 minutes, the fluorescence-staining of the fibers was markedly reduced, and the varicosities disappeared. By two hours, only rarely were the terminals seen except along perivascular structures, although the preterminal processes became more intensely stained. At 24 hours neither preterminal nor terminal processes were seen (99). Administration of 1 mg of $\alpha$-methyl-NE did not unmask any adrenergic nerves indicating actual destruction as opposed to simple depletion (259). Within four to five days of treatment preterminal fibers began to appear on the edge of the iris, in a pattern completely different from original observations, thereby suggesting formation of new preterminal processes. By one week bundles of varicose terminal fibers were observed and these continued to arborize so that at three weeks individual fibers could be seen. Not until the end of the third week did reinnervation begin to occur in the sphincter. By one month the ground plexus was well structured, but still of lesser density than in control irides, and by two to three months there was complete recovery. It was felt that the relatively short time required for recovery of neurons after the lower doses of 6-OHDA is indicative of a temporary displacement or impairment of functional activity rather than a complete destruction (99). However, it must also be considered that the regenerative process is probably delayed since high doses of 6-OHDA cause greater damage to the nonterminal axons as indicated by larger distortion and swellings.

Changes in the CA content of the cat iris have also been observed after a high dose of 6-OHDA (96 mg/kg i.v., divided dose). Such treatment was found to reduce stores by 95% for as long as two weeks after treatment. At four weeks the NE content of the iris was 20% of control and by eight weeks, 80% of control (164).

Because of the resultant development of supersensitivity after chemical sympathectomy, 6-OHDA has a potential usefulness in ophthalmology as an adjuvant in the treatment of glaucoma. Holland and Mims (193, 194) have shown that 6-OHDA, when

applied *via* a corneal bath or injected subconjunctivally, produces sympathetic denervation of the anterior segment of the eye, as determined by histofluorescence microscopy (193, 194), electron microscopy (194), and unresponsiveness of the pupil to hydroxyamphetamine, an indirectly acting sympathomimetic amine (193). In man, initial adrenergic nerve degeneration is indicated by the sympathomimetic responses due to release of intact NE, and is characterized by a decrease in intraocular pressure (IOP) lasting for a period of several days, although this response is clinically unimportant because of its short-lived nature. The clinical utility of 6-OHDA lies in the resultant supersensitivity that develops to exogenous CA or directly acting sympathomimetic agents. Consequently, there is a potentiation of the reduction in IOP by E (192, 196), phenylephrine, and isoproterenol (195) in chronic glaucoma. Although it was originally believed that the reduction in IOP was due to facilitation of outflow of the aqueous humor (193), it is now considered that outflow is unchanged (195, 196) and that the reduction in IOP is due to a decrease in aqueous humor production. In a majority of patients with medically or surgically uncontrolled glaucoma, 6-OHDA pretreatment has made it possible to control the IOP at around 20 mm Hg with only E (2% 2 times daily), while in untreated cases IOP averaged over 35 mm Hg despite maximum medical therapy (193). In a clinical study involving 87 patients with recalcitrant glaucoma, the optimal therapeutic effects of 6-OHDA were seen in cases of open angle glaucoma. In severe hemorrhagic glaucomas, 6-OHDA in limited trials does not appear to be clinically effective. It has been emphasized that 6-OHDA is not considered a substitute for conventional therapy, but offers an alternative to subjects that are medically uncontrollable or surgical failures. Patients have now been treated over a two-year period with no obvious major side effects. 6-OHDA retreatment appears to be necessary about two to three times a year, although in a third of the patients (29 of 87) retreatment has not been necessary for over half a year (197).

*3. Salivary gland.* In the salivary glands of rats relatively low doses of 6-OHDA produce marked depletion of endogenous NE stores (table 3). Higher doses produced an even greater depletion, and these were well correlated with the severity of nerve terminal destruction as seen with the fluorescence microscope. Between two and four weeks after treatment with 6-OHDA (16.5 mg/kg i.p.) regeneration was complete as determined by fluorescence microscopy but still incomplete as determined by NE levels, which were still moderately reduced. Not until eight weeks after treatment were NE levels fully recovered (155). Thus, as evidenced again, the temporal sequence of recovery cannot be fully assessed with measurement of a single index.

The activity of MAO in the rat salivary gland was found to be reduced by 40%, three days after treatment with 6-OHDA (100 mg/kg i.v.) (100). Ekstrom (112) reported that there was a 40 to 50% increase in CAT activity in rat salivary gland one week after final treatment with 6-OHDA (83 mg/kg i.v. 4 times, weekly intervals), identical with that found after surgical removal of the sympathetic supply (283, 284). This was presumed to be a sprouting response of the cholinergic neurons innervating the salivary gland, and one wonders about similar effects occurring in other organs after administration of 6-OHDA.

*4. Spleen.* The sympathetic terminals in the spleen of rats are less susceptible to destruction by 6-OHDA than are the nerve endings in the salivary gland. An intravenous dose of 3 mg/kg of 6-OHDA produces a slight decrease in the NE content of the rat spleen after two hours, but at 24 hours, the NE content is at control levels, so that no apparent lasting effect is produced on NE storage sites (356). This contrasts with a much greater effect on noradrenergic terminals in the salivary gland after a lower dose of 6-OHDA (table 3). The acute decrease in

NE produced by 6-OHDA in the rat spleen is somewhat greater than the reduction in $^3$H-NE accumulation by spleen *in vivo*, and it has been suggested that this latter effect is the result of uptake of $^3$H-NE into residual nervous structures (187, 356). However, two weeks after treatment with 6-OHDA (100 mg/kg i.v.) the accumulation *in vivo* of $^3$H-NE is reduced to the same extent as endogenous NE content (99). It is difficult to make interspecies comparisons on the sensitivity of sympathetic terminals in the spleen because of a lack of data. However, from information presented in table 3 it would appear that no great differences exist. After treatment with a large dose of 6-OHDA (34 mg/kg 2 times plus 68 mg/kg 2 times, i.v., one week interval) the gradual increase of NE in the spleen is a slow process, being only 20% of control after four weeks; this indicates a slow regenerative process, as in the heart (356). However, higher doses of 6-OHDA may possibly cause a more marked destructive effect on nonterminal axons. In such a case, regenerative capacity would be much slower or retarded and not simply reflect recovery of the terminal endings.

*5. Vas deferens.* The sympathetic fibers in the vas deferens appear to be the least susceptible to damage by 6-OHDA. After a dose of about 15 mg/kg of 6-OHDA, the NE content and the fluorescence staining of sympathetic terminals in rat vas deferens is unchanged at 24 hours (151, 262). After doubling the dose, the NE content is depleted by 50% after two days but is back to control values in eight days (239). Only after a high dose of 6-OHDA (>200 mg/kg i.v., total dose) is there a prolonged depletion of NE (356) and decrease in the accumulation *in vitro* of $^3$H-NE (233) and alteration in the number of nerve terminals (109, 140). Furness *et al.* (139, 140) showed that after these high doses of 6-OHDA, transmission of the sympathetic fibers in this organ usually failed within 2.5 hours after treatment, and that the process could be hastened with frequent stimulation of the nerves or duplicated with 5-hydroxydopamine. It appears that the blood supply of 6-OHDA is one of the major factors limiting destruction in this and probably other tissues, since incubation *in vitro* of mouse vasa deferentia with 6-OHDA ($5 \times 10^{-5}$ M, 30 minutes) produced a marked decrease in the numbers of nerves, similar to that seen in other organs. However, the degree of damage was not quite as great as that produced in mouse atria and irides under similar conditions (221), thus suggesting the possibility of other factors in limiting chemical lesioning.

*6. Lung, kidney, gastrointestinal tract.* Studies to date indicate depletion of NE stores by 6-OHDA in various other organs (table 3), such as the kidney, intestinal tract, and lung. Little information is available for the determination of a threshold dose, time-course of recovery and interspecies comparison of susceptibility of nerves in these various tissues. However, it does appear that the threshold dose is lowest for duodenum and colon and highest for the lung (383). Intermediate susceptibility is found in the stomach and kidney (383, 390). In the latter tissue, it has been reported that recovery is quite slow, with single fibers appearing in the renal capsule only three to four weeks after treatment with 6-OHDA (100 mg/kg i.v.) (99).

*7. Adrenal glands.* The adrenal gland appears to be resistant to direct action of 6-OHDA. When the adrenal is assayed for E or NE at various times after different doses of 6-OHDA, it is found in general, that the CA content is unaltered (table 3). One report indicates an increase in NE after a high dose of 6-OHDA but this is accompanied by a decrease in E content (158). Therefore, it appears that the total CA content is relatively unchanged. The nature of the shift in NE and E levels cannot readily be explained.

Although CA content of rat adrenals was unaltered, T-OH activity was increased over 200% and phenylethanolamine-N-methyltransferase (PNMT) increased 25%, 49 hours after treatment with 6-OHDA

(100 mg/kg i.v. 2 times, eight-hour intervals). The increase in T-OH was noticeable within 16 hours (274, 353) and appeared to be induced by increased discharge of the preganglionic nerves, since transection of the splanchnic nerve abolished the effect (273, 352, 354, 355). Such a mechanism is likely to be responsible for the increased adrenal DBH activity (270). Therefore, chemical sympathectomy brings about a compensatory increase in turnover of CA in the adrenals (272, 274). The physiological significance of such a mechanism, particularly in view of the increased sensitivity of various organs to CA after 6-OHDA, was well illustrated by De Champlain *et al.* (101, 149). In dogs that were chemically sympathectomized with 6-OHDA (50 mg/kg i.v., total dose) it could be seen that the adrenals were responsible in large part for maintaining blood pressure, since clamping of the hilar vessels of the adrenals resulted in a marked degree of hypotension (149). This compensatory role of the adrenals was even more obvious in hypertensive rats pretreated with 6-OHDA. The latter treatment alone, altered blood pressure only little, as did adrenalectomy alone. However, the combination of chemical sympathectomy and adrenalectomy resulted in a marked drop in pressure to hypotensive levels, indicating the importance of the compensatory role of the surviving system (101). Therefore, after treatment with 6-OHDA, the adrenals play a major part in maintaining homeostasis in the animals by increasing the turnover of CA. It would appear that the magnitude of this effect decreases as sympathetic nerves begin to regenerate, since T-OH activity in the adrenal gland is the same as in control animals eight days after final 6-OHDA treatment (34 mg/kg 2 times plus 68 mg/kg 2 times i.v., six-day interval) (40).

*8. Blood vessels.* Several studies have reported the effect of 6-OHDA on noradrenergic neurons innervating vascular smooth muscle, and the rapid sequence of recovery of neurons from initial damage (155, 268). The sympathetic supply of vascular smooth muscle in cardiac ventricles appears to be damaged to a greater extent than innervation of vessels coursing through other tissues, such as the submaxillary gland, atria, or vas deferens (155). The vessels sustaining the greatest destruction of their sympathetic nerves are those vessels situated in organs whose adrenergic nerves are most damaged. It appears that no prolonged reduction of T-OH or MAO is produced in blood vessels after treatment with 6-OHDA (34 mg/kg i.v. 2 times plus 68 mg/kg i.v. 2 times) (40), although the amount of enzyme present in nonfunctional, swollen and highly fluorescent preterminal nerve trunks is not known. An accumulation in this region would mask a depletion in the nerve terminals. An accumulation of T-OH after ligation of adrenergic nerve axons has been demonstrated (389). Because of such an effect it appears that histofluorescence microscopy is essential for studying reinnervation. The reason for the resistance of sympathetic nerves in the vasculature is not known. It is possible that less 6-OHDA comes in contact with this nerve network (40), since NE does not easily reach the vascular nerves when introduced into the lumen of the artery (102).

When 6-OHDA was applied to the retrolingual membrane of the frog, so that 6-OHDA could come in close contact with adrenergic neurons over a prolonged period of time, there was marked destruction of the vascular innervation. Neurogenic constrictor threshold was elevated immediately and remained so for at least 21 days, the duration of study. By five days post-treatment, well conducted neurogenic vasoconstriction was absent in all preparations, and at the end of 21 days only 2 of 10 arterioles regained neurogenic responses (322). Thus, with a marked degree of generalized destruction, vascular neurons regenerate at a relatively slower rate than that observed in mammals *in vivo*.

Finch *et al.* (127–129) studied the functional recovery of sympathetic neurons in

the vasculature of rats after treatment with 6-OHDA. It was shown that all adrenergic terminals in the mesenteric arteries displayed degenerative changes one day after injection of 6-OHDA (68 mg/kg i.v. 2 times, six-hour interval). By seven days adrenergic nerves could be seen growing back and by two to three weeks innervation was as dense as in controls (128). Functionally, it could be seen that stimulation of the entire sympathetic outflow in pithed and adrenalectomized rats (151) failed to elevate the blood pressure one day after injection of 6-OHDA (34 mg/kg i.v. 2 times plus 68 mg/kg i.v. 2 times) (128), but by three days responses could be detected, and at 21 days responses were similar to that observed in controls. Responses to tyramine, physostigmine, and periarteriolar nerve stimulation of the renal artery were completely or nearly normal within one week. Recovery was so rapid in the vasculature that supersensitivity to NE did not occur (128, 129). Therefore, it would appear that this rapid rate of regeneration of vascular sympathetic nerves is the reason that 6-OHDA has been unsuccessful in preventing experimental hypertension (see section IX).

*9. Nictitating membrane.* Haeusler *et al.* (161, 164) studied regeneration of adrenergic terminals in cat nictitating membrane up to 14 weeks after administration of 6-OHDA (14 mg/kg i.v. 2 times plus 34 mg/kg i.v. 2 times). The NE content of the nictitating membrane 6 to 10 days after final treatment with 6-OHDA was about 5% of control, the effect of sympathetic nerve stimulation was nearly abolished and sensitivity to NE increased 71-fold. At two to three weeks, NE content was only 20% of control but the effect of sympathetic nerve stimulation apparently was completely restored, while sensitivity to NE was still elevated 38-fold. It should be noted that the maintenance of postsynaptic supersensitivity could have accounted for the normal functional responses to stimulation of fewer nerves which liberate a much smaller total amount of NE than intact control preparations. Seven to eight weeks after injection, when NE content was 70% of control, sensitivity to NE was normal. By 14 weeks, NE had returned to normal levels.

*10. Effect of 6-OHDA on 5-HT content.* Initially it was reported that 6-OHDA increased the 5-HT content of cat ileum and jejunum (387), while no changes in 5-HT were found in atria, ventricles, lung, salivary gland, spleen, nictitating membrane, stomach, duodenum, and colon (383). 6-OHDA appeared to have no effect on mast cells found in various organs of the mouse (221) and rat (155) when seen under the fluorescence microscope. Whether the change in 5-HT occurs in only select tissues in certain species remains to be determined.

*11. Summary of effects of 6-OHDA on sympathetic neurons.* From the results described above, certain generalizations can be obtained. In the various species it appears that the decreasing order of susceptibility of sympathetic terminals in various end organs or sites after treatment with 6-OHDA is cardiac ventricles > salivary gland > whole heart > iris > nictitating membrane > spleen > atria > blood vessels > vas deferens > sympathetic ganglia > adrenal glands. Relatively low doses of 6-OHDA produce NE depletion in cardiac ventricles while the highest doses of 6-OHDA have little or no direct effect on adrenal CA content. During the acute phases of nerve destruction the alterations in morphological appearance of the nerves as seen under the fluorescence microscope, correlate well with reduction in endogenous NE content and decreased $^3$H-NE uptake *in vivo* or *in vitro*. However, during the period of regeneration the nerve density appears identical to control before full recovery of NE content and associated $^3$H-NE uptake capacity. In general, noradrenergic nerves of the cat appear to be affected to a greater degree or at earlier times than those in the mouse or the rat, the latter species appearing to be the least sensitive of the three.

## IX. Cardiovascular Effects of 6-OHDA

The cardiovascular effects of 6-OHDA may be subdivided into different groupings, depending upon the route of administration, the time interval necessary to observe an effect, and the particular response being studied. The cardiovascular effects observed immediately after peripheral injection are very probably due to the indirect sympathomimetic actions of the compound and have been discussed under that subheading. Other studies quantifying the effects of 6-OHDA on specific vascular beds are discussed in section VIII C 8. Those studies dealing with long-term effects of peripherally administered 6-OHDA on blood pressure, heart rate, and baroreceptor mechanisms are primarily designed to study various hypertensive states and the actual development of hypertension, and will be discussed in this section. Finally, those studies involving the injection of 6-OHDA into the cerebrospinal axis, in an effort to determine central control of pressure and pressor reflexes, as well as the involvement of central adrenergic processes in the development and maintenance of hypertension, will also be discussed in this section.

