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TOLERANCE TO THE ACTIVITY-ENHANCING EFFECT OF D-AMPHETAMINE

by

SVETLANA MILLOY

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

TOLERANCE TO THE ACTIVITY-ENHANCING
EFFECT OF D-AMPHETAMINE
by
Svetlana Milloy

Adviser: Associate Professor Stanley D. Glick

The problem of tolerance development to the activity-enhancing effect of d-amphetamine in the mouse and rat was examined in this thesis. The criterion employed to ascertain the existence of tolerance was the shifting of the dose-response curve to the right.

It was demonstrated that tolerance occurred when mice were tested with d-amphetamine in activity boxes following seven days of drug injections. The number of drug injections and the interval between drug injections were shown to be important factors; tolerance failed to develop after three daily drug injections and when seven drug injections were administered twice daily in closely spaced intervals.

Tolerance to d-amphetamine also occurred in mice following seven days of testing with the drug. However, tolerance failed to develop when mice were tested with d-amphetamine daily for three days, and when mice were tested twice daily with the drug for three and a half days.

Behavioral and environmental variables were also shown

to affect tolerance development in the mouse. Seven days of pre-drug experience in the testing apparatus shifted the dose-response curve to the right when the subjects were tested with d-amphetamine. However, three days of pre-drug activity testing were insufficient to induce tolerance development. The effect of pre-drug experience on the sensitivity of mice to d-amphetamine was related to pharmacological tolerance in terms of a common effect on brain catecholamines. It was also found that the injection per se disrupted tolerance development when mice received either drug or saline injections for the first time before being tested with the drug.

In contrast to the results obtained with mice, rats receiving seven daily injections of d-amphetamine, either with or without concurrent activity testing, were found to be more sensitive rather than tolerant to d-amphetamine. This heightened sensitivity was related to a persistent metabolite of d-amphetamine. Rats which were tested without drug for seven days were less sensitive when tested with d-amphetamine than were rats which had received chronic drug injections.

Implications of the results were discussed in terms of the catecholamine hypothesis of affective disorders and amphetamine-induced psychosis in man.

I would like to offer my sincere thanks to my adviser, Stan Glick, for his guidance, inspiration and help; to Joe Goldfarb, for his invaluable insights and time spent with me; to my parents and to Frank, for their encouragement and unflagging faith in me.

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LIST OF ABBREVIATIONS

DDC: diethyldithiocarbamate

DFP: diisopropyl fluorophosphate

FLA-63: bis-(4-methyl-1-homopiperazinyl-thiocarbonyl) disulfide

SKF-525A: 2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride

THC: tetrahydrocannabinol

INTRODUCTION

Amphetamine, or beta-phenylisopropylamine, is one of the most potent central stimulants known, and has sympathomimetic peripheral actions as well. The peripheral effects elicited by amphetamine in animals and man include raising both the systolic and diastolic blood pressure, increasing the pulse pressure, bronchodilatation and contraction of the urinary bladder (Innes and Nickerson, 1971); additional effects include increased excretion of urinary catecholamines in the rat (Lewander, 1968). The central effects of amphetamine in animals include increased locomotor activity (Searle and Brown, 1938; Zieve, 1937), stereotypy (Hasselager *et al.*, 1972; Randrup and Munkvad, 1967; Randrup *et al.*, 1963), anorexia (Carlton and Wolgin, 1971; Tormey and Lasagna, 1960), decreased water intake (Kosman and Unna, 1968; Tormey and Lasagna, 1960), hypothermia (Chiel *et al.*, 1974; Yehuda and Wurtman, 1972) and hyperthermia (Lewander, 1971; Mantegazza *et al.*, 1970).

Unless specifically designated otherwise, the use of the term "amphetamine" in this thesis refers to any of its optical isomers, since their actions are qualitatively but not quantitatively similar.

Mode of action

Although it has long been accepted that amphetamine exerts its peripheral sympathomimetic effects via catecholamines (Burn and Rand, 1958; Trendelenburg et al., 1962), evidence has only recently accumulated to indicate that catecholamines also mediate the central excitatory effects of amphetamine (Carr and Moore, 1970; Glowinski and Axelrod, 1965; Randrup and Munkvad, 1966; Stein, 1964a).

There are several possible modes of action by which amphetamine could exert its central effects. A direct action of amphetamine on the catecholamine receptor was proposed (Smith, 1963, 1965; Van Rossum et al., 1962) to explain data showing that reserpine pretreatment had no effect on, or enhanced, the increased locomotor activity induced by d-amphetamine in mice. It was also hypothesized that amphetamine acts directly on 5-hydroxytryptamine receptors (Gelder and Vane, 1962). Much evidence has accumulated to indicate that amphetamine may have an indirect mode of action. Amphetamine has been shown to act indirectly by releasing central catecholamines (Andén and Svensson, 1973; Carlsson et al., 1966; Carr and Moore, 1970; Glowinski and Axelrod, 1965), by inhibiting uptake of catecholamines into the presynaptic neuron (Glowinski and Axelrod, 1965; Rutledge, 1970; Taylor and Snyder, 1970, 1971) and by inhibition of monoamine oxidase (Glowinski and Baldessarini, 1966).

Studies with catecholamine storage in the central nervous system further support the theory that amphetamine

exerts its central effects indirectly. It has been reported that norepinephrine is stored in at least two different pools in the peripheral sympathetic nerves (Kopin et al., 1968) as well as in the central nervous system (Glowinski and Axelrod, 1965; Schildkraut et al., 1971; Thierry et al., 1970). The existence of a large "storage" pool with a slow rate of turnover and a relatively smaller "functional" pool with a faster rate of turnover has been postulated (Glowinski and Axelrod, 1965).

The experiments of Schildkraut et al. (1971) differentiated the norepinephrine pools in the brain by examining the rates of disappearance and pathways of metabolism of intracisternally administered H^3-norepinephrine as a function of time after administration. It was found that in the pools with rapid turnover rates, the conversion of radioactive norepinephrine to normetanephrine occurred to a greater extent than did the conversion of radioactive norepinephrine to 3,4-dihydroxymandelic acid. These investigators concluded that this might reflect the uptake of radioactive and intracisternal norepinephrine into an intraneuronal pool of newly synthesized norepinephrine. Other experiments have demonstrated that the "functional" pool is the site of continuous catecholamine synthesis and is reserpine-resistant (Glowinski and Axelrod, 1965; Hanson, 1967), while the larger pool is reserpine-releasable (Glowinski and Axelrod, 1965). Since amphetamine was found to release physiologically active norepinephrine while reserpine released only inactive

metabolites of norepinephrine, it was concluded that the central action of amphetamine depended on the availability of newly synthesized catecholamines (Glowinski, 1970; Glowinski and Axelrod, 1965).

Neff et al. (1968, 1970), however, have argued against the existence of two pools. These investigators showed that the use of tracer doses of H^3-norepinephrine results in a single exponential decline of the specific activity of heart norepinephrine, implying the existence of only one pool. These authors have suggested that the biphasic decline obtained by other investigators was an artifact resulting from the use of non-tracer doses of labelled norepinephrine and represented the disappearance of norepinephrine from sites where it is not normally present.

Studies with L-alpha-methyl-p-tyrosine, an inhibitor of tyrosine hydroxylase, strongly support the idea that amphetamine acts indirectly via newly synthesized catecholamines (Dingell et al., 1967; Hanson, 1967; Svensson, 1970; Thornburg and Moore, 1973b; Weissman et al., 1966). At doses which do not affect spontaneous locomotor activity, L-alpha-methyl-p-tyrosine blocks the increased locomotor activity, stereotypy, anorexia and stimulation of non-discriminated avoidance behavior induced by d-amphetamine (Sulser and Sanders-Bush, 1971). L-alpha-methyl-p-tyrosine was also found to decrease the brain content of endogenous dopamine and norepinephrine, and inhibited the formation of ^{14}C-dopamine and ^{14}C-norepinephrine from ^{14}C-tyrosine (Thornburg

and Moore, 1973b). Several investigators (Ahlenius et al., 1973; Randrup and Munkvad, 1966; Sulser and Sanders-Bush, 1971) have found that the administration of L-dopa reversed the anti-amphetamine effect of L-alpha-methyl-p-tyrosine. Others (Enna et al., 1973; Weissman et al., 1966) however, have found no antagonism and have concluded that the central action of amphetamine was not mediated solely by the release of newly synthesized catecholamines. Enna et al. (1973) suggested that L-alpha-methyl-p-tyrosine, in addition to inhibiting tyrosine hydroxylase, also decreased the ability of d-amphetamine to provoke a sustained release of stored brain amines, possibly by blocking amine transfer from a general storage pool to an amphetamine-releasable one. Since L-alpha-methyl-p-tyrosine pretreatment does not alter brain levels of amphetamine or affect the metabolism of amphetamine in rats (Sulser et al., 1968), it appears that the anti-amphetamine action of L-alpha-methyl-p-tyrosine in the central nervous system is not due to dispositional factors.

The results of many studies indicate that a dopaminergic mechanism rather than a noradrenergic one is involved in the mediation of stereotyped behavior (compulsive gnawing and rearing) elicited in animals by large doses of amphetamine (Hasselager et al., 1972; Maj and Przegalinski, 1967; Naylor and Costall, 1971; Randrup and Munkvad, 1966; Scheel-Kruger and Randrup, 1967; Taylor and Snyder, 1970, 1971). However, there is no consensus as to which catecholamine is primarily responsible for mediating the increased locomotor activity

elicited by relatively lower doses of amphetamine.

Costa et al. (1972) demonstrated that minimally effective doses of d-amphetamine which increased locomotor activity increased the turnover of striatal dopamine in rats but failed to affect the turnover of brain norepinephrine. These investigators concluded that dopamine mediates the locomotor activity elicited by d-amphetamine. A similar conclusion was reached by Groppetti et al. (1973), who concluded from their neurochemical and behavioral studies with rats that 1) an indirect action on the noradrenergic neuronal system in the hypothalamus and telencephalon was not required to elicit the enhanced activity seen following the administration of d-amphetamine, and 2), that d-amphetamine elicits hyperactivity by releasing dopamine from the striatal nerve terminals.

The use of pimozide, a dopamine receptor blocking agent, has produced contradictory results. Pimozide does not affect the metabolism of amphetamine or its penetration into the brain (Soudijn and Van Wijngaarden, 1972). It was reported by Schlechter and Butcher (1972) that pimozide had no effect on activity by itself, but antagonized the amphetamine-induced increase in locomotor activity in mice. On the other hand, Maj et al. (1972) found that pimozide decreased the locomotor activity of saline-treated rats and mice. These authors, however, used a dose of pimozide two to four times larger than that used by Schlechter and Butcher (1972).

In another study (Thornburg and Moore, 1973b), FLA-63, a dopamine beta-hydroxylase inhibitor, decreased the brain

level of endogenous and radioactive norepinephrine, but did not alter the locomotor stimulant action elicited by d-amphetamine in mice. These investigators concluded that the central stimulant action of d-amphetamine was mediated by a dopaminergic mechanism, since the blockade of norepinephrine synthesis did not prevent the d-amphetamine-induced hyperactivity.

However, other investigators have found that amphetamine-induced locomotor activity but not stereotyped behavior was blocked by the administration of dopamine beta-hydroxylase inhibitors such as disulfiram (Maj and Przegalinski, 1967) and DDC (Scheel-Kruger and Randrup, 1967); it was concluded that norepinephrine rather than dopamine was primarily responsible for the increase in locomotor activity elicited by amphetamine. Similar conclusions were reached by Coyle and Snyder (1969) and by Taylor and Snyder (1970, 1971), based on the differential potencies of d- and l-amphetamine on the inhibition of uptake by brain of dopamine and norepinephrine and on stereotypy and locomotor activity. However, their studies on reuptake have not been replicated by others (Ferris et al., 1972; Harris and Baldessarini, 1973; Thornburg and Moore, 1973a).

Scheel-Kruger (1972) has suggested that the inhibition of reuptake cannot be a major mechanism responsible for the amphetamine-induced locomotor activity, since the tricyclic antidepressant drugs also inhibit reuptake of catecholamines but do not increase locomotor activity. Additional evidence indicates that the central stimulant effect of amphetamine

is due to the release of newly synthesized catecholamines, since amphetamine can elicit hyperactivity at a dose which does not significantly affect the reuptake mechanism (Carlsson, 1970; Carlsson and Waldeck, 1968; Fuxe and Ungerstedt, 1970; Glowinski, 1970).

The conflicting results obtained with dopamine beta-hydroxylase inhibitors may be attributed to the use of different dopamine beta-hydroxylase inhibitors (FLA-63, disulfiram, DDC) with different routes of administration (i.p., in the diet), different isomers of amphetamine (d-amphetamine, dl-amphetamine), different species (mice and rats) and different methods of measuring locomotor activity.

Jonsson and Lewander (1973) have shown that disulfiram and its metabolite, DDC, not only inhibit dopamine beta-hydroxylase, but also inhibit the para-hydroxylation of d-amphetamine in the rat. This would increase the tissue levels of d-amphetamine in rats, and would possibly confound any result obtained with disulfiram or DDC.