Because of the involvement of the sympathetic nervous system in various hypertensive states and the obvious ability of 6-OHDA to destroy the postganglionic components, numerous attempts have been made to reverse the hypertension state or prevent its development in experimental animals. All initial attempts to prevent DOCA/NaCl hypertension with 6-OHDA proved unsuccessful (81, 273), even when accompanied with adrenal demedullation (130, 131). However, recent studies show that the doses of 6-OHDA employed in all such experiments were ineffective in producing a prolonged diminution in the number of adrenergic terminals in the vascular bed (127–129), so that with regeneration blood pressure will continue to rise and attain the hypertensive levels seen before treatment with 6-OHDA. In one study where functional sympathectomy has been maintained from birth by continued injections of 6-OHDA (100 mg/kg s.c., weekly intervals), development of DOCA/NaCl hypertension was successfully prevented (101). It should be realized, however, that the action of 6-OHDA on central control centers cannot be completely ruled out, as 6-OHDA treatment of neonates has been shown to alter central noradrenergic neurons (see section X). Other studies indicate that 6-OHDA is even effective in abolishing established DOCA/NaCl hypertension when treatment is accompanied by simultaneous removal of the adrenal compensation by adrenalectomy, simply clamping of the adrenal peduncle, or *alpha*-adrenergic blockade (101).

6-OHDA was also reported to have little effect on the development of renal hypertension in rats. Rats received 6-OHDA (34 mg/kg i.v. 2 times plus 68 mg/kg i.v. 2 times) two days before uninephrectomy and adrenal demedullation and were given saline for the following two weeks in place of water. Despite treatment with 6-OHDA, there was only a slight delay in the rate of development of hypertension to maximal levels (130, 133). However, when demedullation followed 6-OHDA (34 mg/kg i.v. 2 times plus 68 mg/kg i.v. 2 times plus 15 mg/kg i.v.) by one week, with both kidneys being left intact and saline omitted from the drinking water, six of eight mature rats with bilateral renal artery clips failed to develop hypertension for at least seven weeks. Without demedullation, no reversal was obtained. When the same procedures (with demedullation) were carried out in two- to three-week-old rats, only 4 of 20 rats (*vs.* 11 of 17 rats without 6-OHDA) developed hypertension over a nine-week period and the severity was much less than in rats without 6-OHDA (158).

In spontaneously hypertensive rats 6-OHDA had no effect on the development of hypertension over an 18-day period. A similar lack of effectiveness of 6-OHDA (80 mg/kg i.v.) was also noted on neurogenic hypertension, produced by denervation of the carotid and aortic baroreceptors (390).

At first, it may appear surprising that 6-OHDA has such a little effect on hypertension in rats, because of the widespread destructive effect of the agent. However, as described previously (section VIII C 8), 6-OHDA failed to produce any long-lasting lesion of sympathetic terminals in various vascular beds (128). Electron microscopic studies showed that regeneration from neuronal damage occurs after one week, even with high doses of 6-OHDA, so that in the above cited studies on hypertension, sympathetic vascular neurons were apparently functional in most cases for almost the entire duration of the studies.

When injected into one of the brain cavities, 6-OHDA causes an immediate fall in blood pressure and decrease in heart rate of cats (159), rats (162), and rabbits (75). Treatment with 6-OHDA (250 μg ivt. 1 or 3 times) is equally effective in normotensive, DOCA/NaCl- and renal hypertensive rats (163). The response appears to be mediated by central *alpha*-adrenergic activation, since phentolamine given intraventricularly successfully antagonizes the chronotropic and hypotensive responses. Because of the ineffectiveness of scopolamine in antagonizing these actions, it appears that the effects are not mediated by increased peripheral parasympathetic tone. Rather, the effects appear to be due to a decrease in peripheral sympathetic tone, as supported by observance of a decrease in spontaneous discharge of the splanchnic nerve and effectiveness of combined peripheral *alpha*- and *beta*-blockade in antagonizing the effects (162). In rabbits, however, the negative chronotropic responses are blocked by atropine, indicating the likelihood of increased vagal (parasympathetic) tone in this species (75). The hypotensive response to 6-OHDA in rabbits was unaffected by atropine and, therefore, was assumed to be due also to reduced sympathetic tone, as in rats (75). In the cat the hypotensive effects were prolonged and did not return to control levels after 6-OHDA (0.5–2 mg ivt.). In this species it was shown that responses of the medullary and hypothalamic vasomotor centers were depressed to both direct stimulation and indirect excitation by occlusion of the carotid arteries (159).

After the acute changes in blood pressure and heart rate there was a secondary fall in both cardiovascular parameters one to two days after 6-OHDA in rats. A moderate reduction in blood pressure was noted after a single treatment with 6-OHDA (250 μg ivt.) in spontaneously hypertensive rats and after two or three successive treatments (250 μg each, 48-hour interval) in normotensive rats. In DOCA/NaCl- and renal hypertensive rats the fall was only slight. Recovery of blood pressure to control levels occurred in five to seven days in all cases, but the bradycardia persisted for at least three weeks in normotensive and spontaneously hypertensive rats (162, 163). However, neurogenic hypertension produced in rabbits by denervation of carotid and aortic baroreceptors, appears to be made more labile with respect to 6-OHDA, since reversal of the hypertensive state occurs within one day of treatment (75). 6-OHDA administered intracisternally or intraventricularly has also been shown to be effective in preventing the development of renal and neurogenic hypertension in both rats and rabbits (73–75). When 6-OHDA (600 μg i.c.) is administered five days prior to denervation of carotid and aortic baroreceptors in the rabbit there is no observed increase in blood pressure in the following two weeks, although in vehicle-treated rabbits hypertension is marked at this time (74, 75). In rabbits with kidneys wrapped in cellophane it was similarly found that 6-OHDA prevented the development of renal hypertension (75). Likewise, treatment of seven-week-old spontaneously hypertensive rats with 6-OHDA (250 μg 2 times, ivt.) effectively prevented development of hypertension for up to 12 weeks (163). In isolated mesenteric artery preparations from DOCA- and renal hypertensive rats the responsiveness to NE was increased, but in animals in which the development of hypertension was

impeded, the responses of mesenteric arteries were the same as in normotensive rats (163).

The above results with 6-OHDA suggest that central adrenergic neurons are involved in the development ("trigger mechanism") but not in the maintenance of DOCA- and renal hypertension. Both the development and maintenance of neurogenic hypertension, however, appear to involve the central adrenergic processes. It appears that activation of central *alpha*-adrenergic receptors through the sympathomimetic action of 6-OHDA inversely affects peripheral sympathetic tone.

## X. Studies with 6-OHDA on Developing Neurons

### *A. Peripheral Injections and the Sympathetic Nervous System*

When 6-OHDA is administered to newborn animals its neurotoxic effects are accentuated. The destructive lesions extend to axonal processes and the perikarya *per se*, thereby bringing about total destruction of the noradrenergic neurons and permanent reduction of NE in tissues or sites normally innervated by these neurons. Thoenen (347) has made the distinction between the "reversible chemical sympathectomy" in adult animals and the "irreversible chemical sympathectomy" in newborn animals after 6-OHDA.

Angeletti and Levi-Montalcini (15, 16) claimed that 6-OHDA, when given to mice or rats during the first several postnatal days of life, was able to irreversibly destroy sympathetic (noradrenergic) neuroblasts. So extensive were the lesions that virtually all of the sympathetic perikarya were reportedly destroyed in the superior cervical, stellate, celiac, mesenteric ganglia, and the entire thoracic paravertebral chain. The effects were much more extensive than those produced by the anti-nerve growth factor (anti-NGF) in newborn animals, since after anti-NGF treatment about 5 to 10% of the perikarya in the paraganglia survived, as well as 15 to 20% in the prevertebral ganglia and virtually 100% of the terminal ganglia (244, 245). The destructive effects of 6-OHDA were not reversed by NGF (60). Glial and other satellite cells are unaffected (15, 16) by 6-OHDA indicating a high selectivity of 6-OHDA for noradrenergic neurons.

A certain degree of differentiation of the neuroblast appears to be a requirement for 6-OHDA activity in the ganglia, since diminishing effects of the compound are seen on neuroblastoma cells with continued treatment, presumably because of de-differentiation with time (14). Initially, 6-OHDA slows the rate of growth *in vitro* of cultured mouse C-1300 (14, 296, 300) and human (14) neuroblastoma cells. The same effects of 6-OHDA are noticed *in vivo* with implanted C-1300 neuroblastoma in mice (14, 300). However, the effectiveness of 6-OHDA in destroying the neuroblastomas becomes diminished with time and remission *in vivo* of the tumor occurs within one week after initial treatment in most cases. However, as confirmation of the relative specificity for monoaminergic components, 6-OHDA did produce its effects on the neuroblastoma cells while the growth rate of HeLa cells, mouse sarcoma 37, and fibroblast cells were unabated. It was conjectured that 6-OHDA might be effective in treating human neuroblastomas, since in some cases they are characterized by a relatively high degree of differentiation and a slow growth rate (14).

When administered to newborn mice and rats, 6-OHDA produces its initial lesions on sympathetic ganglia within an eight-hour period, as evidenced by the appearance of clear cytoplasmic lesions. After 24 hours nuclear pyknosis is observed and axonal processes appear distorted and swollen, with beadings along their course. Virtually all sympathetic cell bodies are destroyed by two to three days, at which time there is extensive connective tissue proliferation in affected ganglia (15–17). It is this latter process which accounts for the initial size increase of the ganglia (16). Monoamine oxidase activity of sympathetic ganglia decreases during this interval (13). From this

point on, sclerotic processes set in and gradually decrease the ganglia to "exceedingly diminutive nodules" (16). Accompanying the reduction in number of noradrenergic perikarya (12, 15, 16, 79, 211) is a decrease in NE content of the ganglia for a period of up to six months (tables 4 and 5).

Tissues normally innervated by the sympathetic neurons (which have their perikarya in the affected ganglia) also exhibit a decreased content of catecholamine. The biochemical data indicate that the number of surviving ganglia is considerably greater than originally reported, or suggest an extensive proliferation of terminal endings of surviving sympathetic neurons, since the NE content of numerous tissues is relatively high several weeks after treatment. In the following discussion on the effects of 6-OHDA on CA content in central and peripheral tissues, all data refer to findings in the rat, unless otherwise stated.

When rat superior cervical and celiac ganglia are assayed for NE 4 or 12 weeks after treatment with 6-OHDA (350 μg/g, total dose) there is a 60% decrease in total NE content. However, the concentration of NE remains unchanged, indicating that the reduction in total NE content is correlated with the reduction in size of the ganglion (211), or identical to the reduction in number of ganglion cell bodies (25, 115). Angeletti (12) first reported a 90 to 95% reduction in NE concentration in the hearts of mice and rats six months after neonatal treatment with 6-OHDA (50 μg/g 4 times). However, more recent studies (79, 211, 246) indicate that treatment with 6-OHDA (50 μg/g 5 times or 50 μg/g 7 times) during the first postnatal week reduces cardiac NE concentration by only 50 to 75% after three to four months. Thus, contrary to original findings, total and complete cardiac sympathectomy is not attained for a prolonged period after treatment with 6-OHDA in the first postnatal week. However, continued treatment with 6-OHDA during the first two to three weeks of life does produce a marked depletion of NE. Total doses of 6-OHDA ranging between 550 to 700 μg/g reduced cardiac NE concentration 85 to 95% between 42 and 104 days (79, 289). Treatment with even higher doses of 6-OHDA (100 μg/g 10 times) during the first week and a half of postnatal life resulted in an even greater reduction in the heart NE concentration, with levels remaining at only about 2% of controls (347). The NE content of rat salivary gland and iris was reduced to the same degree as in cardiac tissue after 6-OHDA was given during the neonatal period (211, 224). With a 350-μg/g regimen a 55 to 60% reduction of NE was seen in each tissue up to 12 weeks, and a total dosage of 1 mg/g resulted in greater than a 95% reduction in NE content.

The noradrenergic innervation to the spleen appears to be destroyed to a much greater degree by 6-OHDA than that of the aforementioned tissues. Low doses of 6-OHDA (5 to 20 μg/g), during the first two postnatal days produces a 50 and 70% reduction in NE content, respectively, for as long as two months after treatment (253). Higher doses (200 to 1000 μg/g) result in an 80 to 99% reduction in NE concentration when given up to six months of age in both mice and rats (12, 211, 347).

The NE content of the pancreas was found to undergo similar profound reductions. Six months after neonatal treatment with 6-OHDA (50 μg/g 4 times) NE levels in this tissue were only 10% of controls in both mice and rats (12). Higher doses of 6-OHDA (100 μg/g 7 times), administered up to day 12, produced only a 70% reduction in the NE concentration of the pancreas-mesentery, while the concentration in the kidneys was unaltered at 10 weeks of age (79).

A total dose of 350 μg/g of 6-OHDA early in postnatal development failed to alter NE concentration in the vas deferens after 4 to 12 weeks (211), while 700 to 1000 μg/g in divided doses produced a 25 to 50% decrease in NE concentration after as long as 10 weeks of age (79, 347).

The adrenal glands appear to be relatively

TABLE 4

*Effect of 6-OHDA on monoamine content (μg/g) of whole brain and brain regions of the rat*

| Tissue | Total Dose of 6-OHDA | Route | Dose | No. of Injections | Age at Time of Treatment | Age at Time of Sacrifice | Per Cent Control | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | NE | DA | |
| | μg/g | | μg/g | | days | | μg/g | μg/g | |
| Whole brain | 100[a] | i.c. | 100 | 1 | 7 | 85–102 days | 20 | 10 | 60 |
| | 100[a] | i.c. | 50 | 2 | 0, 2 | 12 days | 10 | 60 | 253 |
| | | | | | | 24 days | 20 | 60 | 253 |
| | | | | | | 60 | 5 | 55 | 253 |
| | 150[a] | i.c. | 150 | 1 | 14 | 85–102 days | 20 | 10 | 60 |
| | 200[a] | i.c. | 100 | 2 | 0, 2 | 12 days | 10 | 45 | 253 |
| | | | | | | 24 days | 10 | 20 | 253 |
| | | | | | | 60 days | 5 | 20 | 253 |
| | 400[a] | i.c. | 200 | 2 | 0, 2 | 12 days | 10 | 5 | 253 |
| | | | | | | 24 days | 10 | <5 | 253 |
| | | | | | | 60 days | 5 | 15 | 253 |
| | 5 | i.p. | 2.5 | 2 | 0, 2 | 12 days | N.S.[b] | N.S. | 253 |
| | | | | | | 24 days | N.S. | N.S. | 253 |
| | | | | | | 60 days | N.S. | N.S. | 253 |
| | 10 | i.p. | 5 | 2 | 0, 2 | 12 days | N.S. | N.S. | 253 |
| | | | | | | 24 days | N.S. | N.S. | 253 |
| | | | | | | 60 days | N.S. | N.S. | 253 |
| | 20 | i.p. | 10 | 2 | 0, 2 | 12 days | N.S. | N.S. | 253 |
| | | | | | | 24 days | N.S. | N.S. | 253 |
| | | | | | | 60 days | N.S. | N.S. | 253 |
| | 170 | i.p. | 34 | 5 | 0, 1, 2, 3, 4 | 3–4 months | (95)[c] | | 79 |
| | 200 | s.c. | 50 | 4 | 0, 2, 4, 6 | 6 months | 35 | | 11 |
| | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 60 | | 306 |
| Telediencephalon | 240 | s.c. | 83 | 3 | 0, 2, 4 | 1 week | 60 | | 327 |
| | | | | | | 2½ weeks | 50 | | 327 |
| | | | | | | 4 weeks | 75 | | 327 |
| | | | | | | 6 weeks | 50 | | 327 |
| | | | | | | 8 weeks | 70 | | 327 |
| | | | | | | 10 weeks | 70 | | 327 |
| | | | | | | 12 weeks | 65 | | 327 |
| | | | | | | 14 weeks | 50 | (90) | 327 |
| | | | | | | 16 weeks | 60 | | 327 |
| | | | | | | 56 weeks | 70 | | 327 |
| | 900 | s.c. | 100 | 9 | 0, 7, 14, 21, 28, 35, 42, 49, 56 | 10 weeks | 65 | | 207 |
| Cerebral cortex | 170 | i.p. | 170 | 1 | 0 | 2 days | 50 | | 64 |
| Hippocampus striatum | | | | | 6 | 8 days | 35 | (125) | 64 |
| | | | | | 20 | 22 days | (70) | (125) | 64 |
| Cerebral cortex | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 25 | | 306 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | N.S. | | 211 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 10 | | 79 |
| | | | | | | 3 months | 10 | | 341 |
| Frontal cortex | 575 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, | 42 days | 55 | | 289 |
| | | | 75 | 3 | 5, 6, 9, 14, 21 | 104 days | 45 | | 289 |
| Hippocampus | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 3 months | 15 | | 341 |
| Corpus striatum | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 3 months | (85) | N.S. | 341 |
| "Thalamus"[d] | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | (105) | | 341 |
| | | | | | | 3 months | (100) | | 341 |

TABLE 4—*Continued*

| Tissue | Total Dose of 6-OHDA | Route | Dose | No. of Injections | Age at Time of Treatment | Age at Time of Sacrifice | Per Cent Control | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | NE | DA | |
| | μg/g | | μg/g | | days | | μg/g | μg/g | |
| Thalamus-hypothalamus | 170 | i.p. | 170 | 1 | 0 | 2 days | 65 | (120) | 64 |
| | | | | | 6 | 8 days | 50 | (85) | 64 |
| | | | | | 20 | 22 days | (95) | (120) | 64 |
| Hypothalamus | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 60 | | 306 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | N.S. | | 211 |
| | | | | | | 23 weeks | 130 | | 246 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 3 months | (100) | | 341 |
| | 575 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, | 42 days | (100) | | 289 |
| | | | 75 | 3 | 5, 6, 9, 14, | | | | |
| | | | | | 21 | 104 days | (100) | | 289 |
| Brainstem | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | N.S. | | 211 |
| Brainstem[e] | 575 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, | 42 days | 175 | | 289 |
| | | | 75 | 3 | 5, 6, 9, 14, | | | | |
| | | | | | 21 | 104 days | 151 | | |
| Midbrain | 170 | i.p. | 170 | 1 | 0 | 2 days | 65 | (85) | 64 |
| | | | | | 6 | 8 days | 45 | (65) | 64 |
| | | | | | 20 | 22 days | (105) | (85) | 64 |
| Midbrain | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 3 months | 175 | | 341 |
| Lower brainstem | 250 | s.c. | 83 | 3 | 0, 2, 4 | 1 week | (105) | | 327 |
| | | | | | | 2½ weeks | (105) | | 327 |
| | | | | | | 4 weeks | 155 | | 327 |
| | | | | | | 6 weeks | 180 | | 327 |
| | | | | | | 8 weeks | 200 | | 327 |
| | | | | | | 10 weeks | 180 | | 327 |
| | | | | | | 12 weeks | 180 | | 327 |
| | | | | | | 14 weeks | 180 | | 327 |
| | | | | | | 16 weeks | 220 | | 327 |
| | | | | | | 56 weeks | 220 | | 327 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 210 | | 79 |
| | | | | | | 3 months | 200 | | 341 |
| | 900 | s.c. | 100 | 9 | 0, 7, 14, 21, 28, 35, 42, 49, 56, 63 | 10 weeks | 180 | | 207 |
| Pons medulla | 170 | i.p. | 170 | 1 | 0 | 2 days | (75) | (90) | 64 |
| | | | | | 6 | 8 days | 35 | (85) | 64 |
| | | | | | 20 | 22 days | (115) | (95) | 64 |
| Cerebellum | 900 | s.c. | 100 | 9 | 0, 7, 14, 21, 28, 35, 42, 49, 56, 63 | 10 weeks | 30 | | 79 |
| | | | | | | 3 months | 20 | | 341 |
| Spinal cord | 170 | i.p. | 34 | 5 | 0, 1, 2, 3, 4 | 3–4 weeks | 42 | | 79 |
| | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 30 | | 306 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 35 | | 79 |
| | | | | | | 3 months | 35 | | 341 |

[a] Indicates 6-OHDA, μg total, rather than μg/g.

[b] N.S., no significant difference between 6-OHDA- and control groups.

[c] Numbers in parentheses are indicated literature value with undetermined degree of significance from control group.

[d] "Thalamus" includes the midbrain, hippocampus, thalamus, and hypothalamus.

[e] Brainstem includes pons, medulla oblongata, and midbrain.

unaffected by treatment with 6-OHDA early in life. Expressed either as μg/g or μg/tissue, NE and E appear to be unaltered by a total dose of 1 mg/g of 6-OHDA given during the first 10 days of postnatal development. A single report indicates a 30% increase in the CA levels five months after 350 μg/g of 6-OHDA (246). It is noteworthy that an increase in T-OH activity of adrenals of three-day-old piglets was observed in the absence of changes in E content after treatment with 6-OHDA, so that turnover of adrenal CA appears to increase at least initially (333), probably as a compensatory response to reduced sympathetic activity.

From the preceding data in newborn animals it should be noted that 6-OHDA permanently reduces the number of perikarya in some, but not all sympathetic ganglia. Therefore, variable results concerning the NE content in peripheral organs are observed. It would appear that the degree of differentiation of the sympathetic ganglion cell bodies, the dose and time interval of administration of 6-OHDA are critical factors which determine the effectivenss of the neurotoxic agent. Other factors such as regional blood flow and envelopment of perikarya by Schwann cell membranes are probably also involved in the 6-OHDA-induced neuronal cell toxicity.

Little is currently known about the existence of undifferentiated stem cells (neuroblasts, sympathoblasts) in the neonatal ganglia. It would be of interest to learn at what age the neuroblasts lose their potential for ganglion cell formation. It would seem that differentiation is still possible between one to two weeks postpartum. This would explain why many cells were observed in the superior cervical ganglion two months after injection of 6-OHDA (50 μg/g 7 times) into newborns (208).

It has been shown that extra-adrenal chromaffin cells of the abdominal sympathetic paraganglia and superior cervical ganglia of newborn rats undergo marked hyperplasia after administration of glucocorticoids (77, 114, 242). It has been conjectured that stem cells in the ganglia differentiate after the glucocorticoid treatment. A time-dependent decline in the hyperplastic response of chromaffin cells to glucocorticoids was observed (242). Hydrocortisone-induced proliferation of cells in the abdominal paraganglia did not occur if treatment was begun after 12 to 16 days of age. It would seem that the potential for stem cell (pheochromoblast) differentiation to CA-containing chromaffin cells is lost after about two weeks. We would suggest an analogy with the neuroblasts which probably lose their ability to differentiate into sympathetic ganglion cells at about this time. If this situation indeed exists, it would be necessary to administer 6-OHDA to newborn animals over a period of about two weeks at sufficiently high doses (79, 347).

### *B. Peripheral Injections and the CNS*

When 6-OHDA is administered peripherlaly (i.v., i.p., s.c.) to mature animals monoaminergic neurons in the CNS appear to be little affected. In contrast to these findings, 6-OHDA produces marked alterations in central CA-containing neurons when injected into animals early in the developmental stages. Thus, 6-OHDA is capable of crossing the unfully-developed blood-brain barrier of newborn animals thereby affecting noradrenergic neuronal development. A threshold for these effects appears to be above a total of 10 μg/g 2 times (0, 2 days i.p.) since this and lower doses apparently do not alter development of noradrenergic neurons, as indicated by unaltered whole brain NE levels (253). When the brain is dissected into more discrete anatomical regions, it can subsequently be seen that regions containing a predominance of terminal endings of noradrenergic neurons exhibit a depletion of the neurotransmitter, while regions containing a large population of noradrenergic nonterminal axons or cell bodies demonstrate an increase in NE content (table 5). Histochemical observations show a decrease in the number of noradrenergic terminals and a retrograde accu-

TABLE 5

*Effect of 6-OHDA on NE content (μg/g) of various tissues of the rat*

| Tissue | Total Dose of 6-OHDA | Route | Dose | No. of Injections | Age at Time of Treatment | Age at Time of Sacrifice | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|---|---|
| | *μg/g* | | *μg/g* | | *days* | | | |
| Superior cervical ganglion | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 45 | 306 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | N.S.[a] | 211 |
| | | | | | | 12 weeks | N.S. | 211 |
| Coeliac ganglion | | | | | | 12 weeks | N.S. | 211 |
| Heart | 5 | i.p. | 2.5 | 2 | 0, 2 | 12 days | N.S. | 253 |
| | | | | | | 24 days | 80 | 253 |
| | | | | | | 60 days | N.S. | 253 |
| | 10 | i.p. | 5 | 2 | 0, 2 | 12 days | 75 | 253 |
| | | | | | | 24 days | 75 | 253 |
| | | | | | | 60 days | 75 | 253 |
| | 20 | i.p. | 10 | 2 | 0, 2 | 12 days | 50 | 253 |
| | | | | | | 24 days | 75 | 253 |
| | | | | | | 60 days | 60 | 253 |
| | 170 | i.p. | 170 | 1 | 0 | 2 days | 65 | 64 |
| | | | | | 6 | 8 days | 40 | 64 |
| | | | | | 20 | 22 days | 50 | 64 |
| | 170 | i.p. | 34 | 5 | 0, 1, 2, 3, 4 | 3–4 months | 25 | 79 |
| | 200 | s.c. | 50 | 4 | 0, 2, 4, 6 | 6 months | 5 | 11 |
| | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 20 | 306 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | 40 | 211 |
| | | | | | | 12 weeks | 45 | 211 |
| | | | | | | 23 weeks | 45 | 246 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 15 | 79 |
| | 550 | s.c. | 50 | 7+ | 0, 1, 2, 3, 4, 5, 6, | 42 days | 5 | 289 |
| | | | 75 | 3 | 9, 14, 21 | 104 days | 5 | 289 |
| | 1000 | i.p. | 100 | 10 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 | 120–140 grams | 2 | 347 |
| Salivary gland | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | 40 | 211 |
| | | | | | | 12 weeks | 45 | 211 |
| | 1000 | i.p. | 100 | 10 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 | 120–140 grams | 1 | 347 |
| Spleen | 5 | i.p. | 2.5 | 2 | 0, 2 | 12 days | 55 | 253 |
| | | | | | | 24 days | 50 | 253 |
| | | | | | | 60 days | 50 | 253 |
| | 10 | i.p. | 5 | 2 | 0, 2 | 12 days | 40 | 253 |
| | | | | | | 24 days | 35 | 253 |
| | | | | | | 60 days | 35 | 253 |
| | 20 | i.p. | 10 | 2 | 0, 2 | 12 days | 30 | 253 |
| | | | | | | 24 days | 30 | 253 |
| | | | | | | 60 days | 30 | 253 |
| | 200 | s.c. | 50 | 4 | 0, 2, 4, 6 | 6 months | 10 | 11 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | 20 | 211 |
| | | | | | | 12 weeks | 15 | 211 |
| | | | | | | 23 weeks | 5 | 211 |
| | 1000 | i.p. | 100 | 10 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 | 120–140 grams | <1 | 347 |
| Pancreas | 200 | s.c. | 50 | 4 | 0, 2, 4, 6 | 6 months | 10 | 11 |
| Pancreas, mesentery | 170 | i.p. | 34 | 5 | 0, 1, 2, 3, 4 | 3–4 months | (80)[b] | 79 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 30 | 79 |
| Kidney | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | (80) | 79 |

TABLE 5—*Continued*

| Tissue | Total Dose of 6-OHDA | Route | Dose | No. of Injections | Age at Time of Treatment | Age at Time of Sacrifice | Per Cent Control NE | Ref. |
|---|---|---|---|---|---|---|---|---|
| | μg/g | | μg/g | | *days* | | | |
| Vas deferens | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | (120) | 211 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | 55 | 79 |
| | 1000 | i.p. | 100 | 10 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 | 120–140 g. | 75 | 347 |
| Adrenal | 300 | s.c. | 100 | 3 | 0, 1, 2 | 10–14 weeks | 130 | 306 |
| | 350 | s.c. | 50 | 7 | 0, 1, 2, 3, 4, 5, 6 | 4 weeks | N.S. | 211 |
| | | | | | | 23 weeks | 130 | 246 |
| | 475 | i.p. | 68 | 7 | 0, 2, 4, 6, 8, 10, 12 | 10 weeks | N.S.[c] | 79 |
| | 1000 | i.p. | 100 | 10 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 | 120–140 | N.S.[d] | 347 |

[a] N.S., no significant difference between 6-OHDA- and control groups.

[b] Numbers in parentheses are indicated literature value with undetermined degree of significance from control group.

[c] E

[d] NE + E

mulation of NE in the axons and cell bodies. Catecholaminergic axons become intensely fluorescent, swollen and distorted (373). Measurement of whole brain NE levels may reflect a balance of terminal depletion or destruction and axonal accumulation of NE, so that the net amount of NE may be unaltered. With increasing time, it is possible for the axons to lose their large NE stores, with the net effect then being a decrease in central NE levels. Because of the relocation of CA stores it is obvious at this point that simple measurement of whole brain NE levels can in no way be used as a reliable index to demonstrate the central effects of 6-OHDA in newborn animals. It is imperative that the brain be dissected into discrete anatomical regions so that the actions on monoaminergic neuronal development can be ascertaned as accurately as possible. Subsequent paragraphs will describe the effects of 6-OHDA on development of noradrenergic neurons in different areas of the CNS.

When administered during the first week of postnatal life 6-OHDA (100 μg/g 3 times, s.c.) produced a moderate decrease in NE concentration of the telendiencephalon. During a period from one week to one year of age, the NE concentration remained reduced by 25 to 50%, with no tendency toward recovery (327). Such effects were also noted after nine weekly injections of 6-OHDA (207). The DA content appeared to be unchanged at three months of age (327).

Administration of 6-OHDA (350 μg/g, s.c.) during the neonatal period did not alter NE levels in the cortex four weeks after initial treatment (211). However, when three additional doses of 75 μg/g were included during the second and third weeks postnatally NE levels were reduced approximately 50% in the frontal and posterior cortices, 42 or 104 days after initial treatment (289). Uptake capacity for $^3$H-NE in the cortex was reduced to the same degree as endogenous NE levels (224). The most effective schedule to date for producing marked depletion of NE in the neonatal rat cortex is 100 μg/g for seven days during the first two weeks after birth. After 10 weeks (79) or three months (341) NE concentration is reduced 90%, while 5-HT concentration is unaffected at the latter time period. The same dosage schedule results in an 85% decrease in NE concentration in the hippocampus, while 5-HT is unaltered. Neither DA, NE, nor 5-HT is affected in the corpus striatum (341) and the levels of the latter two neurohumors likewise are unaltered in

an area which includes the midbrain, hippocampus, striatum, thalamus, and hypothalamus (79, 341). Levels of NE and 5-HT appear to be little affected in the hypothalamus by 6-OHDA (289, 341), although one report (246) indicates a 30% rise in the hypothalamic NE concentration. The dosage and time-course of drug administration may explain these different observations.

The brainstem, containing the long axonal processes extending from the caudal to the rostral regions of the brain, shows a 50 to 75% elevation in NE levels after high doses of 6-OHDA (289), although lower doses (50 μg/g 7 times) reportedly have no effect (211). The midbrain alone, also shows a 75% rise in NE after a large dose of 6-OHDA (341). In the lower brainstem there is a marked increase in NE levels between 2.5 and 4 weeks after treatment with 6-OHDA (100 μg/g 2 times, s.c.) in the neonatal period (327). During this interval, the concentration of NE increases 50%, and is followed by another 30% increase in the following two weeks. After four months, and through the age of one year, NE concentration stabilizes at 120% above control levels (327). Such a marked rise has also been demonstrated by others (79, 207, 341), and accompanied by unaltered 5-HT levels (341). The cerebellum shows a marked decrease in NE concentration after large doses of 6-OHDA (100 μg/g 7 times, s.c.) from birth (79, 341). The effects of neonatal treatment with 6-OHDA on the spinal cord is almost of the same magnitude as on the cerebellum. Large doses of 6-OHDA (100 μg/g 7 times, i.p.) produce a 65% decline in NE concentration (79, 341), while lower doses have a lesser effect (289). A similar degree of reduction in NE uptake capacity is also noted in the spinal cord after treatment with 6-OHDA (224).

In no instance was there a report of altered DA or 5-HT levels after neonatal treatment with 6-OHDA. DA has been assayed in the telediencephalon (327) and corpus striatum (341). Therefore, the effects of 6-OHDA on neonates appear to be selective for impeding the development of noradrenergic neurons.

### *C. Central Injections*

When 6-OHDA is administered centrally to neonates intracisternally the apparent degree of specificity for noradrenergic neurons is lost, and DA depletion is marked. Doses as low as 100 μg deplete central NE levels by 90%, and DA is lowered by about the same degree (59, 253). One interesting point arising from this neonatal study was the actual demonstration that 6-OHDA administered intracisternally has a greater destructive effect on central monoaminergic neurons when administered soon after birth. Possible explanations for such effects are that 1) less 6-OHDA (constant dose) is present on a μg/g basis as the brain matures and increases in mass; 2) MAO content of the brain increases with maturity (23), thereby limiting the intraneuronal concentration of accumulated 6-OHDA; 3) the concentrating ability of monoaminergic neurons for 6-OHDA decreases with age, since noradrenergic perikarya of mature animals are not destroyed with the same facility as in young animals (253). In addition, little development of noradrenergic neurons has occurred within the first week of postnatal life (60, 250) so that 6-OHDA can be concentrated to a much greater degree by the relatively few developing neurons. (4) The proximity of the intracisternally injected 6-OHDA, to the locus coeruleus (noradrenergic) cell bodies allows for a greater concentration of 6-OHDA to be taken up and to destroy the cell bodies. This would likely affect the cerebellum, thalamus, hippocampus, and cortex.

From the studies with 6-OHDA in neonatal animals, several findings appear to be of considerable relevance. To date, various dosage regimens of 6-OHDA have been utilized in mature animals with the purpose of selectively destroying the majority of central noradrenergic neurons. All attempts have been to little avail, since dopaminergic neurons exhibit destructive lesions with

higher doses of 6-OHDA. The same is also true with combinations of agents, such as pargyline (58) or protriptyline (121), with 6-OHDA. When neonates are treated with 6-OHDA there appears to be a high degree of selectivity of the agent for noradrenergic neurons. Doses which produce an 80 to 90% NE decrease in the hippocampus, cerebral, and cerebellar cortices fail to alter DA and 5-HT levels (79, 341). Naturally, any resultant behavioral and growth abnormalities as of yet are complicating factors. It still remains to be determined whether such alterations are a direct effect of the destruction of noradrenergic neurons or more a reflection of a subsequent state of malnutrition. The latter alteration has been implicated in altered maturation of animal subjects (60, 253). Maintenance of treated and control litters on force-fed diets (no simple task) could resolve this problem. Another consideration of neonatal treatment with 6-OHDA is that NE accumulates in the hindbrain regions, whereas the same agent in mature animals produces a depletion of the amine in these regions. Therefore, it would appear that the effects of 6-OHDA are considerably different, qualitatively, in neonatal and mature animals. The different effects are more likely due to the influence of 6-OHDA on the aminergic cell bodies of the brain. With large doses of 6-OHDA administered intracisternally or intraventricularly in adults (47, 378) or in neonates (60), there is the likelihood that both noradrenergic and dopaminergic cell bodies are destroyed.