Another complicating variable was demonstrated by Thornburg and Moore (1971), who showed that parenteral administration of the dopamine beta-hydroxylase inhibitor FLA-63 led to stressful effects (indicated by increased plasma levels of corticosterone). Stress has been shown to alter brain catecholamine levels (Moore, 1963; Thierry et al., 1968), and normal spontaneous locomotor activity was decreased when FLA-63 was administered intraperitoneally to mice (Svensson, 1970). In addition, Carlsson (1970) has suggested that DDC

has a central depressant action with a rapid onset which may not be related to the depletion of norepinephrine.

Although the arguments for a dopaminergic mediation of amphetamine-induced locomotor activity appear to be stronger than those favoring a noradrenergic mediation, the specific mechanism by which amphetamine elicits hyperactivity remains to be elucidated.

Tolerance

The term "tolerance" refers to an acquired change in an organism's reaction to a drug which renders the organism less sensitive to the drug's effects. Tolerance is said to occur when an increase in the amount of drug is necessary to produce a specific response or when the production of the same response requires an increase in the dose of the drug. Acute tolerance, or tachyphylaxis, usually indicates changes in sensitivity to a drug within the duration of one continuous drug exposure. Chronic tolerance, on the other hand, refers to the changes in sensitivity to a drug following its repeated administration. The term "tolerance" as used in this thesis will refer to chronic tolerance unless stated otherwise. Tolerance to different actions of a drug may develop at different rates; such is the case with amphetamine (Kalant *et al.*, 1971).

Cross-tolerance is said to occur when tolerance to one drug confers tolerance to a related drug; this has been demonstrated among psychotomimetic drugs

(Appel and Freedman, 1968; Winter, 1971) and among depressant drugs (Kalant et al., 1971). Although cross-tolerance commonly occurs in both directions, Sparber and Tilson (1972) have shown that cross-tolerance can develop in one direction only. The bar-pressing behavior of rats was disrupted by intravenously administered d-amphetamine. Following the occurrence of tolerance to d-amphetamine, a similarly administered equipotent dose of mescaline resulted in a behavioral disruption, indicating a lack of cross-tolerance. However, when tolerance to intravenously administered mescaline developed, an equipotent dose of d-amphetamine similarly administered did not result in behavioral disruption, indicating that cross-tolerance had occurred.

Any test for tolerance must take into account several important variables. Pharmacological factors which may affect the development of tolerance to a drug include the size of the dose, the number of doses, the interval between doses and the route of administration. Testing for tolerance with a particular behavior requires the use of doses which span the entire dose-response curve of the behavior; this is especially important when the dose-response curve is nonmonotonic. Many discrepancies in the literature pertaining to the development of tolerance to psychoactive drugs may be due to the fact that many investigators have used only a single test dose at an unknown point in the dose-response curve when attempting to demonstrate tolerance.

The route of administration of the drug is important in

that the gross levels of the drug in blood and brain differ with different routes of administration. Different sites of administration may also cause a differential distribution of drug within the system. This is shown in a study of tolerance to the disruptive effects of amphetamine on bar-pressing behavior in rats. Following the occurrence of tolerance to the intraventricular administration of amphetamine, the behavioral response to peripheral administration of the drug indicated a lack of cross-tolerance between the routes of administration. Conversely, after tolerance was induced by the peripheral administration of amphetamine, intraventricular administration of the drug produced a behavioral disruption indicating a lack of cross-tolerance in both directions (Sparber and Tilson, 1972).

Classically, tolerance has been defined in terms of dispositional and functional mechanisms. Dispositional tolerance includes those changes in drug absorption, metabolism, distribution and excretion which tend to alter the duration of contact between the drug and target tissue and the concentration of drug at the target tissue as well. The concept of functional or pharmacological tolerance implies that the nature of the target tissue itself becomes altered and is rendered less sensitive to the action of the drug. Dispositional tolerance can be theoretically differentiated from functional tolerance by measuring relative concentrations of the drug at the receptor before and after the development of tolerance. The development of dispositional

tolerance does not preclude the simultaneous development of functional tolerance (Kalant et al., 1971). It has been recently demonstrated that dispositional and functional tolerance to pentobarbital develop concurrently in the cat, although at different rates (Okamoto et al., 1975).

Although the metabolism of amphetamine has been studied extensively in many species, the question of whether or not dispositional tolerance to amphetamine occurs has yet to be answered conclusively. Amphetamine is metabolized by the drug-metabolizing enzymes of the liver to p-hydroxyamphetamine in several species including the rat (Axelrod, 1954), and to a minimal extent, in the mouse (Dring et al., 1970).

Two studies have shown that SKF-525A, and inhibitor of the hepatic drug-metabolizing enzymes, decreased the urinary excretion of amphetamine metabolites (Clay et al., 1971; Creaven et al., 1970) and raised the brain and heart levels of amphetamine in the rat (Clay et al., 1971). Pretreatment with phenobarbital caused a significant increase in metabolite excretion in one study with mice (Benakis and Thomasset, 1970), but did not affect metabolite excretion in another study with rats (Groppetti and Costa, 1969a). It has also been reported that ethanol depressed, while phenobarbitone and benzo(a)pyrene did not affect the hydroxylation of d-amphetamine in the rat (Creaven et al., 1970). There is evidence to indicate that the amphetamine aromatic hydroxylase found in the rat liver differs from the nonspecific aromatic hydroxylase of the rat liver (Groppetti and Costa, 1969a).

This suggests that the metabolism of amphetamine cannot be induced by other drugs since a specific enzyme is involved. Lewander (1968) reported data which indicate that tolerance to amphetamine was not due to an increased metabolism of the drug since the pattern of urinary metabolites of amphetamine and the disappearance rate of amphetamine in the brain is not different between chronically amphetamine-treated and drug naive rats.

Another mechanism proposed to explain tolerance to amphetamine is based on the finding that the depletion of norepinephrine stores by a single large dose or by smaller successive doses of d-amphetamine in rats lasts long beyond the time that appreciable amounts of drug remain in the body (Brodie et al., 1970). The rate of repletion of norepinephrine stores after l-amphetamine administration was found to correspond to the normal rate of norepinephrine synthesis while the depletion of norepinephrine stores persisted for more than forty hours following administration of d-amphetamine (Brodie et al., 1970). Since d- but not l-amphetamine is a substrate for dopamine beta-hydroxylase and ultimately converted to p-hydroxynorephedrine in the rat brain (Goldstein and Anagnoste, 1965), it would appear that p-hydroxynorephedrine is responsible for this persistent effect of d-amphetamine. This metabolite has been reported to accumulate in the sympathetic nerve endings of the heart and in the brain of the rat (Groppetti and Costa, 1969b), and is selectively released by d-amphetamine in preference to norepinephrine

(Brodie et al., 1970). Although experiments with disulfiram, an inhibitor of both aromatic and beta hydroxylation in the rat, have shown that p-hydroxynorephedrine is not responsible for the primary catecholamine-depleting action of d-amphetamine (Brodie et al., 1970; Freeman and Sulser, 1972), it has been suggested that the maintenance of the norepinephrine depletion in the rat brain following d-amphetamine administration is related to the uptake of p-hydroxynorephedrine by noradrenergic neurons and its subsequent release as a false transmitter (Freeman and Sulser, 1972).

Although there is evidence that p-hydroxynorephedrine is responsible for tolerance to the peripheral effects of d-amphetamine in the rat (Brodie et al., 1970; Lewander, 1971), tolerance to the central stimulant effect of d-amphetamine was not observed following the intraperitoneal administration of p-hydroxyamphetamine (Lewander, 1971, 1972) and p-hydroxynorephedrine (Brodie et al., 1970). However, conflicting results reported by Clay et al. (1971) indicate that p-hydroxynorephedrine is not the active metabolite responsible for the depletion of norepinephrine in the rat brain, and thus could not act as a false transmitter.

Behavioral tolerance

In several instances the development of tolerance does not appear to depend on either dispositional or pharmacological factors. Behavioral variables have been shown to affect the development of tolerance to psychoactive drugs. Some of

these variables include external factors in the experimental situation such as stimulus intensity (Irwin, 1963), relationship between order of drug administration and behavioral testing (Campbell and Seiden, 1973; Carlton and Wolgin, 1971) and whether or not the effects of the drug are disruptive or facilitative (Schuster et al., 1966). Since the development of tolerance can be influenced by nonpharmacological factors, the existence of a "behavioral" type of tolerance can be postulated.

Kalant et al. (1971) have suggested that behavioral tolerance occurs when the organism acquires new skills to replace those which have been impaired by the effects of the drug. Another theory of behavioral tolerance was proposed by Schuster et al. (1966), who found that rats became tolerant to a single dose of amphetamine when it impaired food-reinforced timing behavior and avoidance of shock but did not become tolerant when amphetamine facilitated these behaviors. They suggested that behavioral tolerance will develop only when the drug acts to impair the animal's ability to effectively cope with its environment, but not when the effect of the drug is such that it enhances or has no effect on responses which are in themselves rewarding.

Kalant et al. (1971) have also proposed, however, that behavioral and pharmacological tolerance are not separate phenomena, but are functions of the same mechanism. They suggest the term "behaviorally augmented" tolerance as a substitute for behavioral tolerance. Evidence in support of

this idea has been demonstrated with respect to ethanol tolerance (LeBlanc et al., 1973). Three groups of rats received ethanol daily on different schedules. Ethanol was administered to the first ("psychological") group before the behavioral test, while the second ("physiological") group received ethanol immediately after the test session. Rats in the third group served as controls and received only saline injections each day. The results showed that rats in the "psychological" group became tolerant sooner than did rats in the "physiological" group, while rats in the control group did not show any evidence of tolerance development. These investigators concluded that the difference between psychological (behavioral) and physiological (pharmacological) tolerance was one of rate only.

Tolerance to the disruptive effects of amphetamine has been shown to occur in rats performing operant behaviors such as bar-pressing on a differential reinforcement of low rate (DRL) schedule (Schuster et al., 1966; Schuster and Zimmerman, 1961), a fixed-ratio schedule (Appel and Freedman, 1968; Sparber and Tilson, 1972), a fixed-interval schedule (Schuster et al., 1966; Tilson and Sparber, 1973) and in a shock avoidance situation (Schuster et al., 1966). Rats have also been shown to become tolerant to amphetamine's anorexigenic effect (Carlton and Wolgin, 1971; Lewander, 1971; Panksepp and Booth, 1973; Tormey and Lasagna, 1960), hyperthermic effect (Lewander, 1971), hypothermic effect (Chiel et al., 1974) and toxic effects (increase in LD_{50})

(Lewander, 1968). The occurrence of tolerance to the activity-increasing effect elicited by amphetamine, however, is still a subject of controversy. There are reports indicating that neither rats (Lewander, 1971; Lu et al., 1972; Nahorski and Rogers, 1975; Schuster and Zimmerman, 1961; Tilson and Rech, 1973b; Tormey and Lasagna, 1960) nor mice (Kosman and Unna, 1968) become tolerant to this effect. Herman et al. (1971), however, demonstrated that rats could be made tolerant to the activity-enhancing effect of amphetamine following three months of drug administration and weekly activity testing. Tolerance to the hyperactivity induced by methamphetamine in rats has also been reported (Bell et al., 1974).

The present investigation

There were several factors which influenced my decision to study the nature of tolerance to d-amphetamine. I first became interested in the phenomenon of tolerance while conducting experiments with delta-9-THC, the active component of marijuana; however, the probable accumulation of this compound in the tissues of rats (Layman and Milton, 1971) confounded the results and rendered subsequent investigation with this drug difficult. To circumvent this problem I decided to examine the effect of d-amphetamine on locomotor activity in the mouse, since d-amphetamine is known to be eliminated in two to six hours in both the mouse and the rat (Benakis and Thomasset, 1970; Brodie et al., 1970). By choosing the mouse as the principal species of study, the problem of a

false transmitter interfering with the development of tolerance would be eliminated since p-hydroxylation of d-amphetamine occurs only minimally in the mouse (Dring et al., 1970). The activity-enhancing effect of d-amphetamine can be easily and consistently measured, and thus was chosen as the behavior with which to study tolerance development.

The failure of many investigators to find evidence of tolerance to the central stimulant action of amphetamine served to further whet my interest in this problem. As indicated above, tolerance has been shown to develop to many of the central effects evoked by amphetamine (i.e. bar-pressing behavior, anorexia, hyperthermia, hypothermia, toxicity). In general, tolerance appears to develop to the disruptive or depressant effects of amphetamine, but not to the facilitative effects. Based on this assumption, tolerance to the activity-increasing action of amphetamine would not be expected to occur; however, since high doses of amphetamine decrease activity, this would be considered a depressant effect. Since tolerance to opposite effects such as the amphetamine-induced hyperthermia and hypothermia has been shown (Chiel et al., 1974; Lewander, 1971), it would seem reasonable to expect that tolerance to both the activity-increasing and decreasing action of amphetamine should also occur.

A cursory examination of the literature pertaining to the effects of amphetamine on locomotor activity revealed that testing for tolerance was conducted using only one dose of amphetamine (Lewander, 1971; Schuster and Zimmerman, 1961;

Tormey and Lasagna, 1960), or with doses which did not cover the entire dose-response range (Tilson and Rech, 1973b). It therefore seemed possible that tolerance to the activity-increasing action of amphetamine might become more apparent if representative doses spanning the entire dose-response curve were employed. Preliminary activity experiments with d-amphetamine revealed differences in locomotor activity changes and in the subsequent development of tolerance in mice and rats, thus demonstrating the possible existence of species differences.