### *D. Maternal Treatment and Neonatal Effects*

To date only a single report indicates that 6-OHDA, administered to a gestating female mouse, is able to cross the placental barrier and affect the developing pup (72). It was found in this study that NE content of the resultant litter was decreased in both heart and whole brain by 20 and 35%, respectively. There was no effect on 5-HT levels in either tissue, so that the specificity of 6-OHDA for noradrenergic neurons appears to be maintained even at late embryonic stages. It is somewhat surprising that a polar phenolic amine as 6-OHDA would cross the placental barrier unmetabolized. This phenomenon, if real, would have marked implications as to the susceptibility of developing noradrenergic neurons to influence by a large group of agents, including indirectly acting sympathomimetic amines.

## XI. Molecular Mechanisms of Action of 6-OHDA

Because the effects of 6-OHDA on NE levels could readily be blocked by tyramine, guanethidine, and metaraminol it seemed that 6-OHDA had a relatively low affinity for the NE-binding sites. However, because of its prolonged duration of action, as indicated by a reduction of NE content in the mouse heart for up to three months after a single low dose (10 mg/kg i.p.), Porter *et al.* (294) postulated that 6-OHDA "destroys or 'irreversibly' alters the binding sites."

When $^{14}$C-6-OHDA was administered to mice, approximately 25% of the initial amount of label found in the heart was still present after 44 days. The amount of radioactivity retained at any time was inversely related to the degree of NE depletion, so that as the NE content was slowly being restored toward control levels, the amount of label present decreased. Based on these results, it was thought that 6-OHDA, or a metabolite, replaced NE from its binding site and was retained throughout the duration of study at these sites (293). However, when 6-OHDA was assayed fluorometrically its concentration in the heart was seen to fall from a maximal level of more than 1 $\mu$g/g at 15 minutes after injection (10 mg/kg) to a negligible amount at one hour. There was a simultaneous fall in the NE content arguing against replacement of NE by 6-OHDA at binding sites. Chromatograms of hearts indicated that phenolic amines and indole amines, possible metabolites, were present in only submicrogram quantities, if at all. The possibility that neutral, acidic and/or chemically unstable

metabolites produced the sympatholytic action was not ruled out (240).

Thoenen and Tranzer (356) demonstrated in a more quantitative manner that 6-OHDA was not merely replacing NE at the level of the binding site. It was shown that as the dose of $^3$H-6-OHDA was increased, the amount of tritium label in the acidic fraction of column eluates of rat spleen and heart homogenates increased. Also, as the interval between injection time and sacrifice increased, the tritium label in the acidic fraction correspondingly increased. In the amine fraction the converse was found since most of the label in this fraction was present as unchanged $^3$H-6-OHDA. Thus, 6-OHDA apparently destroyed its own binding sites, and therefore, the NE binding sites, in confirmation of the original hypothesis of Porter *et al.* (294). Further studies by Thoenen and Tranzer clarified the apparently discrepant results between Laverty (240) and Porter *et al.* (294). In the heart, one week after treatment with $^3$H-6-OHDA (30 mg/kg), metabolites of $^3$H-6-OHDA were detectable [in agreement with Porter *et al.* (294)] but no $^3$H-amines were found (in agreement with Laverty). Thus, it appeared that a long-lasting metabolite of 6-OHDA was retained in the residual nervous tissues. When the heart and spleen of rats given injections of $^3$H-6-OHDA (30 mg/kg i.v.) 2 or 24 hours before sacrifice were removed, homogenized, centrifuged, washed and rehomogenized five times, it was found that 30 to 35% of the tritium label was retained by the particulate matter even though radioactivity was absent from the supernatant after two or three rehomogenizations. It appeared that the $^3$H-6-OHDA was very likely reacting specifically with the adrenergic nerves rather than non-nervous tissue components. Because of the prolonged retention of the tritium label it was suggested that 6-OHDA or one of its metabolites or spontaneous oxidation products underwent covalent bonding with the nucleophilic groups of biological macromolecules of adrenergic nerves.

Saner and Thoenen (311, 312) utilized bovine serum albumin as a model for macromolecular structure and incubated this with $^3$H-6-OHDA under various conditions. The reaction was stopped by addition of 0.4 N $HClO_4$, the resulting precipitate was homogenized, centrifuged, and rehomogenized 10 times in fresh alcoholic 0.4 N $HClO_4$. The amount of radioactivity bound irreversibly to the albumin increased progressively from pH 4 to 7.4. Oxidation of 6-OHDA proceeded at greater rates at higher pH, and the necessity of this change as a prerequisite to binding was indicated by the fact that the antioxidant, metabisulfite, reduced the binding of tritium at optimal conditions, to nearly background levels. Thus, generation of reactive oxygen appeared necessary for the binding. It was also shown that nucleophilic addition was probably involved in the binding processes, since reduction of the number of nucleophilic groups on the albumin molecule by acetylation also reduced the retention of tritium. That nucleophilic groups specifically were of importance in the above process was indicated by the failure of heat denaturation of the albumin to markedly alter the binding of tritium.

Thoenen *et al.* (358) initially indicated that several postulated intermediaries formed from the auto-oxidation of 6-OHDA may be involved in bringing about neuronal destruction, through the binding with nucleophilic groups of biological macromolecules. Studies by Senoh and Witkop (318) and later studies by Saner and Thoenen (311, 312) pointed out the numerous compounds that were thought to be formed by simple auto-oxidation of 6-OHDA. The rapid appearance of a red color immediately after dissolution of 6-OHDA at neutral or alkaline pH gave the first indication of quinone formation from oxidation of 6-OHDA. Ultraviolet (UV) spectral shifts provided a means of detecting the many suspected oxidation products of 6-OHDA.

At pH 3 a solution of 6-OHDA is stable for at least two hours at room temperature (311, 312). Between 10 and 20 hours, additional UV absorption peaks appear at 265

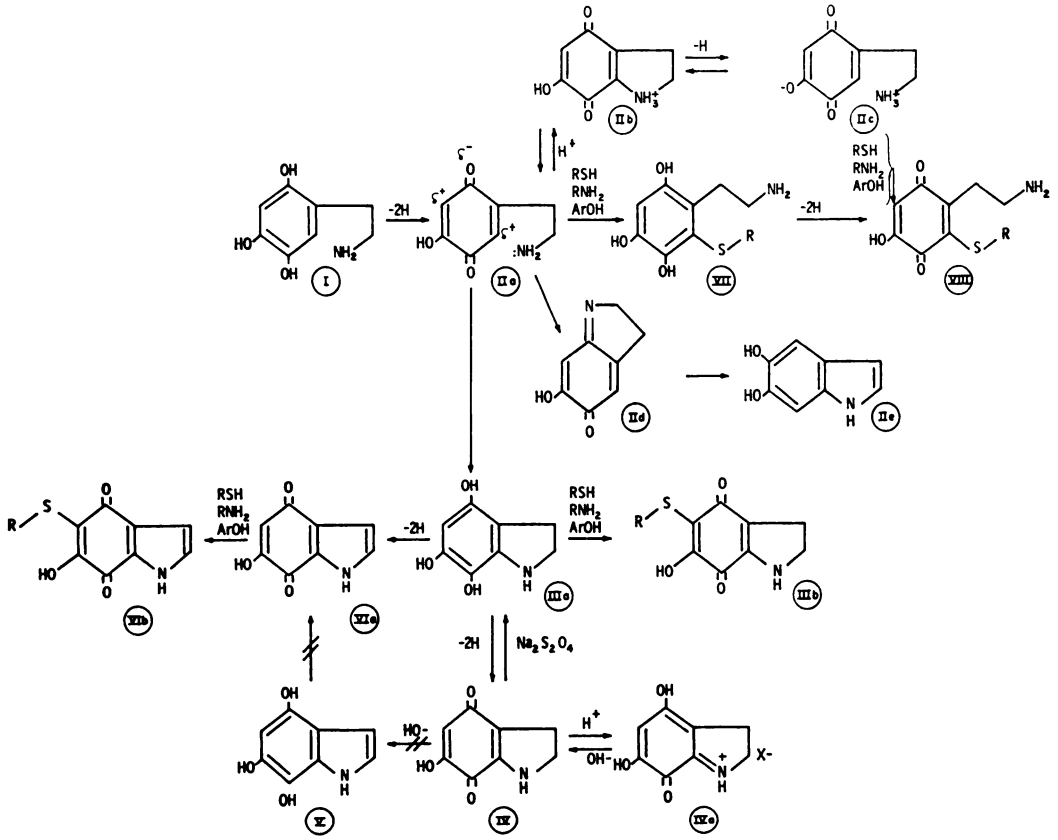

FIG. 1. Suggested intermediary and end products formed by auto-oxidation of 6-OHDA and the proposed reactions of such products with biological macromolecules. The numerical labeling of compounds is in accordance with the convention of Senoh and Witkop (318). The reactions of these compounds with the biological products (RSH, $RNH_2$ and ArOH) is in accordance with the scheme proposed originally by Thoenen *et al.* (358) and later modified by Saner and Thoenen (311, 312). I = 6-Hydroxydopamine. IIa = 6-Hydroxydopamine-*p*-quinone. IIb, IIc = Rearrangement products of II in acidic solution. IId = Aminochrome. IIe = 5,6-dihydroxyindole. IIIa = 4,6,7-Trihydroxyindoline. IV = 4,6,7-Trihydroxydihydroindolequinone or 6-hydroxyindoline-*p*-quinone. V = 4,6,7-Trihydroxyindole. VIa = 6-Hydroxyindole-*p*-quinone. IIIb, VIb, VII, VIII = Suggested types of covalent bonding formed between 6-hydroxydopamine-generated products and reactive groups of biological macromolecules. RSH = Sulfhydryl moiety. $RNH_2$ = Amine function. ArOH = Phenolic grouping.

and 480 nm. This was suggested to indicate formation of an additional compound, thought to be IIa in figure 1. However, subsequent studies indicate another product accounting for the spectral shift may be the semiquinone anion (IIc). Since such solutions *in vitro* generate three equivalents of protons, it is improbable that the aminochrome is responsible for the red color or the spectral shift. At higher pH the auto-oxidation process occurred much more rapidly and at pH 7.4 it was stated that the initial product (IIa) was not observed because of further condensation to IIIa (4,6,7-trihydroxydihydroindole or 4,6,7-trihydroxyindoline), which in turn may have been rapidly oxidized to IV (4,6,7-trihydroxydihydroindolequinone or 6-hydroxyindoline-*p*-quinone). This sequence is that originally proposed by Senoh and Witkop (318) and spectral characteristics described by Saner and Thoenen (311, 312) supported those found by the earlier investigators.

Several lines of evidence indicated that

6-OHDA was converted to a *p*-quinone rather than an *o*-quinone. First, the UV absorption maxima are similar to that of hydroxy-*p*-quinone which absorbs at 260 and 480 to 485 nm. In addition, the polarographic half-wave potential of 6-OHDA, which is determined without interference of slow side chain intramolecular additions is 83 millivolts, also suggestive of *p*-quinone formation. And finally, tritium labeling of the *beta*-carbon of the side chain of 6-OHDA and of the related structure 2-methoxy-4,5-dihydroxyphenethylamine provided evidence that the *p*-quinone was preferentially formed. In the direct formation of the *p*-quinoidal dihydroxyindole (IV) from 6-OHDA, tritium atoms are retained by the parent molecule. However, intramolecular rearrangement of the 2-methoxy-4,5-dihydroxyphenethylamine would result in the formation of an *o*-quinoid aminochrome which tends to further oxidize to the dihydroxyindole, with consequent loss of one of the tritium atoms (fig. 2). Since only background activity was found in the volatile fraction, the product was determined to be a *p*-quinone (318). Later studies showed that the aminochrome IV was relatively stable because of the hydrogen bonding possible between the *o*-oxygen atoms (fig. 2) (98). These studies do not completely rule out the possibility of *o*-quinone formation *in vivo*. It has since been shown that 6-OHDA in the presence of oxygen can directly form a resonant tautomer of both 6-OHDA *o*- and *p*-quinoidal anions. Also, the 6-OHDA-*p*-quinone molecule (IIIa) can be converted to the above tautomeric anions at an alkaline pH (387a). Both the *o*- and *p*-quinones *per se* are capable of bringing about degeneration of sympathetic nerve terminals with both compounds being equally potent, although somewhat less effective than 6-OHDA (182).

The transitory formation of the intermediate IIIa was suggested because of the observation that metabisulfite addition to an acidified solution of IV shifted the absorption peak to 281 nm, which resembles the

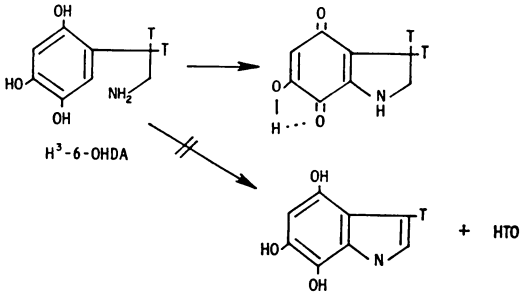


FIG. 2. Fate of the tritium atom on the $\beta$-carbon of the side chain of 6-hydroxydopamine in the formation of 6-hydroxyindoline-*p*-quinone and in formation of a trihydroxyindole, presumably through the rearrangement of 6-hydroxyindoline-*o*-quinone. Note the hydrogen bonding between the *ortho*-oxygen atoms of 6-hydroxyindoline-*p*-quinone.

absorption maximum of a trihydroxybenzene, hydroxyhydroquinone, at 288 nm (318). Similar findings of Saner and Thoenen (311, 312) supported this hypothesis.

An *o*-quinoid-type structure, IVa, was proposed as a by-product of 6-OHDA oxidation, because of a shift in absorption maxima to 262 and 385 nm, after acidification of IV (318). The spectral data are analogous to that of *o*-quinone ($\lambda$ max 390).

Senoh and Witkop (318) concluded that the aminochrome IV was not converted to 6-hydroxyindole *p*-quinone (VI) by addition of alkalai (pH $\sim$ 7.4), because the oxidation-reduction potential presumably was too low for an internal hydrogen shift through the intermediate V. Saner and Thoenen (311, 312) supported this hypothesis but felt that at a lower pH of 5 to 6 there was transformation of IV to VI as indicated by a shift in the peak of IV at 270 nm to maxima at 275 and 293 nm. In addition, it was further suggested that the product III was dehydrogenated to form VI, providing another route for formation of this product (311, 312).

More recently Blank *et al.* (42) have indicated that the internal cyclization of the 6-OHDA molecule proceeded by a one to two condensation rather than by the originally theorized one to four addition. Also, it

was found that 6-OHDA at 25°C would not readily cyclize and would simply be converted to a semiquinone (IIc) by a two electron transfer. Only at a higher temperature was the product 5,6-dihydroxyindole formed to any significant degree *in vitro*. Whether the postulated trihydroxyindolines are formed *in vivo* is not known with certainty, but such products do not appear to be formed *in vitro* (1, 42).

Besides generating quinones, oxidized 6-OHDA was shown 20 years ago to generate 5,6-dihydroxyindole (IIe) (173). Such findings have been confirmed more recently by use of cyclic voltammetry and mass spectrometry (42). It is well known that indoles complexed with proteins, have a yellow fluorescence when exposed to UV light, and it is interesting to note that yellow fluorescence has been observed intraneuronally after high doses of 6-OHDA *in vivo* (87, 368, 371).

Bigler (41) was the first to note peroxide formation from auto-oxidation of 6-OHDA. Heikkila and Cohen (177) demonstrated that 6-OHDA *in vitro* ($5 \times 10^{-4}$ M) is a potent inhibitor of $^3$H-monoamine accumulation by rat brain homogenates and slices, and that it is generated peroxide which is responsible, at least in part, for impaired neuronal function. Suitable control experiments show that the reduction of catecholamine levels is not due to mere oxidation of catecholamines by generated peroxide, but more accurately reflects action at uptake sites. Catalase, an enzyme responsible for destruction of peroxide, was able to partially reverse the inhibition of CA uptake and fully reverse the inhibition of 5-HT uptake by 6-OHDA in brain homogenates. The latter effect is noteworthy, since the 6-OHDA molecule does not compete with 5-HT for uptake sites as it does with NE and DA, thereby implicating a by-product for such an effect. The competition of the 6-OHDA molecule for DA uptake sites was eliminated as a major contributory factor by performing the study with brain slices preincubated with 6-OHDA. In this case catalase restored the uptake capacity of DA to 50% of control, from a low of 6 to 8%. Therefore, experiments *in vitro* demonstrate the capacity of 6-OHDA to impair neuronal function by liberation of peroxide after auto-oxidation.

6-OHDA *in vivo* has also been shown to generate peroxide, as demonstrated by formation of an irreversible complex between catalase and aminotriazole (3-amino-1,2,4-triazole) in red blood cells, a process presumably occurring only in the presence of peroxide. After administration of 6-OHDA, such a process is indicated by a reduction in catalase activity (179).

One noteworthy aspect relating to this phenomenon is the effect of ascorbate on 6-OHDA. Because of the high degree of instability of 6-OHDA in aqueous solutions ascorbic acid is often added as an antioxidant. However, it has been shown recently (179) that ascorbic acid *in vitro* increases the rate of formation and total amount of peroxide liberated by 6-OHDA, and potentiates the inhibitory effect of 6-OHDA on NE or DA uptake by rat brain slices. A suggested reaction sequence to illustrate the mechanism for such a phenomenon is shown in figure 3.

It is interesting to note that 5-hydroxydopamine which is a potent NE depleting agent, does not destroy noradrenergic neu-

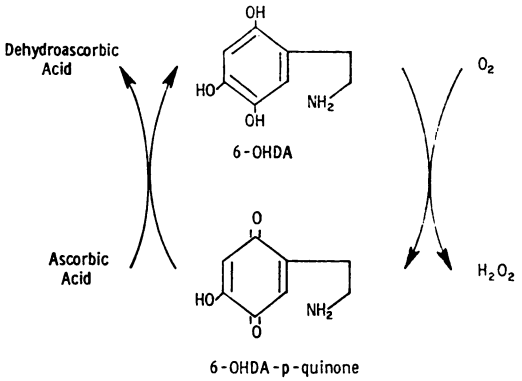


FIG. 3. Suggested reaction sequence whereby ascorbic acid augments the rate of $H_2O_2$ formation by reduction of the quinone form of 6-OHDA, followed by an oxidative recycling (179).

rons as does its analogue, 6-OHDA. Such differential effects may be associated with the capacity to generate reactive oxygen, since 6-OHDA forms peroxide at 12 times the rate of 5-hydroxydopamine. It is also interesting that ascorbate, which reduces the amount of 6-OHDA-*p*-quinone (fig. 3) potentiates the inhibitory effects on CA uptake, thereby supporting the contention that peroxide is an important intermediary or end product in the destructive process of 6-OHDA on monoaminergic neurons. The ascorbate has been shown to bring about such a reduction in quinone-type structures, as evidenced by the diminution of red color in solutions of 6-OHDA and in the prevention of a blackening of tissue slices incubated with 6-OHDA.

In addition to the above by-products suggested to be formed by auto-oxidation of 6-OHDA, it has been found recently that 6-OHDA generates a superoxide radical ($O_2 \cdot^-$) and that this product is involved in the formation of $OH\cdot$ and peroxide according to the scheme:

$$\text{6-OHDA} + O_2 \rightarrow SQ\cdot + O_2 \cdot^- + H^+$$
$$\text{6-OHDA} + O_2 \cdot^- + H^+ \rightarrow SQ\cdot + H_2O_2$$
$$SQ\cdot + O_2 \rightarrow Q + O_2 \cdot^- + H^+$$
$$SQ\cdot + O_2 \cdot^- + H^+ \rightarrow Q + H_2O_2$$
$$O_2 \cdot^- H_2O_2 \rightarrow OH\cdot + OH^- + O_2$$

Any of the highly reactive radicals or the superoxide or peroxide may be responsible for nerve terminal destruction (180).

It has been speculated (178) that the utilization of neuronal oxygen in the formation of peroxide from 6-OHDA, which may be present intraneuronally in amounts as high as $10^{-3}$ M, equal to that calculated for NE (92, 222), results in a relative degree of hypoxia, which of itself may adversely affect neuronal integrity. Also, the peroxide may act to disrupt neuronal structure by reacting with structural lipids, membrane or enzyme sulfhydryl groups (R-SH), protein amino groups ($RNH_2$), or other structural groups as indicated in figure 1. Therefore, from the above series of studies, it appears that 6-OHDA may produce its destructive effect on monoaminergic neurons by the liberation of reactive oxygen which may be present as a quinone, a peroxide, or superoxide.

The possibility was considered that 6-OHDA may be initially converted to 6-hydroxynorepinephrine (6-OHNE), which ultimately would produce neuronal degeneration. However, inhibition of DBH by FLA63, which would inhibit formation of 6-OHNE from 6-OHDA, did not prevent the destructive effect of 6-OHDA, thereby indicating that such a pathway is not of major significance (304).

## XII. Behavioral Alterations Produced by 6-OHDA

### *A. Gross Behavior*

After intracisternal or intraventricular administration of 6-OHDA only slight changes in gross behavior appeared to be produced in rats despite reduction of brain NE and DA to low levels. During the first few hours after administration of 6-OHDA, an effect similar to reserpine was produced in rats, whereupon the animals assumed a posture with hunched back, raised fur (120, 241), and had some diarrhea (184). Food and water intake was initially reported as unchanged after treatment with 6-OHDA (47, 378), or slightly reduced (120) as body weight decreased (58), or was unchanged (241) (see section XII B). The ability of rats to maintain body temperature (241, 377) and to regulate their temperature in a hot or cold environment (47) was reported to be normal (see section XII C). Motor activity and the number of rearings were reduced during the first few days after treatment with 6-OHDA (120, 126, 213) but after 10 days locomotion, as measured in activity cages and in the open field, was normal. At this time, the number of rearings was still reduced 35% (67). The major alterations in behavior appeared to be a lack of self-grooming (47, 58, 126), slightly increased aggressiveness when provoked (47, 213) (see section XII I), mock fighting behavior after D-amphetamine (120), and hypersensitivity to tactile stimulation with animals

preferring to remain isolated from other rats. After one week, treated animals were difficult to distinguish from control animals, although at this time amphetamine was unable to stimulate motor activity in rats treated with 250 μg of 6-OHDA intraventricularly. With lower doses of 6-OHDA, treated rats showed few gross alterations in behavior even during the first week after treatment. In addition, amphetamine was able to elicit increased motor activity (120).

### *B. Food and Water Intake*

Food and water intake was initially reported as normal after administration of 6-OHDA in doses as high as 300 μg i.c. (47) or 500 μg ivt. (241, 378). However, Evetts *et al.* (121) reported a decrease in food and water consumption in the first three days after treatment with 6-OHDA (250 μg 2 times, ivt.) and Breese and Traylor (58) noted a slight decrease in body weight in rats pretreated with pargyline-6-OHDA (200 μg 2 times, i.c.). When pargyline (50 mg/kg i.p., 30 minutes) preceded an intraventricular injection of 6-OHDA the effects on eating and drinking were so marked that many of the treated rats became completely aphagic and adipsic and died unless fed intragastrically (126). Zigmond and Stricker (394) confirmed the 72-hour decrease in food intake after an intraventricular injection of 6-OHDA (200 μg), and showed that the effect (25 to 45% decrease in eating) was not diminished by a second dose of 6-OHDA one week after the first injection. Analysis of brains of rats treated with 6-OHDA showed that NE levels in the telencephalon were lowered by 95%. Pargyline pretreatment, while not modifying the change in NE, further reduced the DA concentration in the striatum from 40 to 5% of control levels (394). Müller *et al.* (275) made similar observations. When treated animals were tested, it was shown that there was little or no increase in food consumption after an acute period of glucoprivation with 2-deoxy-D-glucose (275, 394), but five weeks after treatment with 6-OHDA, food intake was increased when the physiological regulatory demand for food was enhanced by maintaining the rats at lower environmental temperatures (−5° to +15° C) (394). At the end of a 10-week period it was shown that control rats weighed nearly 100 g more than the 6-OHDA-treated (200 μg 2 times) rats although each group averaged 150 g at the start of the experiment. Thus, the growth rate of rats treated intraventricularly with 6-OHDA is considerably less than that of control rats (394).

Ungerstedt (369–371) further implicated dopaminergic neurons as having an important role in feeding behavior, and has suggested that destruction of the nigro-striatal bundle causes the classical "lateral-hypothalamic syndrome" (3, 343, 344). When 6-OHDA (6 to 8 μg) was injected bilaterally into the lateral hypothalamus, animals became completely aphagic and adipsic when the ascending nigro-striatal tract was destroyed, as evidenced by complete disappearance of fluorescent-staining dopaminergic nerve terminals in the corpus striatum. When there was only partial destruction of the tract, recovery of feeding occurred in a number of days (370, 371). After lateral hypothalamic lesioning with 6-OHDA the resultant adipsia was not overcome by hypertonic saline or isoproterenol, both potent dipsogens. Recovery of drinking behavior lagged behind that of eating, with most drinking accompanying ingestion of food, for a period longer than eight weeks (330). Bilateral destruction of the nigro-striatal tract with 6-OHDA in the ventral tegmentum or in the cell body regions of the substantia nigra produced the same deficits in feeding. One consequence of such treatment was that some of the noradrenergic neurons were also destroyed, so that a decreased number of noradrenergic terminals are present in the preoptic and septal regions, as well as in the hypothalamus. This accompanied the diminution in dopaminergic terminals in the olfactory tubercles and nucleus accumbens. That these neuronal components were not the major sites for the

observed eating behavioral changes were indicated by the finding that bilateral destruction of these noradrenergic terminals with a lesion of the ascending noradrenergic tracts at the level caudal to the substantia nigra, or bilateral lesion of the A10 dopaminergic tracts to the nucleus accumbens and olfactory tubercle did not result in the same altered eating responses. A relative specificity of 6-OHDA for monoaminergic neurons in these experiments was indicated by the finding that none of the treated animals became cataleptic, as did a number of rats after electrothermal lesions. Because of the number of different levels at which 6-OHDA produced deficits in the eating response, it appears that the nigro-striatal pathway may indeed play a major role in eating behavior (370, 371). However, nonspecific lesions about 0.3 mm diameter, were often produced at the tip of the injection cannula, so that contributory involvement of other tracts in eating behavior cannot completely be ruled out.

Evetts *et al.* (119) provided evidence for some involvement of these forebrain areas in eating and drinking behavior. When 6-OHDA (0.01 to 16 μg) was injected into the preoptic region of satiated rats, there was a dose-dependent (0.01 to 1.0 μg) increase in eating and drinking lasting for several hours. Repeated injections of 6-OHDA at intervals of several days produced a progressively reduced eating response, indicating an indirect NE releasing action of 6-OHDA, also suggested by the fact that intraventricular pretreatment with desipramine, phentolamine, or sotalol (MJ-1999) blocked the effects. Desipramine and sotalol also reduced water intake after treatment with 6-OHDA, although phentolamine enhanced the response (119). Also, different latency periods were involved for the eating (25 minutes) and drinking (160 minutes) response, so that different neuronal components are likely involved.

Therefore, from these studies it is obvious that the acute effects produced by parenchymal injection of 6-OHDA in the CNS can be due to the indirect sympathomimetic actions of the agent followed by the permanent destructive lesioning with the effect of the latter process depending on the particular nerve tract involved. In general, though, an intraventricular administration of a suitably high dose of 6-OHDA appears to be accompanied by a decrease in eating and drinking and a slowing of the growth rate.

### *C. Body Temperature*

When 6-OHDA (12.5 to 300 μg) is injected into one of the brain cavities of rats maintained at room temperature there follows an immediate dose-related decrease in body temperature (4 to 5°C) with the nadir appearing at one hour after a 300-μg dose and at later time periods for lower doses (56, 279, 324). Recovery to preinjection ambient temperatures is fairly rapid, being complete in a matter of hours (279, 324). Similar experiments performed at 3°C in rats pretreated with desipramine provide essentially the same results (55, 56, 199), except that the hypothermia is much greater (12°C fall by two hours) after 6-OHDA (150 μg i.c.). Pargyline pretreatment of 6-OHDA-treated rats placed in the cold at 3°C produces a 23°C fall in body temperature. The desipramine apparently enhances the responses to 6-OHDA by inactivating the reuptake mechanism for released NE, thereby increasing its effective concentration at the receptor site. Pargyline inhibits monoamine oxidase, thereby increasing the amount and duration of 6-OHDA in the brain, ultimately producing a greater degree of destruction of noradrenergic and dopaminergic nerves.

Because of the acute nature of the response and the recovery to control levels with rats maintained at room temperature, it was suggested that such an effect was related to the sympathomimetic properties of 6-OHDA, *i.e.*, release of endogenous catecholamines from the damaged neurons onto their effector sites. When rats are pretreated with 6-OHDA (300 μg ivt., 250 μg 2 times, ivt. or 200 μg 2 times i.c.) one day to three

weeks prior to study, it was indeed found that a second dose of 6-OHDA did not produce a hypothermic response (54, 56, 199, 253, 279, 324) and a slight elevation in body temperature was noted two to four hours after treatment (324). Also, when rats were pretreated with reserpine the hypothermia produced by 6-OHDA (300 μg ivt.) was inversely related to the dose of reserpine (279). Both pretreatment schedules reduced whole brain NE levels by 80 to 95%, while DA was lowered 65% after administration of 6-OHDA. Tyrosine hydroxylase inhibition with α-methyl-*p*-tyrosine did not alter the hypothermic response to 6-OHDA (237).

In an attempt to further categorize the response as being related specifically to NE or DA release, different pretreatment schedules were applied. With a regimen of 6-OHDA that primarily depletes brain DA stores (56, 59, 120), a hypothermia was still observed after injection of 6-OHDA (250 μg ivt. or 150 μg i.c., preceded 60 minutes by desipramine), in rats kept at 3°C or 22°C (55, 56, 199, 324), although the hypothermic response of desipramine-treated rats at 3°C was less than that of controls (54, 199). In another group of rats pretreated with a regimen of 6-OHDA (25 μg 3 times, ivt., one week before trial) that does not alter brain DA but decreases brain NE content by 75%, 6-OHDA (250 μg ivt.) produces the typical hypothermic response in rats at room temperature (324). However, when the same study was performed in desipramine-treated or pargyline-treated rats, a marked attenuation of the hypothermia was observed (54, 56, 199).

When tyramine (500 μg ivt.) was administered to rats that had been chronically depleted of NE, the usual hypothermic response was absent (228). Injections of NE (20 to 100 μg ivt.) still produced the usual dose-related hypothermia after treatment with 6-OHDA, although it appeared to be less effective, possibly due to receptor damage (228, 279). Likewise, DA (50 to 100 μg, ivt.) also was effective after administration of 6-OHDA, but produced only a slight transient decrease of body temperature (279). Because of this, it would appear that the hypothermic responses of 6-OHDA are due primarily to the release of endogenous stores of NE. This is also supported by the fact that phentolamine (50 to 100 μg, ivt.), an *alpha*-adrenergic blocking agent, inhibited the hypothermia previously observed after treatment with both 6-OHDA and NE, although propranolol (50 to 200 μg, ivt.) had no effect. Also, desipramine (20 to 200 μg, ivt.) and cocaine (20 to 100 μg, ivt.), which inhibit uptake of sympathomimetic amines, including 6-OHDA, into adrenergic neurons, blocked the effects of 6-OHDA while that of NE was still demonstrable, although reduced (279). The agent, chlorimipramine, a 5-HT receptor blocker, effectively antagonized the hypothermia produced by 6-OHDA, indicating a possible role of 5-HT in the response or reflecting a nonspecific receptor blocking action of chlorimipramine (237). Finally, spiramide (10 mg/kg i.p.) a potent blocker of dopaminergic receptors (4) failed to alter the hypothermic response to 6-OHDA (279), while apomorphine, which stimulates dopaminergic receptors (10, 116–118), decreased body temperature (147).

Despite the often marked acute thermal responses to 6-OHDA there appears to be no long-lasting effect on the temperature regulatory center. When "centrally sympathectomized" rats (6-OHDA, 250 μg 2 times, ivt., 12 to 17 days before testing) were placed in a hot (32°C) or cold (15°C, 9°, −5°C) environment for up to four days, body temperature responses were like those of saline controls, although the rise was somewhat less than that of the controls at 32°C (324, 394). Thus, there appears to be no impairment of the ability to regulate body temperature.

Therefore, studies with 6-OHDA indicate that this agent produces an acute hypothermia immediately upon injection, apparently due to release of NE from damaged neurons, with consequent spillover onto receptor sites. It should also be pointed out that although

different pretreatment schedules indicate a selective effect on a single neurohumoral agent, a goal most desirable, one should be wary of the possibility that decreases of one transmitter in one brain region are offset by increases of the same or other amine in the other brain regions. Also, the size of the affected site may be so minute in proportion to the tissue under assay, that detection of such an alteration is extremely difficult. Thus, the effects of 6-OHDA at the temperature-regulation center in the hypothalamus cannot be known for certain. The possibility of a sprouting phenomenon of another aminergic system, such as serotonergic nerves, after treatment with 6-OHDA, should be seriously considered.