This thesis attempts to clarify and resolve the nature of tolerance to d-amphetamine by examining a number of variables which may affect its development. Such variables include the number of drug injections, the interval between drug injections, the temporal relationship between drug administration and behavioral testing and the species of animal.

GENERAL METHODOLOGY

Subjects

The subjects of this study were naive adult CF_1 female mice weighing approximately twenty grams and naive adult Sprague-Dawley female rats weighing between 240 and 260 grams. Mice were housed in groups of twelve and rats were housed in groups of six. All subjects were provided with food and water *ad lib*. and were maintained on a regular twelve hour day-night cycle throughout the course of the study.

Testing apparatus

Six Lehigh Valley photocell activity apparatuses were used to measure locomotor activity. This apparatus is a cylinder twenty-four inches in diameter and eighteen inches in height, and contains six photocells. Interruptions of the photocell beams by movements of the subjects were automatically recorded on Sodeco counters.

Procedure

Each test session consisted of a subject being placed individually in a photocell box for thirty minutes. At the end of the test session the total number of activity counts was recorded and the subject was returned to its home cage. Each group of subjects was tested at approximately the same time each day.

D-amphetamine sulfate was made up in physiological saline solution and administered to mice in doses of 0.5, 1.0, 2.5, 5.0, 7.5 and 15.0 mg/kg i.p. and to rats in doses of 0.5, 1.0, 2.5 and 5.0 mg/kg i.p. The injection volume in all cases was 0.1 ml. Saline solution, 0.1 ml i.p., was administered to control groups of mice and rats.

Experimental groups

The subjects were assigned randomly to different experimental groups; each group of mice consisted of ten to twelve animals and each group of rats consisted of five to six animals. Six basic experimental paradigms were employed in this study. Variations of the basic experimental designs will be described in the procedures paragraph of the appropriate experiment. A general description of these six basic groups is presented below, and a summary appears in Table I.

Chronic Saline-No Testing group (CSN): the subjects were injected daily with saline for a given number of days, referred to in this and the other groups as the pre-test days. On the test day, which immediately followed the pre-test days, the subjects received saline or a given dose of d-amphetamine fifteen minutes before being placed in the photocell boxes for a thirty minute activity test session.

Chronic Drug-No Testing group (CDN): the subjects were injected daily with a given dose of d-amphetamine and a control group received saline on the pre-test days. On the test day, animals in this group were injected with the same dose

of d-amphetamine that they had received on the pre-test days, and tested fifteen minutes later for thirty minutes. Control animals which had received saline injections on the pre-test days were also administered saline on the test day.

Chronic Saline-With Testing group (CST): the subjects received daily saline injections and were tested fifteen minutes later for thirty minutes on each of the pre-test days. On the test day, these animals were injected with a given dose of d-amphetamine fifteen minutes before the thirty minute activity test session. Control animals were treated similarly, but received saline on the test day instead of d-amphetamine.

Chronic Drug-With Testing group (CDT): the subjects were injected with a given dose of d-amphetamine and were tested fifteen minutes later for thirty minutes on each of the pre-test days and the test day. Control animals were tested similarly with saline on the pre-test days and the test day.

Chronic Saline-Post Testing group (CSP): the subjects were tested daily for thirty minutes on each of the pre-test days, and received an injection of saline immediately following the test sessions on the pre-test days. On the test day, these animals received a given dose of d-amphetamine fifteen minutes before the thirty minute test session. Control animals received saline before the test session on the test day.

Chronic Drug-Post Testing group (CDP): the subjects

were tested daily for thirty minutes on each of the pre-test days, and received a given dose of d-amphetamine immediately following the test sessions on each of the pre-test days. On the test day, these animals were injected with the same dose of d-amphetamine which they had received on the pre-test days, and fifteen minutes later were tested for thirty minutes. Control animals were injected with saline on the pre-test days and the test day.

Statistical methods

All pairs of dose-response curves were analyzed using a two-way analysis of variance with dose of d-amphetamine as one factor and group treatment as the second factor. A pair of dose-response curves was compared only if the baseline responses to saline in the two curves did not differ significantly (Student's *t*-test at $p < .05$).

Two dose-response curves were considered to be shifted with respect to one another if, in addition to a significant treatment and/or interaction effect in the analysis of variance, their peaks were shifted. For establishing peak effects, the Student's *t*-test was used to determine whether or not the difference between a point near the peak of the dose-response curve was significantly larger (at $p < .05$) from two points on either side of the peak. The use of additional statistical methods to determine whether or not a dose-response curve is shifted will be described as they appear in the text.

TABLE I

EXPERIMENTAL GROUP	TREATMENT (PRE-TEST DAYS)	TREATMENT (TEST DAY)*
Chronic Saline-No Testing (CSN)	saline injections only	drug injection activity test session
Chronic Drug-No Testing (CDN)	drug injections only	drug injection ** activity test session
Chronic Saline-With Testing (CST)	saline injections before activity test sessions	drug injection activity test session
Chronic Drug-With Testing (CDT)	drug injections before activity test sessions	drug injection ** activity test session
Chronic Saline-Post Testing (CSP)	saline injections after activity test sessions	drug injection activity test session
Chronic Drug-Post Testing (CDP)	drug injections after activity test sessions	drug injection ** activity test session

* All drug injections were administered before the activity test session on the test day.

** Drug dose on test day is the same dose used for pre-test days.

RESULTS

Pharmacological factors affecting tolerance development

The intent of the experiments in this section was to determine whether or not tolerance to the activity-enhancing effect of d-amphetamine could be elicited in mice following chronic drug administration. Different treatment conditions affecting this pharmacologically-induced tolerance were also investigated.

Tolerance is a shifting of the dose-response curve to the right. In a monotonic dose-response curve, there is a decreased response to the same dose of drug with repeated administration. However, in the case of a nonmonotonic dose-response curve, an increased response to the same dose of drug may also occur. The dose-response curve for amphetamine has been shown to be nonmonotonic and in the shape of an inverted-U (Kelleher and Morse, 1968). The theoretical curves illustrated in figures 1 (a) and 1 (b) represent nonmonotonic dose-response curves from tolerant and nontolerant animals; these dose-response curves have the shape of an inverted-U. Implicit in the following discussion is the assumption that the entire dose-response curve represents the same mechanism of action of the drug and that the tolerant and nontolerant curves shift in parallel.

In figure 1 (a) the tolerant curve is shifted to the right and represents a situation in which all subjects

were treated with the same dose of drug for a specific number of pre-test days and subsequently tested with different doses of drug on the test day.

In figure 1 (b) the tolerant curve is also shifted to the right and represents a treatment condition in which the subjects received the same dose of drug on the pre-test days and the test day. Since tolerance develops more rapidly with larger doses of a drug (Kalant et al., 1971), chronic administration of the larger doses would lead to the greater shift seen in the descending portion of this curve.

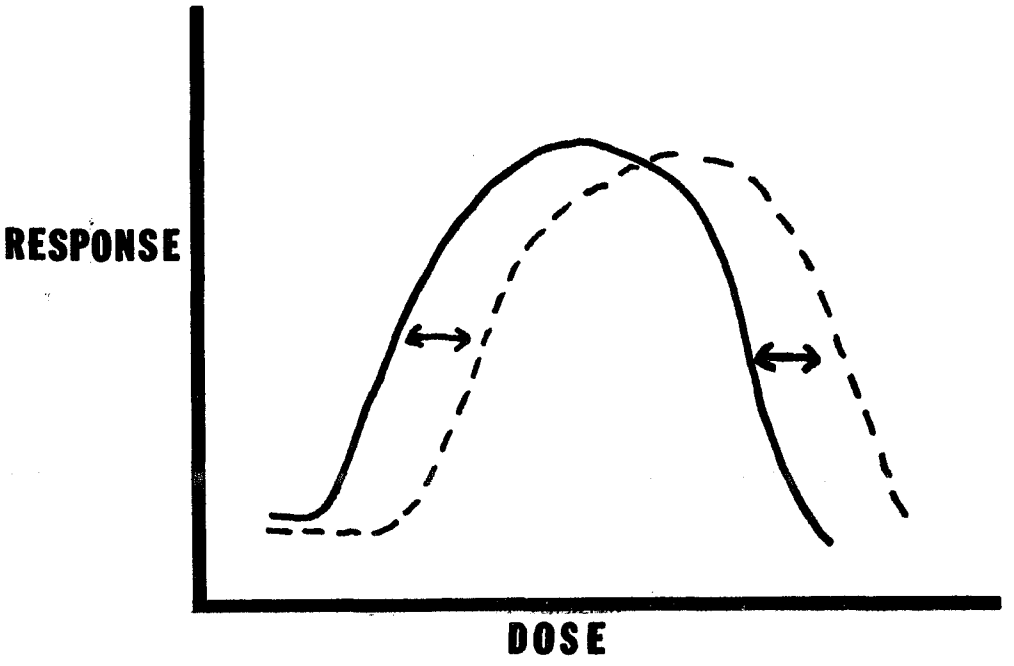
All the subjects in the experimental groups of this study received the same dose of drug on the pre-test and the test days, therefore the experimental dose-response curves presented in this thesis should resemble those in figure 1 (b).

The development of tolerance in an inverted-U function can be demonstrated by plotting the progressive changes in response for each dose of drug over time. A family of curves representing various doses generated in this manner would resemble those shown in figure 2. In the rising portion of the inverted-U curve, tolerance would be manifested by a decreased response to the same dose (A) of drug, as in a monotonic dose-response curve. However, in the descending portion of the inverted-U curve, tolerance would be seen as an increased response to the same dose of drug (B) with repeated administration. With a dose of drug (C) occurring in the region where the tolerant and nontolerant curves cross, an

Figure 1 (a): Theoretical dose-response curves representing the test day activities of nontolerant (solid line) and tolerant (dotted line) subjects. Tolerant subjects were treated with one dose of drug on the pre-test days, and received different doses of drug on the test day.

Figure 1 (b): Theoretical dose-response curves representing the test day activities of nontolerant (solid line) and tolerant (dotted line) subjects. Tolerant subjects were treated with the same dose of drug on the pre-test and the test days.

a.



b.

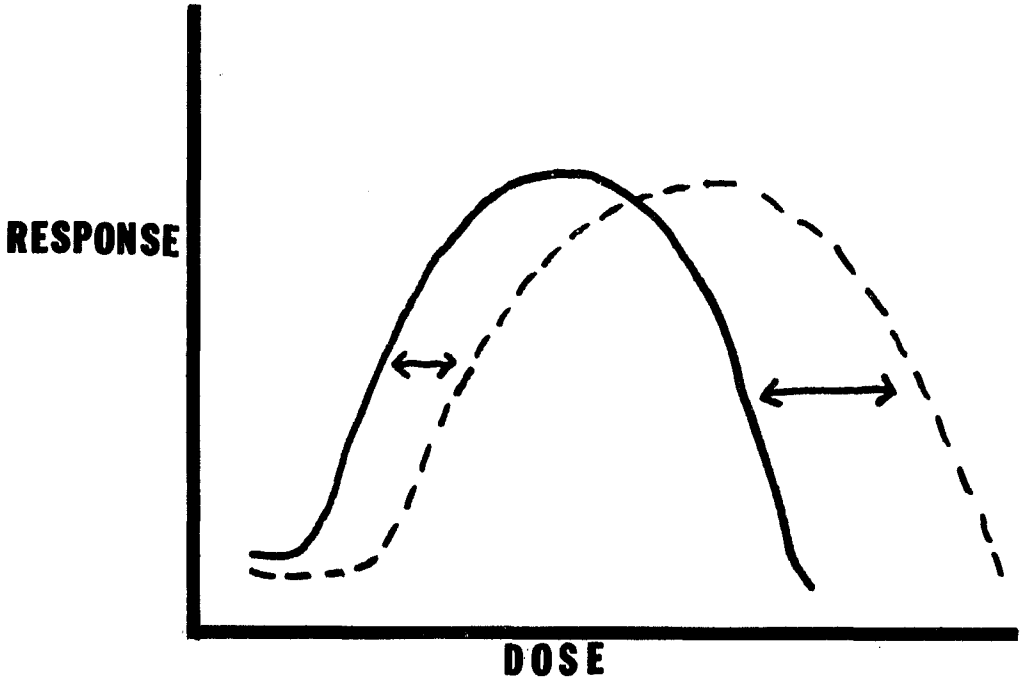
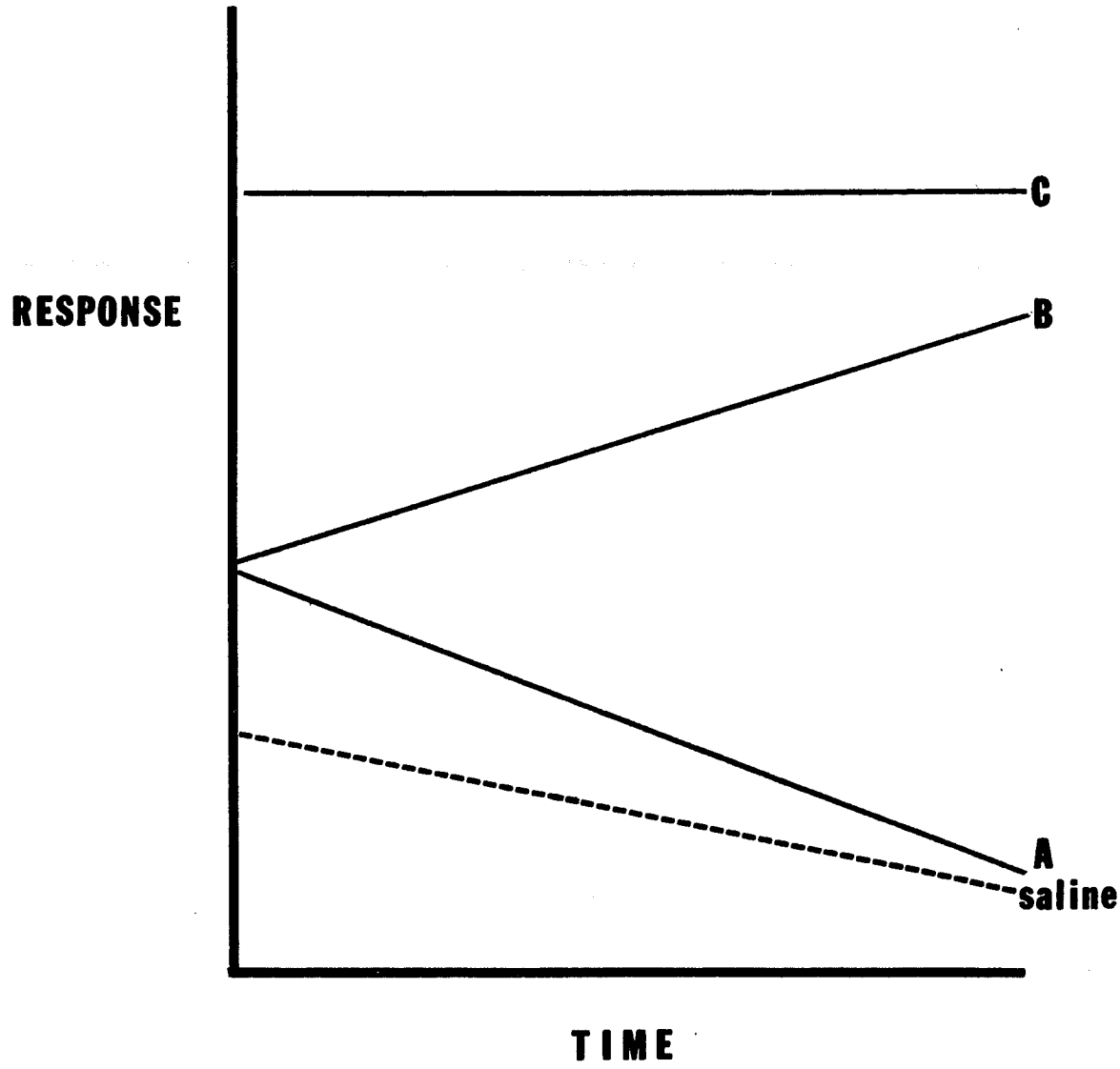