### *D. Locomotor Activity*

When injected intraventricularly 6-OHDA produces slight transient decreases in spontaneous locomotor activity lasting for a period of hours to days, as determined in activity cages and open field tests (121, 126, 184, 213, 279). Nocturnal activity on a treadwheel is likewise decreased for a period of days, and the threshold of 6-OHDA for this parameter is less than for the previously mentioned motor activities (241). Exploratory activity, as measured in a Y-runway and by the number of rearings, is markedly reduced by 6-OHDA for much longer time periods (67, 184, 213, 241, 342). The number of rearings in treated animals decreases for a much longer period of time (67, 213) and this has loosely been termed as a reduction in exploratory activity (184). Intraventricular injections of NE produce a similar reduction in motor activity (280), and it is possible that the direct release of NE by 6-OHDA in the CNS produces this response acutely. However, desipramine is able to prevent whole brain depletion of NE but does not alter the motor responses produced by 6-OHDA (342). While it can be argued that 6-OHDA produces changes in behavior but not release of CA, one must utilize caution when comparing changes in whole brain levels of CA with an action produced in discrete areas of the brain. After several days, it is unlikely that a sympathomimetic discharge is still occurring, and it is possible that increased turnover of, or supersensitivity to, monoamines at certain sites in the brain is responsible for behavioral alterations, if CA are involved in prolonged effects. Such an increased turnover of CA and development of supersensitivity has been demonstrated or suggested previously (372, 374, 376, 379, 380). It was also suggested that the development of supersensitivity in the CNS accounted for the enhanced locomotor responses to L-dopa in 6-OHDA-pretreated rats, when L-dopa was preceded by a dopa decarboxylase inhibitor (379).

When 6-OHDA treatment was preceded by administration of a monoamine oxidase inhibitor completely different responses were observed. Spontaneous locomotion increased as much as 15-fold over a period of up to 12 hours, with the temporal duration dependent on the dose of 6-OHDA (126, 382). The response appeared to be mediated by release of newly synthesized CA, since *alpha*-methyl-*p*-tyrosine was capable of blocking the response. Granule pools of monoamines did not appear to play a major part in the response, since reserpine pretreatment reduced, but was not effective in blocking the increased motor activity. Since pargyline potentiated CA depletion by 6-OHDA only in the caudate nucleus after three to four days, it was postulated that increased release of newly synthesized DA was responsible for the increased motor response (126). However, it should be noted that DA levels were studied in the aforementioned investigations only after three to four days. In rat striatum DA levels initially increase approximately 2-fold after 6-OHDA over a period of about 24 hours (35), which would coincide with the time period in which increased motor activity was observed. Presumably, DA turnover decreased (after 6-OHDA alone) in this interval since T-OH activity was found to be unaltered. Whether DA

turnover increases after pargyline-6-OHDA combination still remains to be determined.

Vetulani *et al.* (382) made similar observations that nialamide-6-OHDA would increase motor activity in rats. During the peak of the response, at two hours, animals were sacrificed and it was found that DA was elevated by 60% in the rat brain, indicating a decrease in DA turnover. However, spiroperidol, a dopamine receptor antagonist, blocked the motor response (4). Spiroperidol also produced a mild cataleptic state in the experimental animals, so that the blockade of the motor response could have been due more to a nonspecific sedative effect. Actual measurement of DA turnover is necessary to clarify the mechanism of the effect.

Ungerstedt (368) demonstrated that 6-OHDA, injected unilaterally into the substantia nigra, would cause degeneration of the nigro-neostriatal bundle and induce ipsilateral rotational activity, similar to that produced by electrothermally lesioning the striatum of rats (190). This effect was later quantified (376) and it was shown subsequently that supersensitivity to DA developed on the lesioned side. Apomorphine, L-dopa, and amphetamine reversed the direction of rotation in lesioned rats, apparently because of the increased number of stimulated receptors in the striatum on the side treated by injection (279, 372, 374). Haloperidol and spiroperidol, DA receptor antagonists (4), effectively blocked the ipsilateral rotation induced by amphetamine, as did treatment with a T-OH inhibitor, $\alpha$-methyl-*p*-tyrosine (375).

When 6-OHDA is injected bilaterally into the substantia nigra there is a marked hypoactivity and consequent loss of exploratory activity. Rats become aphagic and adipsic and die in a matter of days unless they are force-fed (370). After five to seven days, the limbs exhibit obvious rigidity and the *alpha*-activity is greater than the *gamma*-activity (225). A less severe degree of hypoactivity is produced in rats when 6-OHDA is injected at different sites along the medial forebrain bundle (330), but this is likely to be unrelated to action on dopaminergic neurons because of the anatomical location of the respective tracts (7, 96, 373, 375). In cats, 6-OHDA (10 $\mu$g) injected into the ventral tegmentum resulted in destruction of 70% of the neurons in the substantia nigra and led to subsequent sensorimotor impairment. Cats displayed rotational motor activity, truncal ataxia, staggering, and falling as well as "somersaulting and nosediving" when moving from a higher to a lower place (137).

Studies with 6-OHDA also show that striatally-lesioned rats fail to develop stereotypy after iproniazid-amphetamine, which increases locomotion in treated rats, but decreases locomotion in controls because of development of stereotypy (94).

### *E. Audiogenic Seizures*

6-OHDA (200 $\mu$g ivt. 2 times, 48-hour interval), when administered to audiogenic seizure-susceptible rats, increased the severity of seizures for a period of at least 12 days (50). Because reserpine produced a similar effect (22) it appears that the change in seizure threshold produced by 6-OHDA is the result of depletion of CA and not 5-HT stores, although the site in the brain associated with this physiopathological response is not known.

### *F. Effects of 6-OHDA in Morphine-treated Mice*

In both morphine-tolerant and nontolerant mice treatment with 6-OHDA (28 $\mu$g i.c.) reduced the analgesic response to morphine. The rate of development of tolerance was unchanged, but precipitated abstinence, as measured by naloxone-induced withdrawal jumping, was enhanced (136). The Straub tail phenomenon was also reduced (392). Rats with chronic morphine treatment were found to be less irritable after treatment with 6-OHDA (34 or 204 $\mu$g ivt.) and DA levels were reduced to a much lesser extent. It was postulated that morphine may directly or indirectly inhibit 6-OHDA up-

take by dopaminergic neurons in the CNS (278).

### G. Sleep

Buguet *et al.* (66) showed that 6-OHDA (5.5 μg), injected directly into the regions of the pons tegmentum immediately reduced the pontine-geniculo-occipital (PGO) discharge activity of the thalamus and cortex of cats, and by five to six hours, cortical discharges were nil. Additional studies in cats demonstrated that 2.5 mg of 6-OHDA injected intraventricularly immediately caused an increase in PGO discharges, cortical arousal, and agitation. The latter two responses were believed to be caused by NE discharge onto receptors, since the effects were blocked by α-methyl-*p*-tyrosine, a T-OH inhibitor. The prolonged PGO discharges lasting for approximately five days, were thought to be mediated by 5-HT release, since chlorimipramine effectively blocked the response. Following such activity, there was a permanent or semipermanent decrease in the frequency of PGO discharge, and a dose-dependent reduction in paradoxical sleep. During this period there was no change in cortical synchronization. After chlorimipramine, 6-OHDA initially reduced paradoxical sleep for a period of six days followed by a return to control. After a short time the duration of paradoxical sleep decreased in this group of cats. These changes occurred in the absence of any change in PGO discharge activity, and appeared to be related to reduced CA content (237, 291).

When rats were treated with 6-OHDA (500 μg i.c., divided dose) and tested for sleep patterns at intervals up to three months postinjection, it was found that animals deprived of desynchronized sleep for 72 hours recovered immediately after removal from the island. Desynchronized sleep increased markedly in the ensuing eight-hour period, while waking time decreased. Synchronized sleeping time remained unchanged (174). Similar effects on sleep were observed in chicks after 6-OHDA (78).

### H. Reproductive Physiology

When 6-OHDA (250 μg, ivt.) was introduced to the CSF during the estrus or diestrus I phase, spontaneous ovulation was blocked in 80% of rats (226, 301). When administered during diestrus II, 6-OHDA effectively blocked ovulation in only 20% of rats. Because reserpine also inhibited ovulation (26) and this response was reversed with MAO-inhibitors (276) by preventing CA depletion, there is strong implication of involvement of the tuberoinfundibular dopaminergic neurons (142–145) in mediating luteinizing hormone (LH) release from the pituitary. The above findings with 6-OHDA appear to add more fuel to the controversy concerning whether such dopaminergic neurons exert a positive (267, 269, 314) or negative (146) control on LH release from the pituitary.

Kalnins and Ruf (226) and Ruf (301) have shown that the effects of 6-OHDA are reversed by exogenous LH or LH-releasing factor or electrical stimulation of the basal hypothalamus, thus giving support to the hypothesis that the tubero-infundibular tract exerts a positive control on gonadotropin-releasing factors. However, one should also be mindful that the initial effect of 6-OHDA is a sympathomimetic action, which may be of major importance in the above experiment. Also, because of the intraventricular route of administration, 6-OHDA affects the entire distribution of CA-containing neurons in the CNS and therefore, possibly exerts a nonspecific effect on this response.

An intraventricular injection of 250 μg of 6-OHDA causes only a small decrease (about 20%) in the intensity of the CA fluorescence in the median-eminence as observed after seven days by the Falck-Hillarp method (personal observation by D. M. Jacobowitz). The DA-containing arcuate nucleus cell bodies in the male rat brain remain intact. However, the fluorescence intensity of CA-containing nuclei adjacent to the third ventricle (*e.g.*, paraventricular, periventricular, dorsomedialis) is markedly reduced at this

time period. Also, local administration of 6-OHDA (250 μg) into the third ventricle caused a marked decrease (about 75%) of the fluorescence intensity of the DA-containing external layer of the median eminence. Selective destruction of the tract by local administration of 6-OHDA appears to be an ideal approach in studying the role of this pathway on release of the pituitary hormones.

It would appear that intracisternal injection of 6-OHDA does not exert a lasting influence on the reproductive cycle of rats, since they are able to become pregnant, maintain a 22-day gestation period, and deliver a normal number of pups. 6-OHDA administered intraperitoneally to mice does not appear to affect the estrus cycle nor the capacity to conceive and maintain a pregnancy, although the litter size is significantly decreased. Rabbits, however, do not become pregnant after treatment with 6-OHDA (72). When administered to newborn rats 6-OHDA does not appear to impede reproductive behavior, except for delaying vaginal opening (157).

### *I. Irritability and Aggression*

After intraventricular injection of 6-OHDA, the behavior of rats is permanently altered (>4 months) insofar that they appear to exhibit an increased "irritability" or reactivity to exogenous nociceptive (nonpainful) stimuli. Rats display exaggerated responses to blowing air on their backs, or touching the back with a glass rod, touching their whiskers with a rod, or simply holding them by hand. Responses range from panic jumping and running to crying, biting, and severe attack (121, 184, 279, 280, 337, 382). The degree of irritability appears to be correlated to the degree of NE depletion in the hypothalamus, pons medulla and residual parts of the brain. Meprobamate, diazepam, and chlordiazepoxide decreased the irritability of rats and also normalized the $^3$H-NE decay rate toward control levels in both the brainstem and residual brain (280). Because of this effect, it would appear that the increased turnover rate of remaining noradrenergic neurons and/or receptor supersensitivity is associated with the increased irritability observed in the treated rats and not to the generalized decreased CA content throughout the brain. Such an hypothesis remains to be tested.

Possibly related to the above behavior is the facilitated aggression observed in the 6-OHDA-treated rat. When placed in pairs in isolated cells, shock-induced fighting in rats after treatment with 6-OHDA is markedly increased (111, 337, 345, 346). The effect is noted even when only one of the pair of rats is treated with 6-OHDA. Jump-threshold and mouse-killing behavior is unaltered so that the effect is rather selective and not related to a change in pain threshold. Both NE and DA are markedly reduced in rats after a single injection of 6-OHDA, during the time when aggression is apparent. With a second dose of 6-OHDA, 5-HT is reduced by 55% and NE and DA by 75 and 90% respectively; aggressive behavior is enhanced after such treatment (111). Whether the aggression is related to a generalized decrease in CA or due to changes in turnover of monoamines or to receptor supersensitivity is still undetermined. However, with desipramine pretreatment, 6-OHDA-treated rats fail to display the shock-induced fighting. In this group, brain NE is reduced only 25%, as compared with an 80% reduction in rats treated with 6-OHDA alone (345, 346). Therefore, it is possible that noradrenergic neurons play a greater role in this behavior than dopaminergic neurons. On the other hand, it has recently been shown that morphine-tolerant rats are much less irritable after treatment with 6-OHDA (204 μg ivt.), and in such rats the DA content of the brain is reduced less than 20%, while NE depletion is identical to that produced in nontolerant rats. Therefore, the irritability induced in rats by 6-OHDA also seems to involve dopaminergic neurons (278).

### *J. Operant Activity*

When 6-OHDA (140 μg) was administered intraventricularly to rats it was found that conditioned-avoidance (pole climbing, foot shock) responding markedly decreased for the first two days postinjection. Within the following week, most rats recovered to pretreatment levels of activity, although by 32 days a few animals failed to respond consistently (241). In active-avoidance testing there was an observed tendency for chronically treated rats (500 μg 6-OHDA ivt., divided dose) to require a greater number of trials for acquiring the acquisition-avoidance response (89, 335, 337). Acquisition of a passive avoidance task was found to be unaltered after intracisternal injection of 6-OHDA (88). When treatment with pargyline preceded injection of 6-OHDA (400 μg i.c., divided dose), resulting in a greater destruction of CA-containing neurons, rats failed to acquire active avoidance responding for a period of over two months. A major component of this effect was attributable to loss of noradrenergic neurons, since partial protection from 6-OHDA afforded by desipramine resulted in a normal number of responses. In groups of rats where DA content of the brain was reduced only 25%, with NE reduced to a moderate degree by 6-OHDA (100 μg i.c. divided doses), rats were able to acquire the shuttle-box avoidance response. The number of responses was far greater than that observed in the control groups (88, 89). In the "NE-down" group reserpine or α-methyltyrosine slightly decreased the number of avoidance responses, but the effect was most marked in the "DA-down" group. Therefore, although both noradrenergic and dopaminergic nerves modify active-avoidance behavior, dopaminergic neurons appeared to be considerably more important (88).

It is noteworthy that intraperitoneal administration of 6-OHDA retarded the rate of acquisition of a passive avoidance task (107) and significantly decreased escape and avoidance responding on a difficult task (108). Responses were not markedly altered when the avoidance task was easy to acquire (108). Such studies illustrate the importance of the sympathetic nervous system in behavior elicited by aversive stimuli.

In other studies where rats were trained on a schedule of water reinforcement (fixed ratio), it was found that 6-OHDA decreased the number of responses initially, but within a few days, rats returned to the initial rate of activity (316). With a variable-interval schedule, characterized by a very high number of bar-presses, 6-OHDA-treated (250 μg 2 times, ivt.) rats increased the number of responses 4-fold over controls, with a leveling off of activity about seven weeks after treatment. In rats treated with 6-OHDA after training, the same type of behavioral alterations were noted.

In rats chronically implanted with electrodes aimed at the medial forebrain bundle and trained to bar-press, 6-OHDA (200 μg i.c., pargyline pretreatment) was found to decrease the self-stimulation rate by 30 to 50% for as long as one month. Administration of a second dose of 6-OHDA (200 μg i.c.) at this time virtually eliminated bar pressing in nearly all subjects (55). Similar findings were also reported by Stein and Wise (334, 335) who speculated on the involvement of 6-OHDA as part of the etiology of schizophrenia, Parkinsonism, and possibly other affective disorders. Such speculation has been debated (18, 51, 340).

## XIII. Miscellaneous Actions of 6-OHDA

### *A. Effect on Skin Grafts*

In neonatal mice that were treated with 6-OHDA (50 μg/g i.p.) it was found that skin grafts survived for at least 25 days, compared to untreated mice that rejected the homotransplant after about eight days. This finding indicates that the immunological process may be markedly altered by 6-OHDA treatment (336). Alternatively, it may represent an improved blood flow to the grafted region because of the absence of sympathetic vasoconstrictor tone, and thereby, better oxygenation of the grafted tissue (282).

### *B. Effect on Melanophores*

When injected into the teleost fish, *Gasterosteus aculeatus*, 6-OHDA (20 μg/g i.p.) causes an immediate concentration of pigment grains followed by pigment dispersion for a period of three weeks. During this time the ability to adapt to a white background is markedly suppressed, although the speed of adaptation slowly increases in this interval. It has been proposed that 6-OHDA may be producing the contraction phase because of its sympathomimetic nature, as injection of NE produces a similar effect (134).

### *C. Effect on Corticosterone Levels*

When corticosterone levels in the blood of rats treated with 6-OHDA (25 μg/g i.c. 2 times, 48-hour interval and preceded by pargyline) were measured, it was found that the usual diurnal variation of corticosterone was absent in rats whose brain NE levels were reduced 70% (367).

## XIV. Biological Effects of 6-Hydroxydopa

The biological actions of 6-hydroxydopa (6-OHDOPA) in mice were first reported by Ong *et al.* (287). It was shown that 6-OHDOPA was able to deplete NE stores not only in peripheral end organs but also in the brain after simple parenteral administration (i.v., s.c., i.p.). Therefore, 6-OHDOPA has a distinct advantage over 6-OHDA, insofar that the former agent is capable of crossing the blood-brain barrier to affect noradrenergic neurons. Described below is a summary of the actions of 6-OHDOPA on monoaminergic neurons, with the topics of discussion arranged in the same order as those outlined for this review on 6-OHDA.