Figure 2: Theoretical curves indicating the progressive change in activity of three doses of drug and a saline control group (zero drug dose) in an inverted-U dose-response curve.

A: indicates dose of drug in the ascending portion of the dose-response curve.

B: indicates dose of drug in the descending portion of the dose-response curve.

C: indicates dose of drug at the intersection of the nontolerant and tolerant dose-response curves.


C
RESPONSE
B
A
saline
TIME

apparent straight line would result suggesting that tolerance did not occur.

In all dose-response curves presented in this section, the abscissas represent the dose of d-amphetamine and the ordinates represent activity counts. Each point on the dose-response curves represents the mean thirty minute activity count of a group of ten to twelve mice per dose of d-amphetamine.

Experiment I

The first experiment was done to determine whether tolerance to the effect of d-amphetamine on activity would develop in mice following seven days of drug administration.

One group of mice received daily injections of d-amphetamine for seven pre-test days (Chronic Drug-No Testing-8)*. The second group of mice served as controls and received saline injections daily for seven pre-test days (Chronic Saline-No Testing-8). Dose-response curves representing the test day activities were determined for both groups of mice on day 8, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

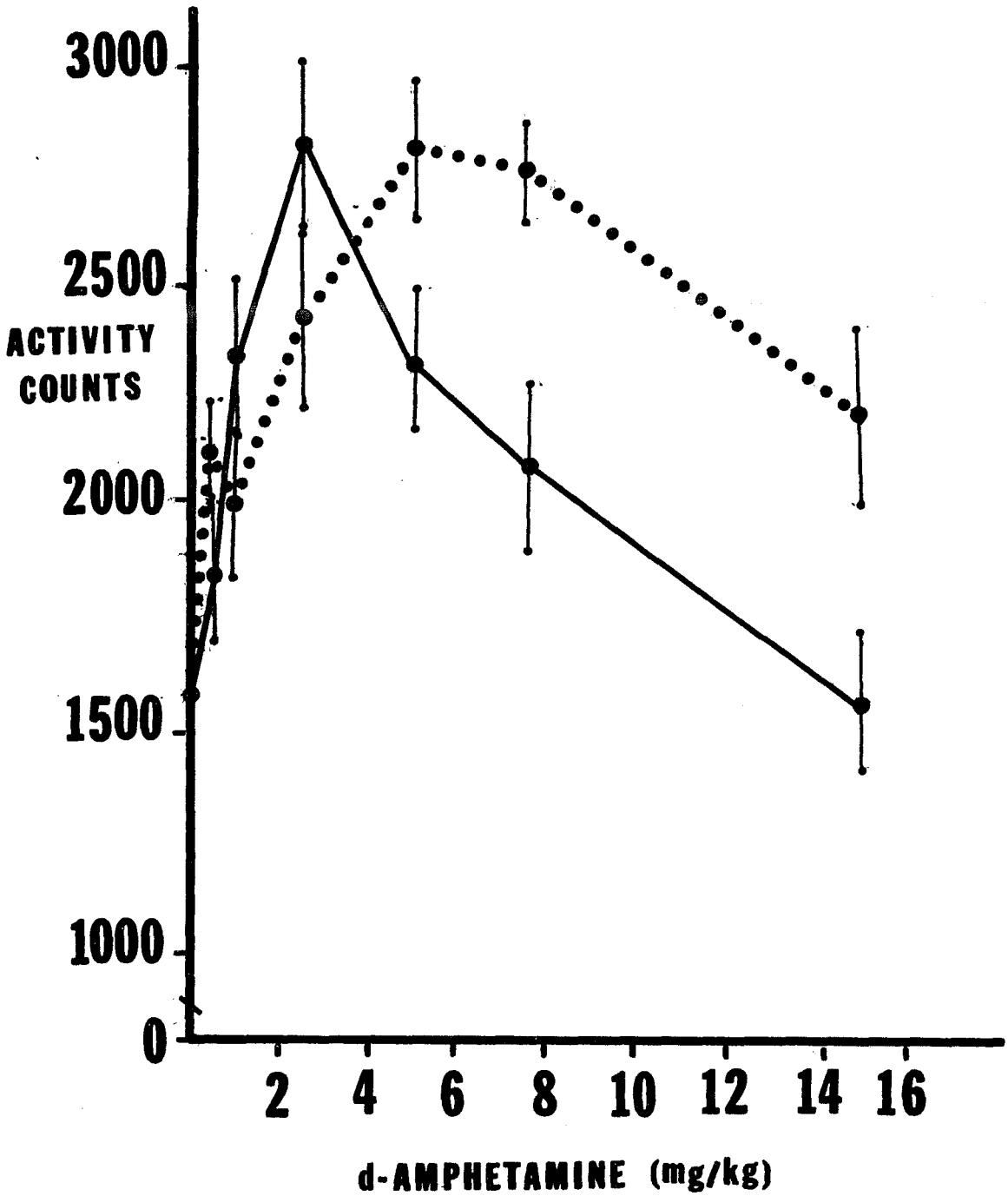
Figure 3 shows that chronic drug administration shifted the dose-response curve representing the test day activities

* The number following the group abbreviation refers to the test day.

Figure 3: Dose-response curves representing the test day activities on day 8 of mice in the Chronic Saline-No Testing-8 (solid line) and the Chronic Drug-No Testing-8 (dotted line) groups.

Each point on the graphs represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

of the Chronic Drug-No Testing-8 group of mice to the right (see Table II), indicating that a pharmacologically-induced tolerance had developed.

Experiment II

Experiment I showed that tolerance to the effects of d-amphetamine on activity developed following seven daily injections of the drug. The present experiment attempts to determine whether or not tolerance will develop when the number of drug injections is decreased to three.

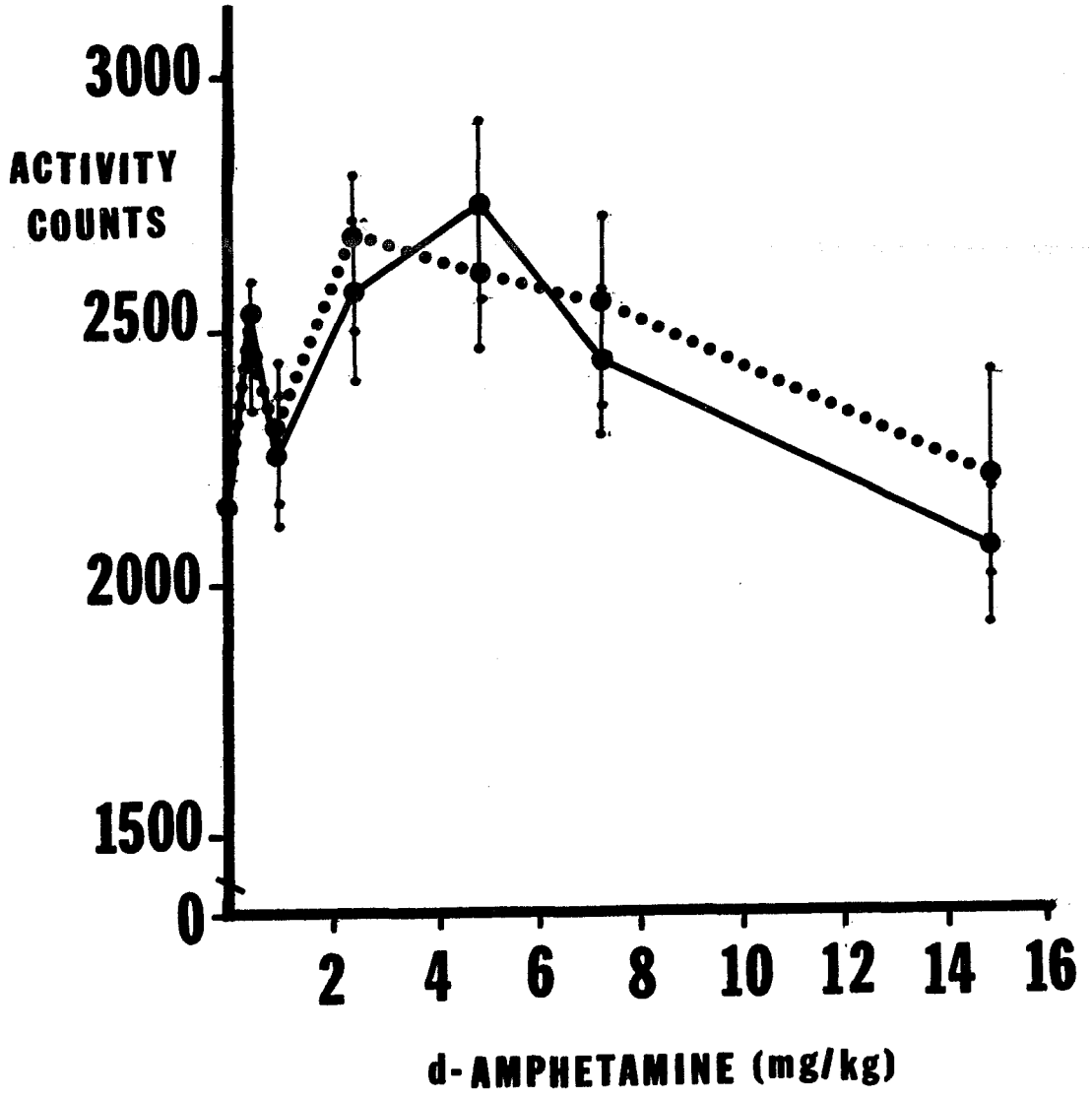
One group of mice received injections of d-amphetamine once a day for three pre-test days (Chronic Drug-No Testing-4). The second group of mice served as controls and received daily saline injections for three pre-test days (Chronic Saline-No Testing-4). Dose-response curves representing the test day activities were determined for both groups of mice on day 4, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 4 shows that the dose-response curves of the Chronic Drug-No Testing-4 and the Chronic Saline-No Testing-4 groups of mice are not shifted with respect to one another (see Table II). This shows that three injections administered on three successive days are insufficient to produce tolerance. The data do not allow for the differentiation between the minimum requirements of dose and duration

Figure 4: Dose-response curves representing the test day activities on day 4 of mice in the Chronic Saline-No Testing-4 (solid line) and the Chronic Drug-No Testing-4 (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

of treatment which are necessary to produce tolerance.

Experiment III

Experiments I and II showed that tolerance develops to the activity-increasing action of d-amphetamine when the drug is administered daily for seven days, but not when it is administered daily for three days. In the present experiment, the effect on tolerance development of administering seven injections of d-amphetamine in three and a half days is examined.