It has become evident through studies *in vivo* (91, 231, 232, 287, 305) and *in vitro* (287) that 6-OHDOPA derives its pharmacological activity from metabolic conversion to 6-OHDA. Studies with several decarboxylase inhibitors, including N-DL-seryl-2,3,4-trihydroxybenzylhydrazine (287), 2,4-dihydroxyphenyl-α-hydrazino-α-methylpropronic acid (MK-485) (91, 305), L-α-hydrazinemethyldopa (carbidopa, MK-486) (231, 232), NSD 1055 (231, 232), and Ro 4-4602 (308), have shown that 6-OHDOPA loses its ability to deplete NE stores when the decarboxylase enzyme is inhibited. It appears that the decarboxylation of 6-OHDOPA occurs extraneuronally, since several agents such as D- and L-amphetamine, chlorpromazine, and desipramine, known to act in a competitive manner for the neuronal uptake-1 sites (see section III A), effectively antagonize the actions of 6-OHDOPA on the neuron (231, 232). These agents would be expected to have little effect on the neurotoxic actions if the decarboxylation occurred intraneuronally. Furthermore, the selectivity of 6-OHDOPA for noradrenergic neurons, described below, also suggests extraneuronal conversion to the amine, 6-OHDA, followed by selective uptake of the product by the neuronal amine uptake pump. One interesting finding was that MK-486, a peripherally-acting dopa decarboxylase inhibitor, effectively antagonized the actions of 6-OHDOPA on sympathetic terminals in the heart, but potentiated the NE depleting action of 6-OHDOPA on the brain (231, 232, 287). It would appear that protection of the 6-OHDOPA molecule from enzymatic conversion in the peripheral nervous system allowed for a greater availability of the molecule in the CNS. Such a study illustrates a potential usefulness of 6-OHDOPA for selectively altering central noradrenergic function while leaving peripheral noradrenergic neurons intact. When MAO was inhibited with nialamide (305, 308) or tranylcypramine (231, 232), there was a potentiation of the neurotoxic actions of 6-OHDOPA, indicating that the generated by-product, 6-OHDA, is a substrate for MAO. The enzyme, COMT, appears to have no major role on 6-OHDOPA metabolism, since inhibition of the enzyme with β-isopropyltropolone (thujaplicin) does not significantly modify the actions of 6-OHDOPA (231, 232).

Berkowitz *et al.* (39) have synthesized

the (−)- and (+)-stereoisomers of 6-OHDOPA and have shown that (−)-6-OHDOPA is far more effective than (+)-6-OHDOPA in depleting brain and heart NE stores. Such a finding would be expected, if uptake-1 processes were responsible for accumulation of the neurotoxic metabolites by noradrenergic neurons, since the uptake pump is distinctly stereospecific for the (−)-isomers. It is interesting that the (+)-form also has the capacity for producing at least a transient decrease in NE stores in both the central and peripheral nervous system.

Studies comparing different routes for administration of 6-OHDOPA have shown an intravenous route to be far superior to an intraperitoneal or subcutaneous route in decreasing CNS levels of NE. A single 150 mg/kg dose of 6-OHDOPA, administered intraperitoneally or subcutaneously produced a 40% decrease in mouse whole brain NE levels at three hours and only a 20 to 25% decrease at 24 hours, while an equimolar intravenous dose decreased brain NE by 70 and 55% respectively, at 3 and 24 hours (232). Time-course studies demonstrate the capacity of 6-OHDOPA for producing a long-lasting depletion of NE at both the 100 and 150 mg/kg doses (i.v.). After two months, whole brain NE is still far below control levels, although there is a significant recovery toward normal, indicating regenerative phenomena in the brain. Histochemical observation of brains of treated animals have shown evidence of such regeneration after only two weeks in the preoptic area, septum, hypothalamus, and pons medulla (210). It has also been shown after 6-OHDOPA (100 mg/kg 3 times, i.p., nialamide two hours) that uptake of $^{3}$H-metaraminol increases nearly 2-fold in cortical slices, between 16 hours and 28 days, indicating regeneration and/or sprouting of adrenergic neurons. However, in this same study it was also noted that both the NE content and the number of nerve terminals observed with fluorescence microscopy from smears of the neocortex was reduced to the same extent at four weeks as at 16 hours after injection (308). One wonders, in this regard, whether the initial NE depletion in the CNS is due mainly to displacement of NE in noradrenergic storage sites by 6-OHDOPA and its metabolites or whether the decrease is actually a reflection of impairment of neuronal function or caused by nerve terminal destruction. It is, indeed, felt that with a lower dose of 6-OHDOPA (20 mg/kg i.v.) the major mechanism for producing NE depletion is simple displacement by a false transmitter, since brain NE is reduced 25 to 30% three hours after treatment and is back to control levels by 24 hours (232). Such a process may also account for part of the depletion at higher dose levels (50 to 150 mg/kg i.v.), but because recovery is gradual over a prolonged period it is felt that the major depleting mechanism is due to degeneration of the terminals, since pseudotransmitters have a relatively short half-life in neurons (210). Also, the initial decrease in NE content is well correlated with a reduction in the uptake capacity of $^{3}$H-NE and $^{3}$H-metaraminol, suggestive of actual destruction of the terminals. Such an observation has indeed been made by use of histofluorescence microscopy (210, 308) and by other light and electron microscopic techniques (230), as will be described subsequently.

When brains of mice treated with 6-OHDOPA (100 mg/kg i.v.) were subdivided, it could be seen that the NE content was reduced by 50 to 55% in the telencephalon and cerebellum, 14 days after treatment. In the brainstem NE was reduced only 20% after (±)-6-OHDOPA (100 mg/kg i.v.) (210) and 35% after (−)-6-OHDOPA (100 mg/kg 2 times i.p.) (39). The DA content of the telencephalon remained unaltered (210). These findings, in conjunction with histochemical data, indicate that noradrenergic neurons in the cortical regions of the brain are most susceptible to damage by 6-OHDOPA. However, it is realized that damage of terminals, as measured by NE depletion, could be masked in

the brainstem because of the retrograde accumulation of NE in axons coursing through the region.

When 6-OHDOPA was injected intraventricularly directly into the CSF the same selectivity for noradrenergic neurons was noted. Telencephalic DA content remained unaltered 48 hours after doses of 6-OHDOPA as high as 180 μg. Time-course studies also showed DA to be unaltered at intervals from 2 to 70 days after treatment with 90 μg of 6-OHDOPA. Telencephalic NE was reduced 20 to 35% 48 hours after graded doses of 6-OHDOPA (45 to 180 μg). In the hindbrain NE levels were decreased in a dose-dependent manner by 6-OHDOPA at this time period, with the reduction ranging between 15 and 45%. The same situation prevailed in the diencephalon, with the 45 to 180 μg sequence of 6-OHDOPA producing a 25 to 55% decrease in NE. In the cerebellum there appeared to be a threshold dose of about 90 μg 6-OHDOPA, which reduced NE content by 40%. A dose of 60 μg produced no effect on the NE content of this region (299).

As was noted after intravenous administration of 6-OHDOPA, recovery of NE toward control levels occurred in certain regions of rat brain after intraventricular injection. Telencephalic NE which was reduced 30 to 35% up to two weeks after 6-OHDOPA (90 μg ivt.), was unaltered 10 weeks after treatment. Brainstem NE increased gradually over this period and was also at pretreatment levels 10 weeks after 6-OHDOPA. Diencephalic NE, which was 60% of control two days after treatment with 6-OHDOPA (90 μg ivt.), decreased progressively over the next 12 days, at which time NE content was only 45 to 50% of control. However, by 10 weeks after injection NE was reduced only 30%. In the cerebellum, NE decreased 70% in the first two weeks after injection, and still was reduced 60% after 10 weeks. Thus little, if any, recovery occurred in this region. The NE content of the spinal cord progressively decreased 65% in the first two weeks after treatment with 6-OHDOPA (90 μg ivt.). The decreased CA content was associated with a decrease in the duration of clonic-tonic convulsions following decapitation. The convulsions exhibited a mild tonic tremor lasting only several seconds, and this effect was consistent through the 10-week study. It could also be seen that the effect on decapitation convulsions was dose-dependent when studied 48 hours after administration of 6-OHDOPA (45 to 180 μg ivt.) (299).

When brain DA levels were measured after treatment with 6-OHDOPA it was found that alterations were produced only after higher doses of 6-OHDOPA (100 or 150 mg/kg i.v.). It was also demonstrated that intraperitoneal or subcutaneous routes of administration of 6-OHDOPA (150 to 400 mg/kg) did not produce such an effect (82, 91, 210, 232) so that dopaminergic neuronal involvement is notably dependent on critical blood levels of 6-OHDOPA. Unlike the initial short-lived increase in brain DA found after treatment with 6-OHDA (35), no consistent increase in DA was found after treatment with 6-OHDOPA. In fact, the usual observation was a slight decrease in brain DA. The reason for the qualitative difference between 6-OHDA and 6-OHDOPA is still not known, although the possibility exists that 6-OHDOPA metabolites may produce a greater displacement of DA from pools in dopaminergic neurons, since the reduction in DA is only short-lived (210, 232). At no time did 6-OHDOPA decrease uptake capacity of $^3$H-metaraminol by the neostriatum (305, 308). It was noted that two months after 6-OHDOPA treatment (150 mg/kg i.v.) DA content in the brain increased by 25% thereby suggesting a possible sprouting response to noradrenergic destruction (210). Such a response of dopaminergic neurons has not been noted after low doses of 6-OHDA which selectively destroy noradrenergic neurons (see section V A).

It is interesting that 6-OHDOPA failed to decrease T-OH activity in various regions

of the rat brain one and seven days after treatment with 6-OHDOPA, although NE content was reduced 45 to 75% (348). Such a finding appears to indicate that the NE depletion is unrelated to neuronal destruction. However, the preterminal axons in the CNS of 6-OHDOPA-treated animals become markedly swollen and acquire an intense fluorescence, possibly due to blockade of axoplasmic transport to the damaged terminals. If a build-up of T-OH occurs in these axons, as occurs after ligation of adrenergic nerve trunks (389), then a destructive effect could be masked when T-OH is used as an indicator of damage.

Earlier studies have shown that 6-OHDOPA will bring about the actual destruction of sympathetic terminals in the heart of rats. Perfusion *in vitro* of hearts of 6-OHDOPA-treated rats with 1 μg/ml NE failed to unmask noradrenergic terminals, as observed by histofluorescence staining, thus indicating absence of neurons. More recently destruction of noradrenergic terminals in the CNS has been observed with electron microscopy and Fink-Heimer-stained preparations. Fink-Heimer staining has also revealed degenerating axonal fibers, while cell body damage in the locus coeruleus has been demonstrated with both light microscopic and electron microscopic techniques (230). Therefore, 6-OHDOPA is capable of destroying the entire noradrenergic neuron in mature animals.

When the brains of mice treated with 6-OHDOPA (100 or 150 mg/kg i.v.) were observed by histofluorescence microscopy it was noted that within 24 hours the number of terminal varicosities decreased in regions known to receive noradrenergic projections. At the same time, nonterminal smooth axons in the reticular formation of the pons medulla and mesencephalic regions became intensely fluorescent (210). Pretreatment with monoamine oxidase inhibitors resulted in a still more striking increase in the fluorescence intensity of the axons (210, 305, 308), similar to that observed after electrolytic lesions (6, 7), axotomy (97, 286) or pharmacological manipulation (141), including treatment with 6-OHDA (368, 371, 373). After treatment with 6-OHDOPA, the fluorescence staining of dopaminergic regions appeared to be unchanged, supporting the biochemical data that indicated selective destruction of noradrenergic neurons (210, 308). Such selectivity of action accompanied by nonterminal fluorescence accumulation provided the opportunity to map the noradrenergic fiber system of the mouse brain (210). The fluorescence build-up in the axons was apparent for less than two weeks, at which time the fluorescence intensity and number of noradrenergic terminals appeared identical with that found in most regions of control brains. Therefore, in most regions regeneration was able to occur. In the cerebral cortex, hippocampus, and cerebellum, the number of terminal varicosities was still far below that seen in control animals (210). Fluorescence build-up in brainstem noradrenergic axonal processes were evident up to 42 days after injection (308).

After intraventricular injection of 6-OHDOPA to rats, essentially the same histochemical pattern in the brain was observed as in mice after intravenous injection of 6-OHDOPA (299). Swollen and distorted intensely fluorescent axonal bundles from the brainstem to more rostral regions of the brain could be seen (209, 299, 346), again allowing detailed mapping of the brain (209). The swollen and fluorescent catecholamine-containing axons were still present 14 days after injection of 6-OHDOPA (90 μg ivt.) but had largely disappeared by 70 days. Regeneration of terminal processes could be observed within several days. Sprouting of varicose nerve fibers were seen budding off the main trunks of noradrenergic neurons within four days, becoming more prominent by 7 to 14 days after injection. By 70 days, regions of the hypothalamus, preoptic area, septum, medial forebrain bundle, and medulla, which had displayed marked reductions in the number of varicose terminals at one week, now appeared identi-

cal with that observed in control brains (299). Thus, regeneration, through axonal and/or terminal sprouting of noradrenergic neurons, is able to occur in the CNS after treatment of 6-OHDOPA.

The majority of evidence presented indicates that the primary effects of 6-OHDOPA are localized to noradrenergic neurons. Histochemical visualization of neurons in brains of mice and rats suggests selective action of 6-OHDOPA (209, 210, 299). Brain levels of DA are only transiently affected, at most, after injection of 6-OHDOPA by several routes (82, 91, 209, 232, 299, 346). The 5-HT content of the brain has been reported to be unchanged after injection of 6-OHDOPA (39, 82), although a slight reduction in 5-HT content of the neocortex was noted after nialamide-6-OHDOPA (308). In the latter study, uptake of $^{14}$C-5-HT was 35% above the suitable control, so that depletion of 5-HT was probably not due to a destructive action of 6-OHDOPA on serotonergic nerves (308). In rats treated with 6-OHDOPA in the neonatal period no measurable decrease of 5-HT in several brain regions, including the neocortex, was observed up to three months after treatment (393).

Choline acetyltransferase activity in mouse telencephalon, brainstem, and cerebellum was unaltered 2 and 14 days after injection of 6-OHDOPA (150 mg/kg i.v.), indicating that central cholinergic neurons are not directly altered by 6-OHDOPA, nor secondarily affected. The data suggest that there is no gross compensatory response of cholinergic neurons to noradrenergic neuronal destruction. However, acetylcholinesterase activity was reduced 20 to 30% in mouse telencephalon 2 and 14 days after injection of 6-OHDOPA (150 mg/kg i.v.) (232). It is possible that the enzyme is associated, in part, with noradrenergic neurons in this region and the reduction in enzyme activity is related to the disappearance of the adrenergic terminals in cortex and/or striatum. MAO activity was unaltered in all brain regions studied at 2 and 14 days, but COMT activity was slightly reduced in the brainstem 14 days after treatment with 6-OHDOPA (150 mg/kg i.v.) (232). The same selectivity for noradrenergic neurons is seen even after intraventricular injection of 6-OHDOPA. However, it is realized that the selectivity of 6-OHDOPA may be related to the dose employed. Low doses of 6-OHDA also are selective for noradrenergic neurons in the CNS (324, 378). Nevertheless, in doses producing marked CNS alterations of NE levels (82, 210, 232), 6-OHDOPA does not permanently affect DA content, so that the selectivity may be real, and related in part to the rate of formation and delivery of 6-OHDA formed from 6-OHDOPA.

In the peripheral nervous system, 6-OHDOPA, like 6-OHDA, produces differential effects on sympathetic neurons in various end organs. Noradrenergic terminals in the heart appear to be the most sensitive to destructive effects of 6-OHDOPA. It has been shown, furthermore, that 6-OHDOPA *via* intravenous injection produces a greater degree of depletion of NE (90%) in mouse heart after 24 hours, than after intraperitoneal (80%) or subcutaneous (65%) administration. The effects of 6-OHDOPA in the heart are dose-dependent (20 to 150 mg/kg i.v.), at 3 and 24 hours after injection. Part of the depleting action appears to be due to displacement of NE stores by 6-OHDOPA or metabolites, since a 55% reduction in NE content is noted after three hours in the cardiac ventricles (20 mg/kg) while at 24 hours NE is at control levels. However, with higher doses of 6-OHDOPA (100, 150, 200 mg/kg) the major component of the depleting action appears to be that of destruction of sympathetic terminals (231). Uptake of $^{3}$H-NE and $^{3}$H-MA by atria 16 hours after 6-OHDOPA (100 mg/kg 3 times i.p., nialamide, two hours) is reduced 50 to 75%, similar to the degree of NE depletion in the heart (308). This finding would suggest actual loss of the uptake capacity or degeneration of the nerve terminal. It has also been shown by histofluorescence microscopy

that noradrenergic terminals are virtually absent in the hearts of rats treated with a large dose of 6-OHDOPA (200 mg/kg, divided dose), even after perfusion *in vitro* with a concentration of NE (1 μg/ml) bordering on the uptake-2 threshold. At this level any neurons with even minimal uptake function would have accumulated NE and become visible. Therefore, lack of terminal processes indicates that 6-OHDOPA produces actual destruction. Recovery of NE to pretreatment levels occurs in two to three weeks after a 100 mg/kg intravenous dose of 6-OHDOPA, and somewhat less than six weeks after a 150 mg/kg intravenous dose (231). With a higher dose of 6-OHDOPA (100 mg/kg 3 times i.p., nialamide, two hours), recovery, determined by NE content and $^3$H-metaraminol uptake capacity in atria, was found to be about 80% complete after four weeks (305, 308), with recovery of both indices occurring at the same rate during this interval (305).