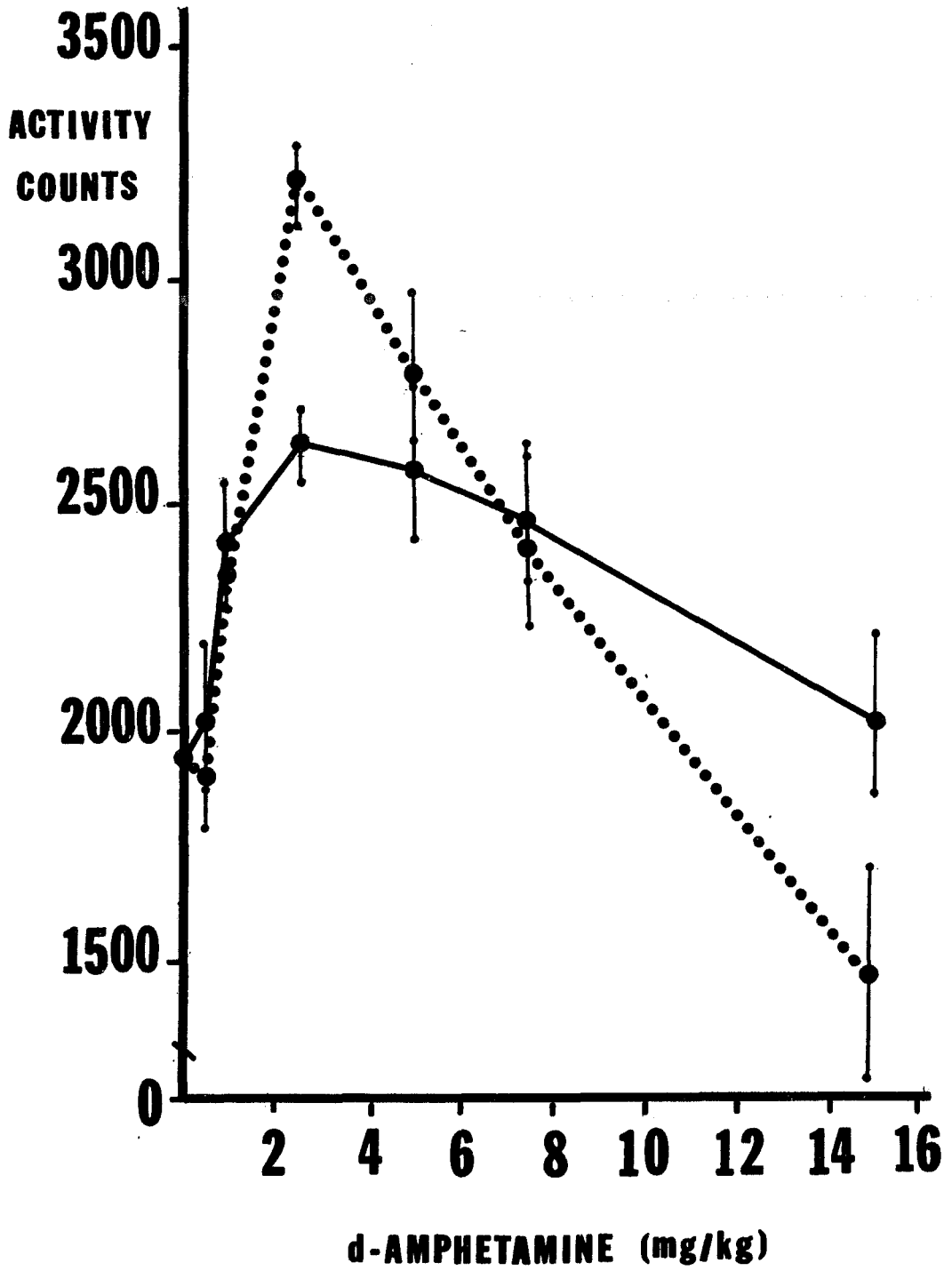
One group of mice received injections of d-amphetamine twice daily for three and a half pre-test days, with the drug injections spaced four hours apart each day (Chronic Drug-No Testing-4,2x). The second group of mice served as controls and received saline injections spaced four hours apart each day (Chronic Saline-No Testing-4,2x). The second session of day 4 was regarded as the test day for both groups. Dose-response curves representing the test day activities for both groups of mice were determined on the second session of day 4, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 5 shows that the dose-response curves of the Chronic Drug-No Testing-4,2x and the Chronic Saline-No Testing-4,2x groups of mice were not shifted with respect to one another (see Table II), but the peak (2.5) of the former dose-response curve is significantly higher than that

Figure 5: Dose-response curves representing the test day activities on day 4 (second treatment session) of mice in the Chronic Saline-No Testing-4,2x (solid line) and the Chronic Drug-No Testing-4,2x (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

(1.0-7.5) of the latter dose-response curve (*t*-test, $p < .005$). This shows that the results in Experiment II could not be accounted for solely on the basis of lower chronic amounts of d-amphetamine since seven drug injections in three and a half days also failed to produce tolerance. It should also be noted that not all the alterations in the dose-response curve are necessarily related to tolerance development.

Summary of Experiments I, II and III

It was demonstrated on pages 25, 26 and 28 that in the case of an inverted-U dose-response curve, tolerance can be shown by shifting the dose-response curve to the right. The experiments in this section were designed to examine pharmacological tolerance.

Experiment I showed that the shape of the dose-response curve representing the effect of d-amphetamine on activity is that of an inverted-U. It was also demonstrated that tolerance to this action of the drug developed following seven daily drug injections. In Experiment II, however, tolerance failed to develop following three daily drug injections.

In addition, Experiment III demonstrated that tolerance also did not develop when the drug was administered seven times within three and a half days. This result indicates that the number of drug injections *per se* is not as crucial to the development of tolerance as is the interval between injections.

TABLE II

FIGURE	TWO-WAY ANALYSIS OF VARIANCE	T-TEST
3	Main effect of dose significant at $p < .01$; interaction between dose and treatment significant at $p < .01$.	Peak effects at 2.5 mg/kg (CSN-8) and between 5.0-7.5 mg/kg (CDN-8).
4	Main effects of dose and treatment insignificant; interaction between dose and treatment insignificant.	Peak effects greater than 1.0 mg/kg (CSN-4) and between 2.5 and 5.0 mg/kg (CDN-4).
5	Main effect of dose significant at $p < .01$; interaction between dose and treatment significant at $p < .05$.	Peak effects at 2.5 mg/kg (CDN-4, 2x) and between 1.0 and 7.5 mg/kg (CSN-4,2x).

The interaction between pharmacological and behavioral factors involved in tolerance development

The experiments in this section attempted to determine what effect, if any, chronic testing with d-amphetamine had on the development of tolerance to the activity-enhancing action of d-amphetamine. The effect of behavioral testing *per se* on the development of tolerance was also examined.

In all the dose-response curves presented in this section, the abscissas represent the dose of d-amphetamine and the ordinates represent activity counts. Each point on the dose-response curves represents the mean thirty minute activity count of a group of ten to twelve mice per dose of d-amphetamine.

Experiment IV

The first experiment was done to determine whether or not tolerance to d-amphetamine would develop when chronic drug administration was combined with activity testing on each of the seven pre-test days.

One group of mice was tested daily in the photocell boxes with d-amphetamine for eight days (Chronic Drug-With Testing-8). The progressive change in activity from day 1 through day 8 was investigated using three doses of d-amphetamine and a saline control (zero drug dose).

Figure 6 shows the progressive changes in activity of mice in the Chronic Drug-With Testing-8 group from day 1 through day 8 for doses of 0.5, 5.0 and 15.0 mg/kg of

d-amphetamine and a saline control group. The abscissa represents days 1 through 8 and the ordinate represents activity counts. Each point on the graph represents the mean thirty minute activity count of a group of ten to twelve mice. The same group of mice was used for each dose of d-amphetamine on each of the eight days.

The activity of the control mice which received saline injections only on each of the eight days was significantly lower on day 8 as compared to day 1 (t-test, $p < .05$). Mice receiving a dose of 0.5 mg/kg of d-amphetamine daily for eight days had a significantly higher activity than the saline control mice on day 1 (t-test, $p < .05$), but on day 8 the activities of the two groups were nonsignificantly different (t-test, $p > .05$). Mice receiving a daily dose of 5.0 mg/kg of d-amphetamine for eight days showed no significant change in activity from day 1 to day 8 (one-way analysis of variance, main effect of days, $p > .05$), but their overall level of activity was significantly higher than that of the saline control mice (t-test, $p < .05$). The activity of mice receiving a dose of 15.0 mg/kg of d-amphetamine daily for eight days significantly increased from day 1 to day 8 (t-test, $p < .05$).

The progressive decline in activity seen in the saline control group was due to the habituation of these subjects to the activity apparatus. The data suggest that tolerance occurs following a chronic dose of 0.5 mg/kg of d-amphetamine. However, it is not the decrease in activity from day 1

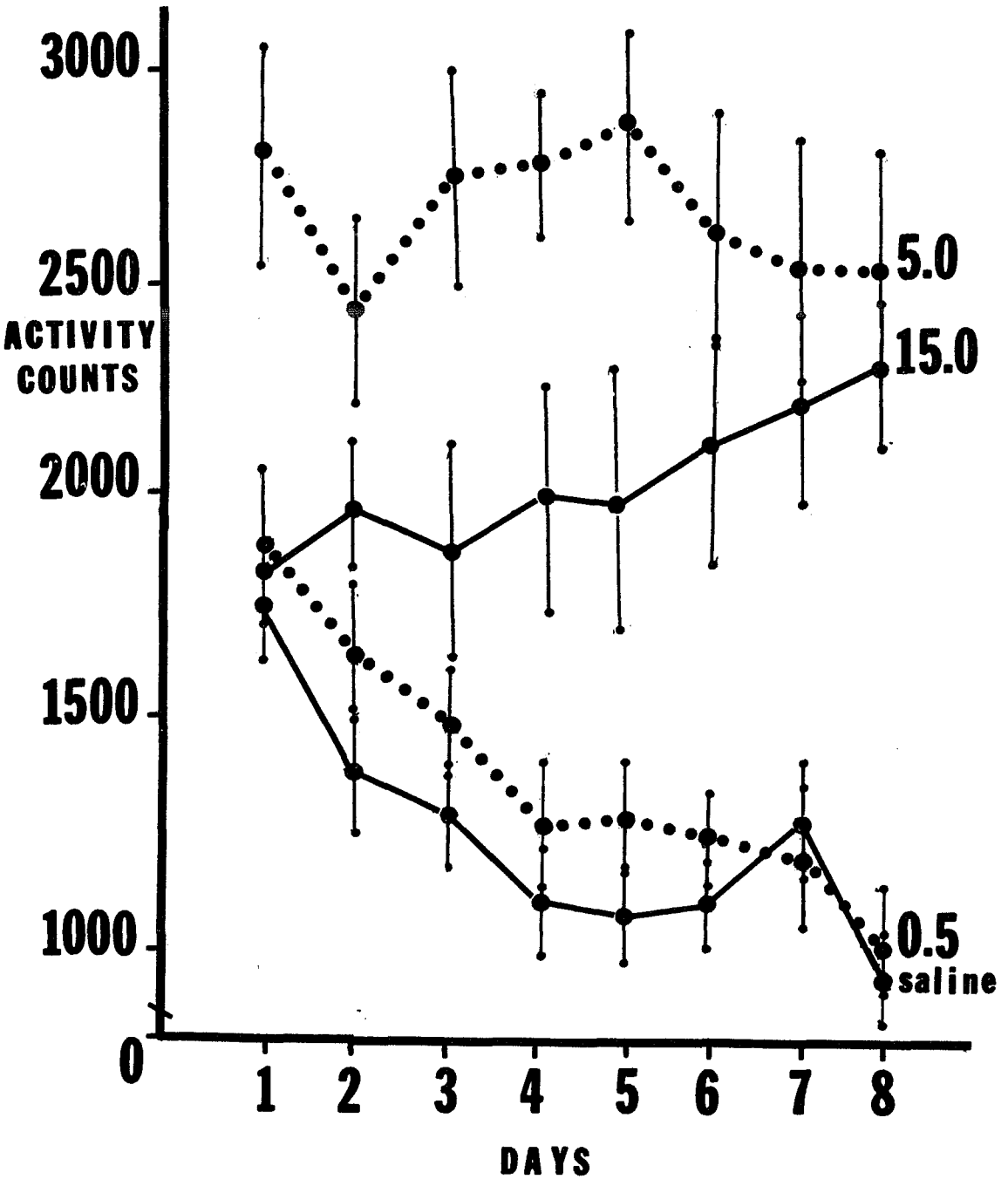
Figure 6: Curves indicating the progressive change in activity of three doses of d-amphetamine and a saline control group in an inverted-U dose-response curve.

0.5 mg/kg occurs in the ascending portion of the dose-response curve.

5.0 mg/kg occurs in the intersection between a nontolerant and a tolerant dose-response curve.

15.0 mg/kg occurs in the descending portion of the dose-response curve.

Vertical lines indicate the standard errors of the mean.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saline

to day 8 at this dose which demonstrates the occurrence of tolerance, but rather that the difference between the activities of the subjects in the saline control group and the 0.5 mg/kg group significantly decreases. The activity of mice receiving a dose of 5.0 mg/kg of d-amphetamine was significantly higher than that of the saline control mice on day 1, but did not change significantly from days 1 through 8, suggesting that no tolerance had occurred. After chronic injections of the highest dose of d-amphetamine (15.0 mg/kg), the response increased significantly from the first to the last day, whereas the activity of the saline control group of mice decreased over the same period of time. This would be evidence of tolerance if the shape of the dose-response curve were that of an inverted-U.

Experiment V

In the following experiment, the shape of the dose-response curve representing the test day activities of the mice from Experiment IV was determined. This dose-response curve was then compared to one representing the test day activities of a control group of mice which had been tested with the drug on the test day only.

One group of mice was tested daily in the activity boxes with d-amphetamine for seven pre-test days (Chronic Drug-With Testing-8). The second group of mice was tested daily in the activity boxes with saline for seven pre-test days (Chronic Saline-With Testing-8). Dose-response curves

representing the test day activities were determined for both groups of mice on day 8, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 7 shows that the dose-response curves representing the test day activities of mice in the Chronic Drug-With Testing-8 and the Chronic Saline-With Testing-8 groups are in the shape of inverted-Us, and are not shifted with respect to one another (see Table III). The data show that mice which had been tested both with and without drug for seven days became tolerant to d-amphetamine by day 8. This implies that testing alone can induce the development of tolerance to the activity-enhancing effect of d-amphetamine.

Experiment VI

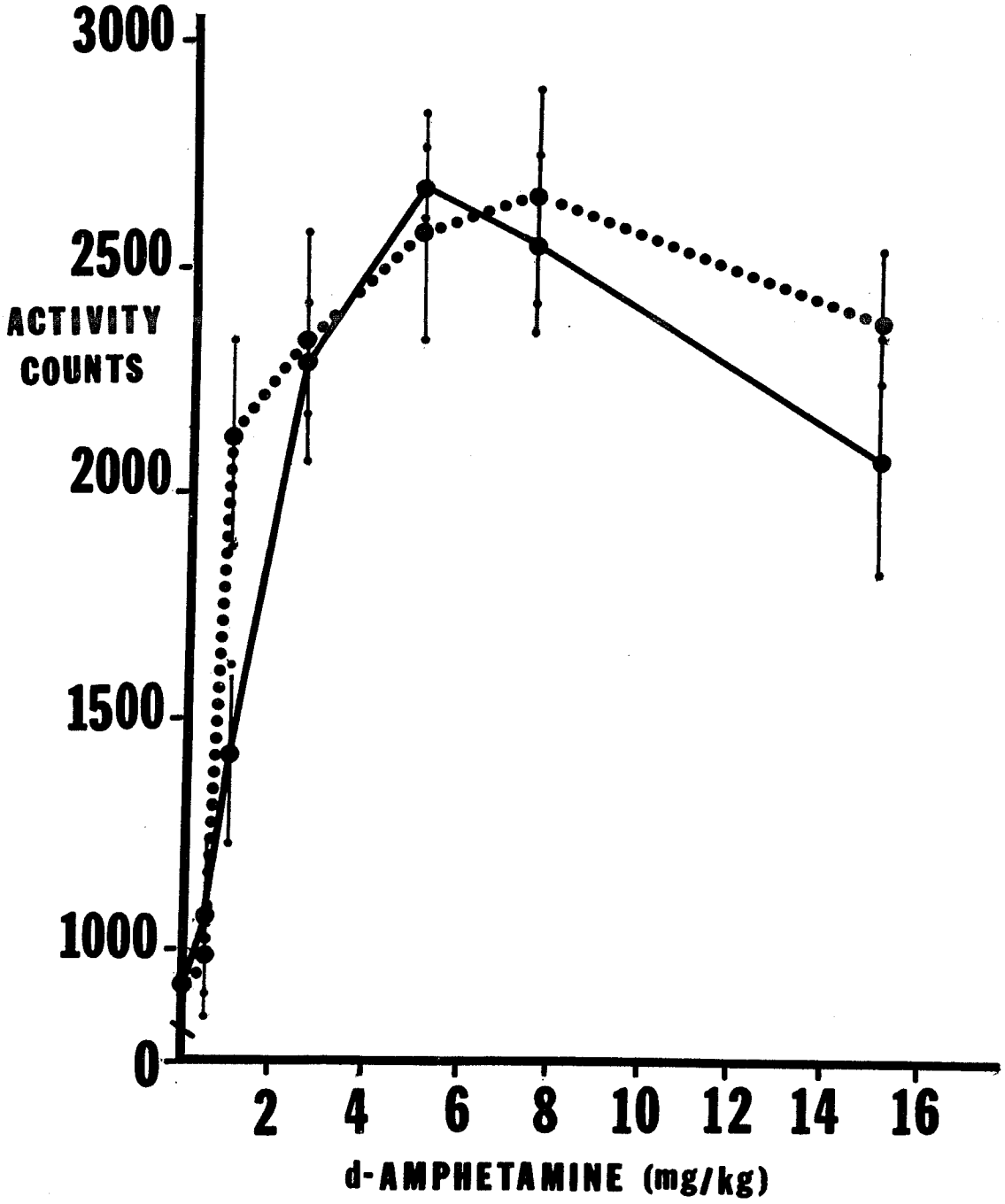
The results of Experiment V showed that seven days of testing without drug appeared to induce the development of tolerance to the activity-increasing action of d-amphetamine. In the present experiment, the possibility that tolerance would occur in mice following three days of testing without drug was investigated by comparing the subjects to nontolerant mice which were tested once with the drug.

One group of mice was tested daily in the activity boxes with saline for three pre-test days (Chronic Saline-With Testing-4). The second group of mice was tested once in the activity boxes with d-amphetamine (Drug-With Testing-1).

Figure 7: Dose-response curves representing the test day activities on day 8 of mice in the Chronic Saline-With Testing-8 (solid line) and the Chronic Drug-With Testing-8 (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

Dose-response curves were determined for the test day activities on day 4 for the first group, and for the activities on day 1 for the second group; on their respective test days, all subjects were tested in the activity boxes with d-amphetamine.

Figure 8 shows that the dose-response curves for the Chronic Saline-With Testing-4 and the Drug-With Testing-1 groups of mice were not shifted with respect to one another (see Table III). Since mice did not become tolerant to d-amphetamine following three days of testing without the drug, it appears that between three and seven days of activity testing are necessary for tolerance to develop.

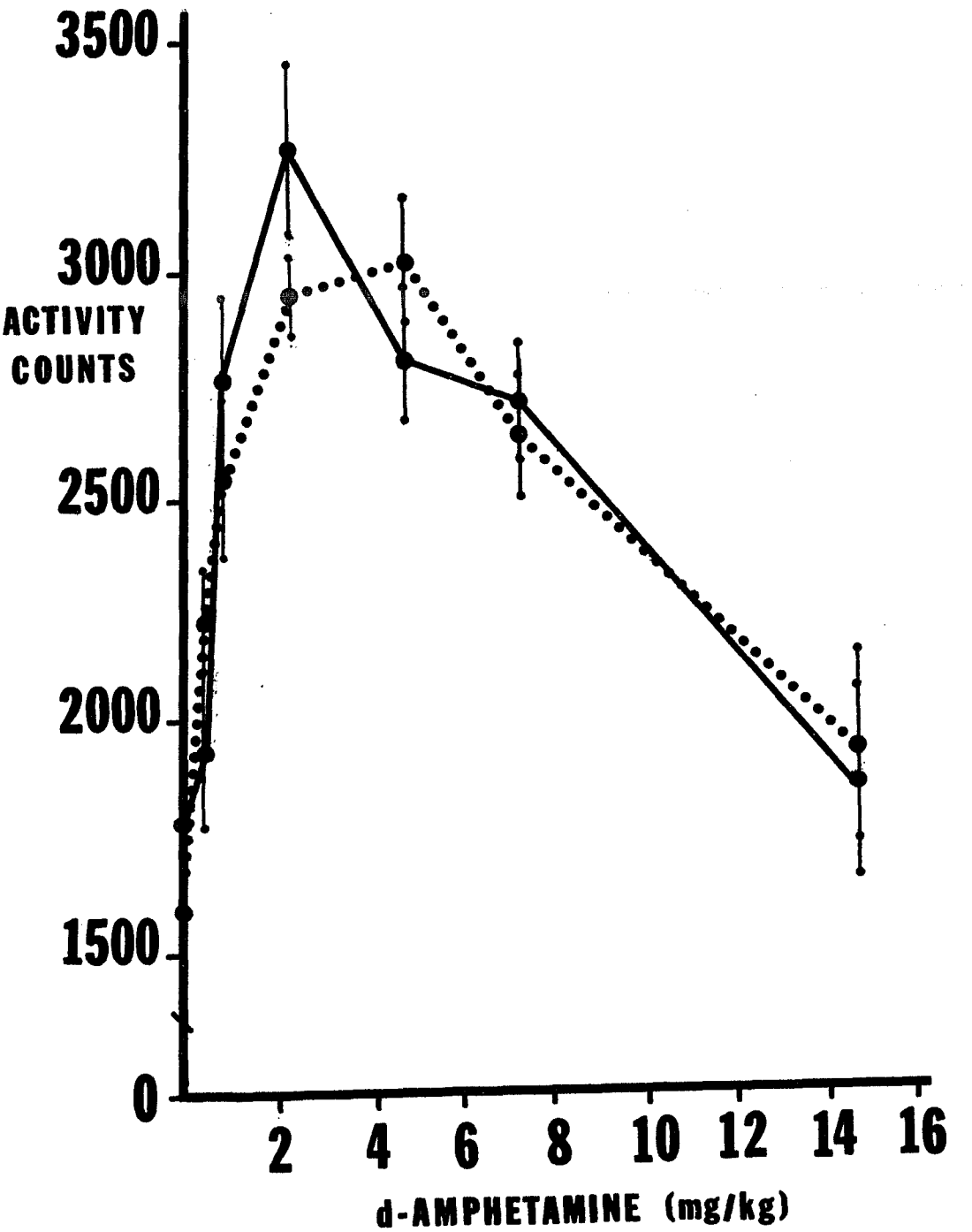
Experiment VII

Experiments IV and V showed that tolerance developed to the activity-increasing action of d-amphetamine following seven days of testing with and without drug. Experiment VI demonstrated that tolerance failed to develop following three days of testing without drug; Experiment II showed that tolerance did not develop in three days when drug administration was the sole variable. The present experiment examined the possibility that tolerance would develop in a shorter period of time when both the pharmacological and behavioral variables were combined. This was accomplished by comparing the test day activities of mice which had been tested for three days with d-amphetamine to those of nontolerant mice which had been tested with drug only once.

Figure 8: Dose-response curves representing the test day activities on days 1 and 4 of mice in the Drug-With Testing-1 (solid line) and the Chronic Saline-With Testing-4 (dotted line) groups, respectively.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

One group of mice was tested daily in the activity boxes with d-amphetamine for three pre-test days (Chronic Drug-With Testing-4). The second group of mice was tested once in the activity boxes with d-amphetamine (Drug-With Testing-1). Dose-response curves were determined for the test day activities on day 4 for the first group, and for the activities on day 1 for the second group; on their respective test days, all subjects were tested in the activity boxes with d-amphetamine.

Figure 9 shows that the dose-response curves representing the test day activities of the Chronic Drug-With Testing-4 and the Drug-With Testing-1 groups of mice are not shifted with respect to one another (see Table III). This result is in agreement with the results of the longitudinal dose studies (see Day 4, Figure 6, p.45). Since mice did not become tolerant following three days of testing with d-amphetamine, it appears that between three and seven days of testing with drug are required for the development of tolerance.