In mouse salivary gland a similar dose-dependent relationship to the NE depleting action of 6-OHDOPA (20 to 150 mg/kg i.v.) is noticeable at 3 and 24 hours. However, the maximal depletion produced is much less than in the heart, being only 60% below control 24 hours after a 150 mg/kg intravenous dose of 6-OHDOPA. Recovery of NE to pretreatment values occurred in about three weeks after this dose of 6-OHDOPA (231).

Mouse irides show only a moderate decrease in the number of noradrenergic terminals innervating the sphincter and dilator muscles three hours after 6-OHDOPA (100 mg/kg i.v.). However, larger doses of 6-OHDOPA (200 mg/kg, divided dose) markedly reduce the fluorescence intensity of noradrenergic varicose terminals, while the preterminal processes in the iris becomes swollen and highly fluorescent (231). With still a greater dose of 6-OHDOPA (100 mg/kg 3 times i.p.; nialamide, two hours) the number of terminal fibers in the irides of mice is markedly reduced after 16 hours. After one month the number of terminals is still diminished about 25%, and many of those present are less varicose. Preterminal fibers can still be seen as swollen and strongly fluorescent (305). During this period, $^3$H-MA uptake by irides of 6-OHDOPA-treated mice paralleled the reduction in NE content (308).

In the spleen, 6-OHDOPA (20 to 150 mg/kg i.v.) produced a slight decrease in NE at 3 hours, but this reduction appeared to be due primarily to displacement of NE, since NE levels were at control values by 24 hours. No depletion of NE in mouse vas deferens was produced at any time period studied, even after a high dose of 6-OHDOPA (150 mg/kg i.v.) (231).

The (−)-stereoisomer of 6-OHDOPA (100 mg/kg 2 times, i.p.) has been found effective in reducing NE content in major blood vessels of rats, and an 80% decrease in NE was found in the mesenteric vein and vena cava. In the mesenteric artery NE was decreased by only 20% (40). Unlike 6-OHDA, both the (−)- and (+)-isomers of 6-OHDOPA were reported effective in decreasing rat adrenal CA content, 24 hours after treatment. The (−)-isomer was found to be much more potent, decreasing CA levels by 45%, while (+)-6-OHDOPA reduced CA by only 25%. Although the nature of this specific alteration is unknown, the differences in sensitivity of other end organs to 6-OHDOPA would be expected to be similar to that of 6-OHDA, and this is discussed in section VIII.

It has recently been shown that 6-OHDOPA, injected into neonatal mice and rats, is capable of producing a long lasting effect on noradrenergic neurons in the brain (230, 308, 393). In mice, sacrificed six weeks after neonatal treatment with 6-OHDOPA (100 μg/g 3 times i.p.; nialamide, two hours), uptake of $^3$H-NE by the neocortex was reduced 60% (308). Brains of rats treated as neonates with 6-OHDOPA (50 μg/g 4 times s.c.) showed analogous reductions in NE content. NE stores in the telediencephalon were reduced 20% and 65%, respectively, at

two weeks and one to seven months of age. The NE content of the hypothalamus was reduced only about 30% through the period of study, while the brainstem exhibited approximately a 60% rise in NE content. The cerebellum was little affected throughout the study (393), while the spinal cord content of NE showed a 50% reduction at one month (230).

Both light and electron microscopic studies of brains of neonatal rats which received an identical dose of 6-OHDOPA (50 μg/g 4 times i.p.), from birth, showed marked degeneration of noradrenergic neurons. Terminals were observed to be in various stages of degeneration in numerous regions of the brain, including the neocortex, hippocampus, hypothalamus, and brainstem. Perikarya in the locus coeruleus, were also undergoing degenerative changes, indicating the susceptibility of the entire noradrenergic neuron to 6-OHDOPA. Therefore, the alterations in central noradrenergic function appear to be related to degenerative changes in the neuron (230).

The DA content of brains of 6-OHDOPA-treated neonatal rats appears to be unaltered between one to three months, postnatal age (230, 393). Even as early as one week after 6-OHDOPA or as late as one year, DA content of the striatum is unaltered (R. M. Kostrzewa, unpublished observations). However, one report has shown a 20% decrease in $^3$H-NE uptake by the striatum of mice treated neonatally with 6-OHDOPA (308). This may be due to the effect of the drug on the noradrenergic neurons passing through the caudate-putamen, *via* the internal capsule fibers, to terminate in the cortex (209). Adult levels of 5-HT were likewise unchanged in rats after 6-OHDOPA-treatment early in postnatal development (393).

One interesting study has shown 6-OHDOPA to be capable of crossing the placental barrier, so that injection of 6-OHDOPA (100 mg/kg 2 times) during gestation (days 18 and 20) effectively retarded noradrenergic development of the rat fetus. Alterations in the CNS were qualitatively similar to those reported after 6-OHDOPA treatment of neonates, although not as marked. The NE content of the telediencephalon was reduced nearly 50% while that in the hypothalamus was lowered by only 20%. The brainstem showed a 125% elevation in NE while NE in the "rest of the brain" was reduced 50% after 40 to 100 days of age. At this time NE content of the heart and salivary gland was unaltered, indicating no permanent alteration in sympathetic function (212, 393).

Behavioral alterations of mature rats treated with 6-OHDOPA have been studied, and these changes are reported below. After intraventricular injection of 6-OHDOPA (90 μg), mature rats become anorexic and adipsic. Water consumption markedly decreases for the initial three days after treatment, while food consumption is reduced for another two days. Body weight decreases 15% during this period and remains below that of saline-treated rats for a period of six weeks. The effects of 6-OHDOPA (45 to 180 μg, ivt.) on body weight are dose-dependent, with a 180-μg dose causing a 25% decrease in body weight after 48 hours (299).

Locomotor activity in the open-field is reduced for the first five days after 6-OHDOPA (90 μg ivt.), but returns to control levels afterwards and remains unaltered during the following week. However, during this entire period latency to initiation of open-field locomotion is markedly elevated (299).

Rats become more irritable after 6-OHDOPA (90 μg ivt.) (299), and the level of emotionality, quantified by a modification of the rating scale of Brady and Nauta (52), was markedly elevated within two days of treatment. Emotionality scores remained at high levels between 6 and 10 weeks after 6-OH-DOPA (299). It was also found that facilitated aggression of 6-OHDOPA-treated (90 μg ivt.) rats was progressively increased between four and six days after injection. The number of fighting bouts rose from an

average of 5 to 40% after treatment. Such effects appear to involve the destruction of noradrenergic neurons, as indicated by the previously reported biochemical data, but other additional factors may be involved because of the latency of shock-induced fighting, possibly related to a supersensitivity of NE receptors in the brain (346).

These studies with 6-OHDOPA indicate that alterations in behavior are similar to those produced after treatment with 6-OHDA. One distinct advantage in the 6-OHDOPA studies, however, is the relatively selective effect that is produced on noradrenergic neurons. These findings, then, suggest that resultant behavioral changes observed after 6-OHDOPA, are due to alterations in central noradrenergic neurons. Subsequent studies should lead to possible implication of noradrenergic neuronal mediation of other behavioral processes.

## XV. Other Agents Causing Possible Degeneration of Monoaminergic Neurons

The peripheral NE depleting action of 6-aminodopamine (6-ADA) was noted a decade ago, along with $\alpha$-methyl-6-OHDA and $\alpha$-methyl-6-ADA. The potency of all three compounds was about the same, approximately 50% of that for 6-OHDA (339). Recent studies have shown that 6-ADA also reduces the NE and DA levels in mouse brain (43). Electron microscopic studies have indicated that 6-ADA, like 6-OHDA, produces an actual destruction of noradrenergic neurons, and the effects appear to be relatively selective (323). Both 6-ADA and 6-OHDA form 5,6-dihydroxyindole *in vitro*, a product presumed to be responsible, at least in part, for bringing about the degenerative events. It would be of interest to determine more about the mechanism of action of the methylated derivatives, as well as the spectrum of their action in the CNS and peripheral end organs or sympathetic ganglia.

Baumgarten *et al.* (30, 33) have shown that another agent, 5,6-dihydroxytryptamine (5,6-DHT) is capable of bringing about the destruction of serotonergic neurons in the CNS. However, there is a low degree of selectivity, since high doses of the agent also deplete central NE stores (31) and are capable of producing a chemical sympathectomy, *i.e.*, destruction of peripheral postganglionic sympathetic (noradrenergic) neurons (32). More recently 5,7-dihydroxytryptamine was found to selectively destroy serotonergic neurons (34).

Ideal neurotoxic agents are now being sought with relatively selective destructive actions on certain types of neurons. Perhaps an agent will be found with pharmacological properties similar to 6-OHDA which will prove to be superior because of differences in stability, potency or selectivity of action, possibly affecting dopaminergic neurons more specifically.

## XVI. Summary

From the previous discussions, it is apparent that 6-OHDA produces a relatively selective effect on sympathetic nerve terminals. The selectivity of this action appears to be related to its accumulation within noradrenergic neurons by uptake-1 transport mechanisms. Once inside the neuron, 6-OHDA is bound in a granular storage pool and can be released by nerve stimulation, thus acting as a false neurotransmitter. In sufficiently high amounts, apparently more related to the concentration in the cytoplasmic pool, 6-OHDA generates highly reactive products, suggested to be peroxides, superoxides, hydroxyindoles, and quinones. These products react nonspecifically with neuronal structures and eventually destroy the neuron. MAO appears to be important in the metabolism of the 6-OHDA molecule and this enzyme, as well as granule storage, may serve as protective mechanisms. The actual subcellular component most altered by 6-OHDA, and most involved with maintaining neuronal function, is not known. However, the endoplasmic reticulum, outer limiting membrane, nucleus, mitochondrion and other structures have been suggested as

sites for the primary lesion, and 6-OHDA has been shown *in vitro* to uncouple oxidative phosphorylation in mitochondria. During the process of degeneration the nerves first lose their ability to conduct action potentials, NE stores become depleted and sympathomimetic responses may be observed. Uptake mechanisms become incapacitated and the nerve membrane ultimately is phagocytized, as evidenced by the intense neuroglia reaction. Accompanying the loss in the adrenergic nerve active amine uptake mechanism is the appearance of presynaptic supersensitivity at a host of sites. Development of postsynaptic supersensitivity has been suggested to occur with time in certain structures. In the peripheral nervous system the terminals regenerate, and it has been observed that functional activity is restored at early times, when the amine levels are still reduced and while the terminal network is far from being fully regenerated.

In the peripheral nervous system, 6-OHDA alters noradrenergic terminals to various degrees in different end organs. Time-course studies, following various routes of administration, have determined the threshold for terminal destruction in various organs of several species. In general, the increasing order for threshold of the destructive action of 6-OHDA in various end organs is cardiac ventricles > salivary glands > whole heart > iris > nictitating membrane > spleen > atria > blood vessels > vas deferens > sympathetic ganglia > adrenal glands. In regard to the adrenals it bears mentioning that they apparently are unaffected by direct 6-OHDA action, but respond to diminished sympathetic function by compensatorily increasing CA turnover. Terminals in all organs studied regenerate at a steady rate.

In the CNS of mature animals 6-OHDA produces marked alterations of both noradrenergic and dopaminergic neurons after injection into the parenchyma of the brain or into one of the brain cavities. Early studies showed that 6-OHDA in moderate doses could deplete the brain of NE in the absence of ultrastructural damage. Also, DA stores are initially increased after 6-OHDA and are depleted only as a consequence of damage to dopaminergic neurons. The regional effects of 6-OHDA on noradrenergic neurons in the brain vary according to the parameter under study. Different regional variations are found when either NE content, NE uptake or tyrosine hydroxylase activity is measured. The degeneration of the central noradrenergic neurons occurs in different phases, classified as primary and secondary degeneration. The primary phase is related to the direct destructive action of 6-OHDA on the neuron and occurs in about the first 48 hours after treatment. The secondary phase occurs over a period of weeks and appears to be the result of retrograde degenerative events subsequent to terminal or axonal damage.

Higher doses of 6-OHDA produce less of a specific effect, as is the case with any other pharmacological agent. Damage extends to noncatecholamine-containing neurons, and may even include non-neuronal cells. Possibly related to this type of unspecific damage is the observed depletion of 5-hydroxytryptamine in certain brain regions of different species. However, in reasonable amounts, 6-OHDA will destroy catecholaminergic neurons with a high degree of selectivity.

The histochemical method shows that 6-OHDA brings about alterations in the appearance of CA-containing nerves, similar to those observed after axonal section or ligation. Nerve terminal varicosities decrease in number, the nerve network stains less intensely, and finally, the number of terminal processes decreases. After a low dose of 6-OHDA the fluorescence intensity of nerves can be restored by subsequent treatment with a CA. However, after a high dose of 6-OHDA nerves are not unmasked by CA treatment, indicating that the initial

depletion is followed by actual destruction. Correlations have been shown between histochemical alterations and reductions in endogenous CA levels, CA-uptake capacity, and endogenous DBH and T-OH activity. Electron microscopic studies have provided the ultimate confirmation of nerve terminal destruction. While the terminal network is undergoing these functional changes, the axonal processes become highly fluorescent, swollen and highly irregular in appearance. Evidence to date indicates that axoplasmic flow is impeded, so that NE, being transported down the axon, accumulates in a retrograde manner. The above alterations are seen in both noradrenergic and dopaminergic neurons.

In newborn mice and rats, 6-OHDA causes extensive damage of sympathetic postganglionic neurons, and the destructive lesion includes the entire neuron. Ganglia at prevertebral and paravertebral sites are destroyed and subsequent development is impaired throughout adulthood. As a consequence, numerous end organs normally innervated by sympathetic fibers, show a reduction of NE content due to the loss of the nerve network. In addition, central noradrenergic neurons are damaged, since 6-OHDA has the capacty of passing through the unfully-developed blood-brain barrier of the neonates. The pattern of this chemical lesion is different from that observed in mature animals.

Numerous behavioral parameters are altered by 6-OHDA. Initial behavioral changes are likely to result from NE or DA release from central CA-containing neurons after treatment with 6-OHDA, while more permanent alterations appear to be a better reflection of central neuronal destruction. Immediately after injection 6-OHDA decreases food and water consumption, lowers body temperature, and decreases locomotion. More permanent effects are manifested by altered sleep patterns, increased irritability and aggression, and altered operant activity. Numerous studies have been performed to characterize the observed alterations to a specific region in the brain and also to the specific neurohumor involved.

Studies with 6-OHDOPA indicate that it is metabolically decarboxylated to 6-OHDA, which then destroys CA-containing neurons. It appears to be useful for selective destruction of noradrenergic neurons. One distinct advantage of 6-OHDOPA is its ability to cross the blood-brain barrier, so that both central and peripheral noradrenergic neurons may be destroyed after a single dose. Although 6-OHDOPA is far less potent than 6-OHDA in mature animals, the central neurotoxic actions in neonates are quite similar. However, unlike 6-OHDA, 6-OHDOPA does not appear to produce permanent alterations of all adrenergic neurons. Thus, as the animals develop one may be able to study the effects of centrally impaired noradrenergic development in the absence of peripheral effects. The limited studies to date indicate that 6-OHDOPA produces alterations in behavior that are similar to those produced by 6-OHDA.

Studies with 6-OHDA have illustrated the utility of this compound in investigating the function of both noradrenergic and dopaminergic neurons. Much has been learned about basic functional processes, such as uptake and storage mechanisms, axoplasmic transport, and the influence of central regulation of ongoing noradrenergic nerve development and function. It is now more meaningful to attempt to characterize the role of noradrenergic and dopaminergic neurons in different types of behavior. The search for other neurotoxic agents continues and perhaps others will be found with more desirable actions. However, the studies with 6-OHDA have provided new insights into innumerable areas of neuroscience.

*Acknowledgments.* The excellent secretarial assistance of Ms. Rene Atkinson and Ms. Lana Robinson during the preparation of this review is greatly acknowledged.

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1. *Date of Filing:* October 1, 1974.
2. *Title of Publication:* Pharmacological Reviews.
3. *Frequency of Issue:* Quarterly.
4. *Location of known Office of Publication:* 428 E. Preston St., Baltimore, Md. 21202.
5. *Location of the Headquarters or General Business Offices of Publisher:* 428 E. Preston St., Baltimore, Md. 21202.
6. *Publisher:* The Williams & Wilkins Company, 428 E. Preston St., Baltimore, Md. 21202.
   *Editor:* Dr. Marion deV. Cotten, Route 3, Box 229, Sylvania, Ga. 30467.
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