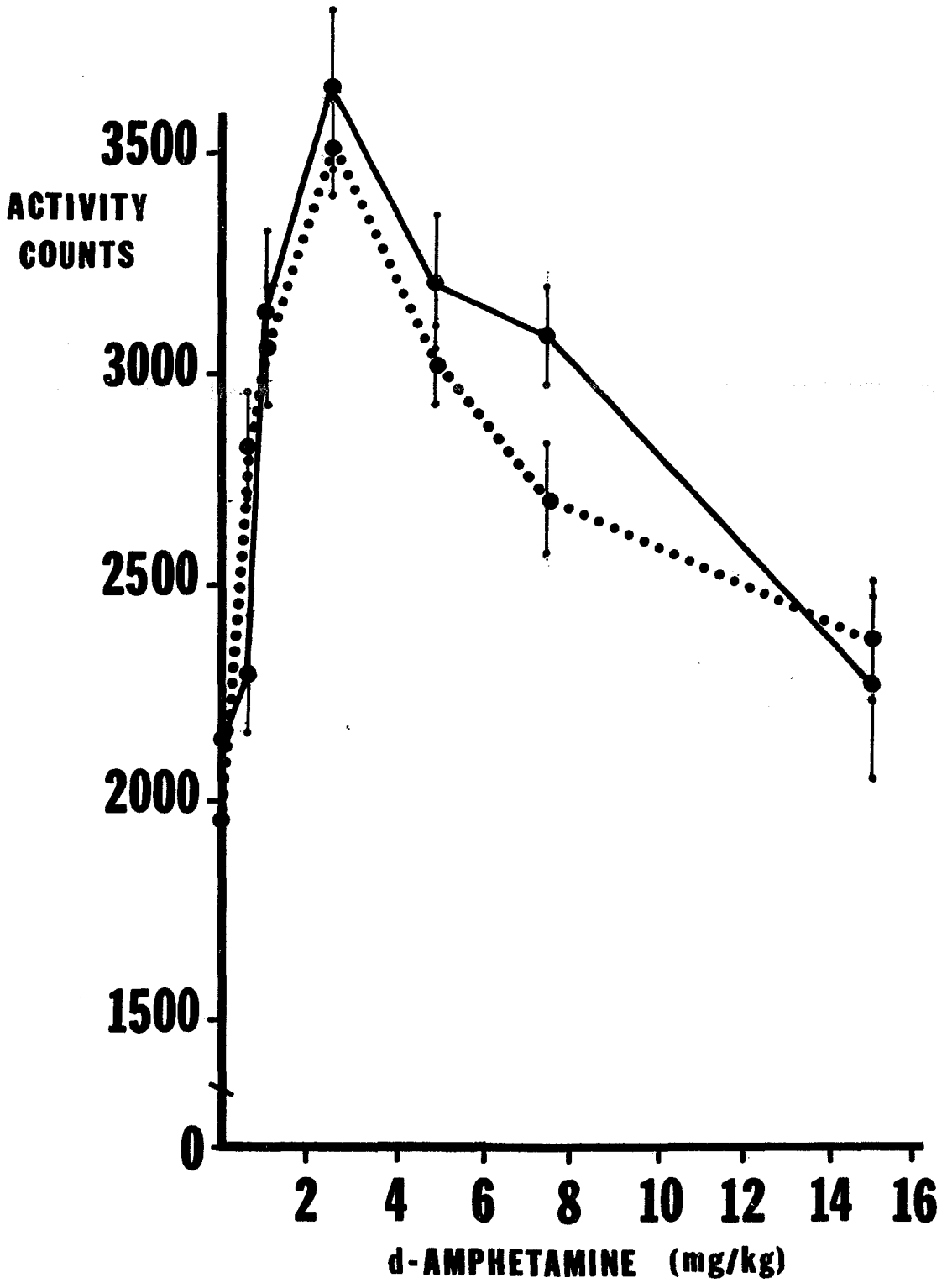
Experiments VI and VII demonstrated that tolerance to the activity-increasing effect of d-amphetamine failed to develop in three days, when testing was conducted both with and without drug. By comparing the dose-response curves of the subjects from Experiments VI and VII, it was determined whether or not these dose-response curves differed from one another.

Mice in the Chronic Drug-With Testing-4 group had been tested with d-amphetamine for three pre-test days. Mice in the Chronic Saline-With Testing-4 group had been tested with

Figure 9: Dose-response curves representing the test day activities on days 1 and 4 of mice in the Drug-With Testing-1 (solid line) and the Chronic Drug-With Testing-4 (dotted line) groups, respectively.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE (mg/kg)

saline for three pre-test days. Dose-response curves representing the test day activities were determined for both groups on day 4, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 10 shows that the dose-response curves for the Chronic Drug-With Testing-4 and the Chronic Saline-With Testing-4 groups of mice are not shifted with respect to one another (see Table III), and therefore are not different from each other.

Experiment VIII

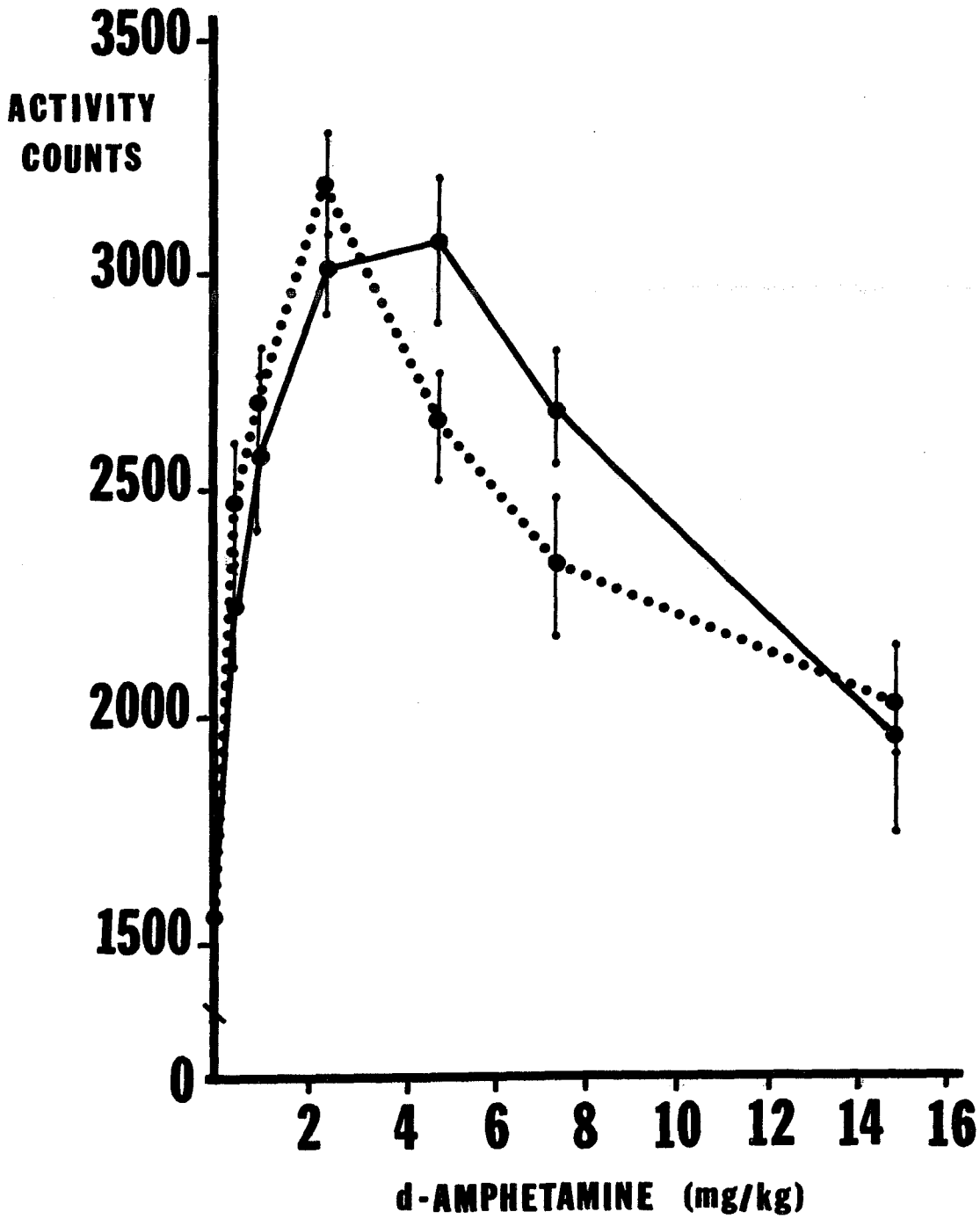
The previous experiments showed that testing once a day with d-amphetamine was not significantly different from testing once a day with saline as far as tolerance development was concerned. In the present experiment, the effect on tolerance development of decreasing the inter-treatment interval was investigated using two groups of mice, one tested with d-amphetamine, the other without drug.

One group of mice was tested with d-amphetamine twice daily for three and a half pre-test days, with the drug injections and test sessions spaced four hours apart each day (Chronic Drug-With Testing-4,2x). The second group of mice was tested with saline twice daily for three and a half pre-test days with the saline injections and test sessions spaced four hours apart each day (Chronic Saline-With Testing-4,2x). The second test session of day 4 was regarded as the test day

Figure 10: Dose-response curves representing the test day activities on day 4 of mice in the Chronic Saline-With Testing-4 (solid line) and the Chronic Drug-With Testing-4 (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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for both groups. Dose-response curves representing the test day activities for both groups of mice were determined on the second test session of day 4, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 11 shows that the dose-response curves of the Chronic Drug-With Testing-4,2x and the Chronic Saline-With Testing-4,2x groups of mice are not shifted with respect to one another (see Table III), but the peak (5.0) of the dose-response curve of the former group is significantly higher than that (5.0-7.5) of the latter group (*t*-test, $p < .05$). It should be noted that the increased peak response seen in this case is similar to that observed in mice which received d-amphetamine only twice a day for three and a half days (Experiment III, p.37). Although it is not possible from the data to determine whether tolerance occurred, it appears that closely spaced injections of d-amphetamine, whether accompanied by testing or not, affect the shape of the dose-response curve.

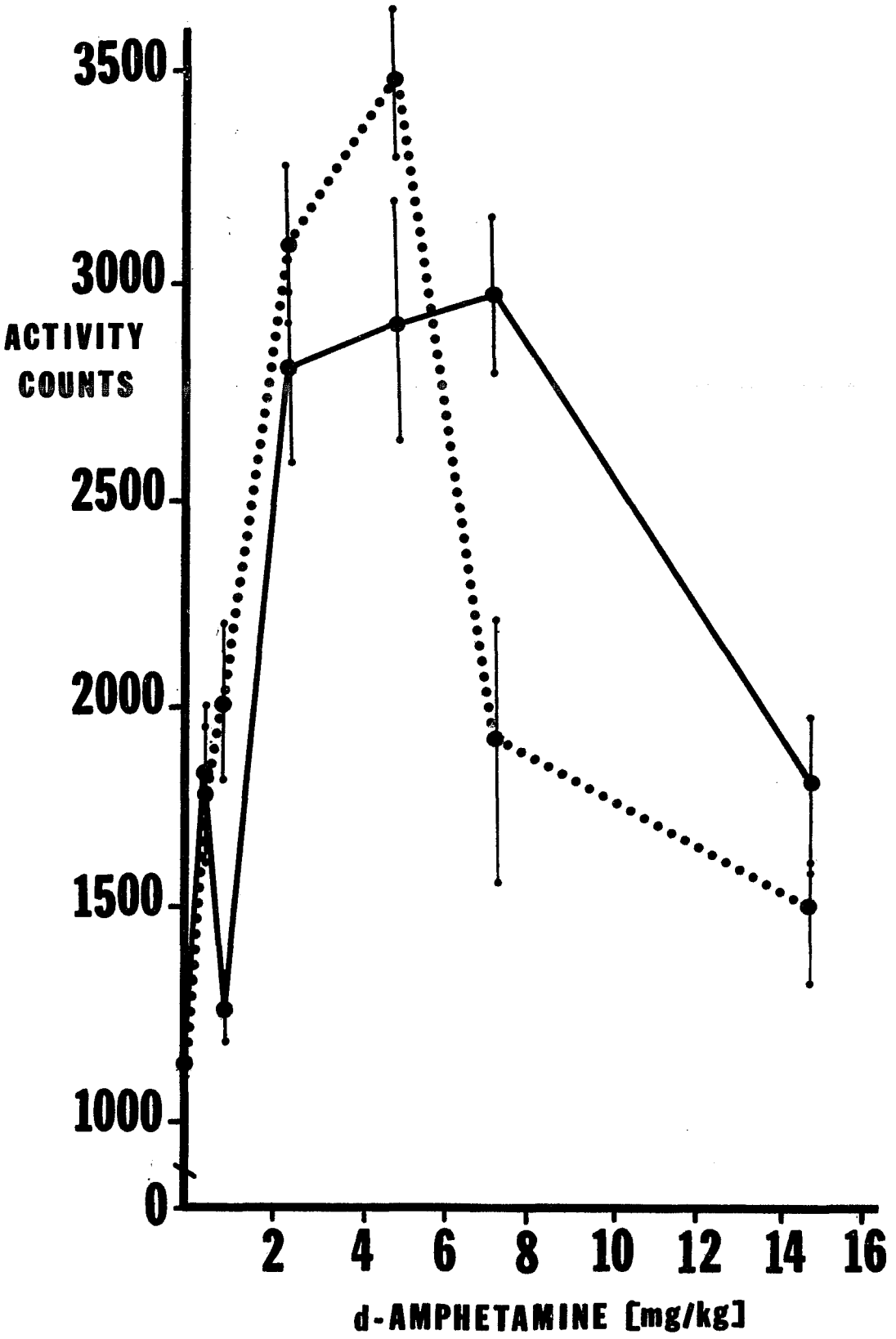
Summary of Experiments IV, V, VI, VII and VIII

By the use of a family of curves showing the progressive changes in activity of single doses of d-amphetamine, Experiment IV demonstrated that tolerance to the activity-enhancing effect of d-amphetamine developed following seven days of testing with the drug. These curves showed that when

Figure 11: Dose-response curves representing the test day activities on day 4 (second treatment session) of mice in the Chronic Saline-With Testing-4,2x (solid line) and the Chronic Drug-With Testing-4,2x (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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d-AMPHETAMINE [mg/kg]

compared to a saline control group, repeated low doses of d-amphetamine caused activity to decline, repeated high doses of d-amphetamine caused activity to increase, and with the chronic administration of doses between the high and low doses, activity remained unchanged. These kinds of changes in activity are consistent with those that would be shown by a curve having an inverted-U shape, such as that found for the activity-increasing action of d-amphetamine. However, when an attempt was made to establish the occurrence of tolerance by comparing dose-response curves representing the activities of mice which had been tested for seven days with and without d-amphetamine, the two curves were not shifted with respect to one another. This result showed that testing without the drug can also produce tolerance to d-amphetamine, and suggested that the addition of the drug does not further decrease the sensitivity of the subjects to d-amphetamine on the test day.

In order to examine the time course of development of tolerance to d-amphetamine with chronic testing with or without drug, two groups of mice were tested with and without drug for three days. In Experiments VI and VII, these two groups were compared to a nontolerant group of mice which had been tested with d-amphetamine once. The results showed that tolerance failed to develop in either group. When the two groups were compared to each other, no significant difference was found between them.

The implications of the preceding experiments are two-fold: 1) from three to seven days are required in order for tolerance to develop to the activity-enhancing effect of d-amphetamine when mice are tested, regardless of whether the subjects received d-amphetamine or not, and 2), testing with d-amphetamine daily is not different from testing daily without d-amphetamine, whether tolerance develops or not.

In Experiment VIII, the effect of administering seven injections of d-amphetamine or saline with seven test sessions in three and a half days was examined in two groups of mice. The occurrence of tolerance could not be ascertained from the data since there was no appropriate control group available; however, it was shown that closely spaced injections of d-amphetamine affect the shape of the dose-response curve.

TABLE III

FIGURE	TWO-WAY ANALYSIS OF VARIANCE	T-TEST
7	Main effect of dose significant at $p < .001$; interaction between dose and treatment significant at $p < .001$.	Peak effects between 5.0 and 7.5 mg/kg (CST-8) and greater than 2.5 mg/kg (CDT-8).
8	Main effect of dose significant at $p < .005$; interaction between dose and treatment significant at $p < .01$.	Peak effects between 1.0 and 7.5 mg/kg (DT-1) and (CST-4).
9	Main effect of dose significant at $p < .001$; interaction between dose and treatment significant at $p < .01$.	Peak effects at 2.5 mg/kg (CDT-4) and between 1.0 and 7.5 mg/kg (DT-1).
10	Main effect of dose significant at $p < .01$; interaction between dose and treatment significant at $p < .01$.	Peak effects at 2.5 mg/kg (CDT-4) and between 1.0 and 7.5 mg/kg (CST-4).
11	Main effect of dose significant at $p < .001$; main effect of treatment significant at $p < .05$; interaction between dose and treatment significant at $p < .01$.	Peak effects between 2.5 and 5.0 mg/kg (CDT-4,2x) and between 2.5 and 7.5 mg/kg (CST-4,2x).

The relationship between order of drug administration and activity testing and its effect on tolerance development

The intent of the experiments in this section was to determine if the relationship between the order of drug administration and activity testing affects the development of tolerance to d-amphetamine in mice.

In all the dose-response curves presented in this section the abscissas represent the dose of d-amphetamine and the ordinates represent activity counts. Each point on the dose-response curves represents the mean thirty minute activity count of a group of ten to twelve mice per dose of d-amphetamine.

Experiment IX

This experiment was conducted to see whether tolerance to d-amphetamine can be elicited in a group of mice when the drug is administered immediately after the test sessions for seven days. This group of mice was then compared to a nontolerant group of mice which showed the same baseline activity, i.e. response to saline on the test day, as did the experimental group.

One group of mice was tested daily for seven pre-test days and received injections of d-amphetamine immediately following the test sessions each day (Chronic Drug-Post Testing-8). The group of mice chosen for comparison was found to be nontolerant in a previous experiment (see Experiment VII, p.53), and was tested daily with d-amphetamine

for three pre-test days (Chronic Drug-With Testing-4). Dose-response curves representing test day activities for both groups of mice were determined for days 8 and 4 respectively, when all subjects were injected with d-amphetamine fifteen minutes before the activity test session.

Figure 12 shows that the dose-response curves of the Chronic Drug-Post Testing-8 and the Chronic Drug-With Testing-4 groups of mice are not shifted with respect to one another (see Table IV); however, the peak (2.5) of the dose-response curve representing the activities of the former group is significantly higher than that (2.5) representing the activities of the latter group (t-test, $p < .05$). These results show that administration of d-amphetamine after the activity test sessions for seven days apparently blocks the development of tolerance, although tolerance should have occurred following seven days of testing (see Experiment V, p.47). In addition, the data show that d-amphetamine injections lead to an increased peak response when the drug is administered before the test session for the first time.

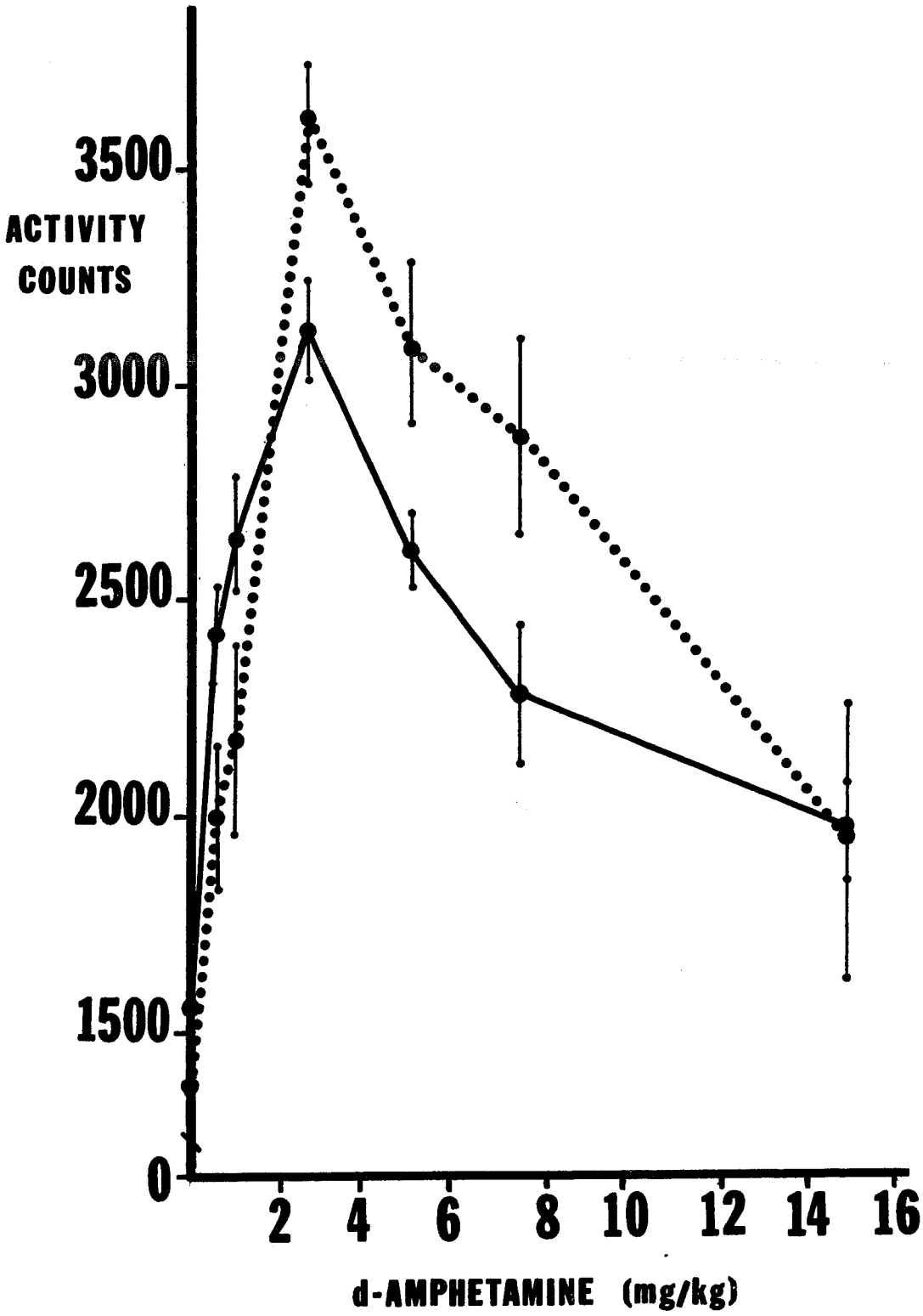
Experiment X

Experiment IX showed that when d-amphetamine was administered immediately after the activity test sessions for seven days, tolerance failed to develop. The present experiment attempts to determine if 1) tolerance will develop, and 2), whether or not the increase in peak height seen in Experiment IX will occur when saline rather than d-amphetamine

Figure 12: Dose-response curves representing the test day activities on days 4 and 8 of mice in the Chronic Drug-With Testing-4 (solid line) and the Chronic Drug-Post Testing-8 (dotted line) groups, respectively.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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is administered to the subjects immediately following the test sessions for seven days.

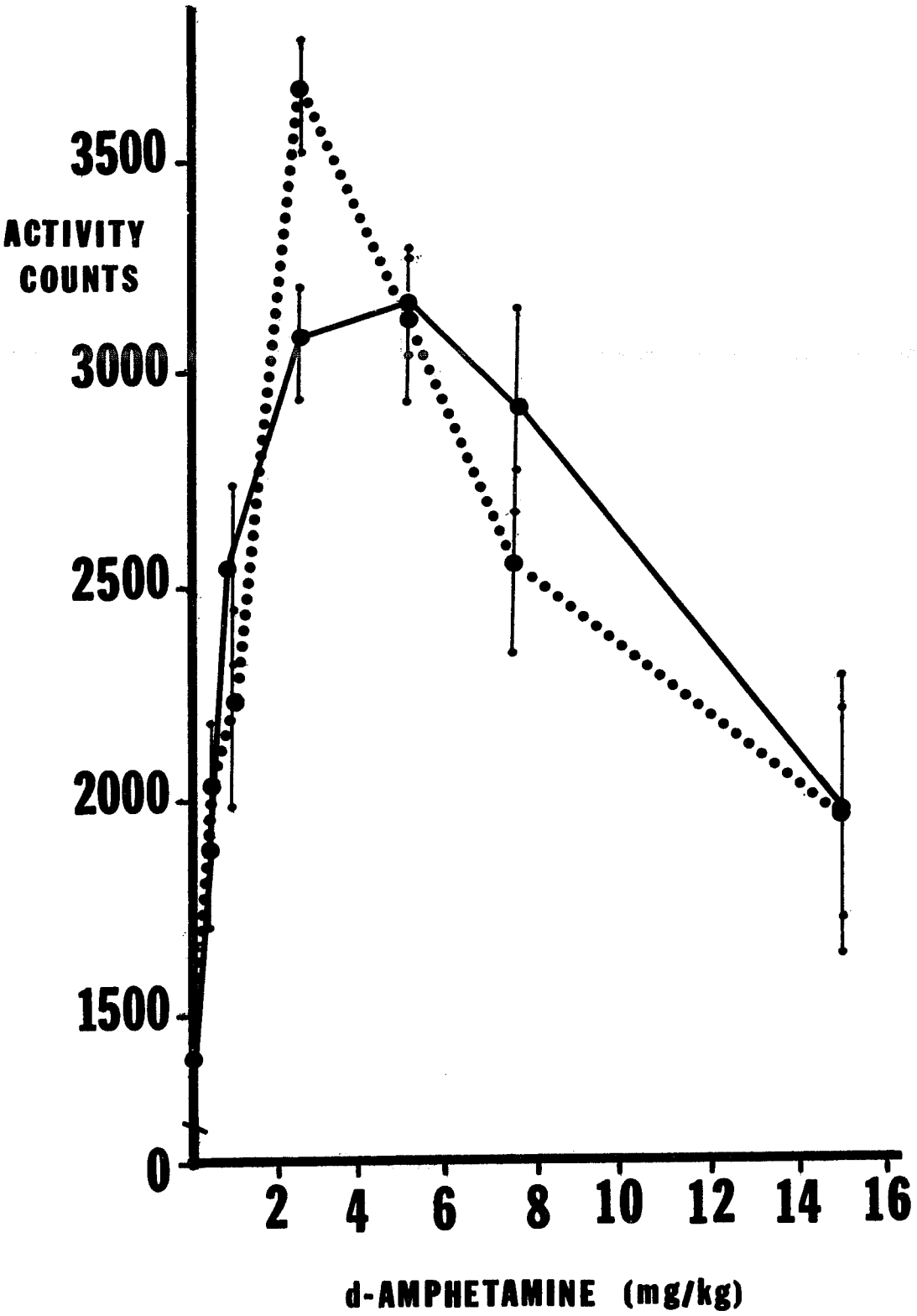
One group of animals (also used in the previous experiment) were tested daily for seven pre-test days, and received injections of d-amphetamine immediately after the test sessions each day (Chronic Drug-Post Testing-8). The second group of mice was tested for seven pre-test days, but received injections of saline immediately following the test sessions each day (Chronic Saline-Post Testing-8). Dose-response curves representing the test day activities were determined for both groups of mice on day 8, when all subjects were injected with d-amphetamine fifteen minutes before the activity test session. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 13 shows that the dose-response curve representing the activities of the Chronic Drug-Post Testing-8 and the Chronic Saline-Post Testing-8 groups of mice are not shifted with respect to one another (see Table IV); however, the peak (2.5) of the dose-response curve representing the activities of the former group is significantly higher than that representing the activities of the latter group (2.5-5.0) (t-test, $p < .05$). The results indicate that neither administration of d-amphetamine nor saline after the test sessions for seven days leads to the development of tolerance. In addition, the post-test session administration of drug appears to affect the subjects' peak activity.

Figure 13: Dose-response curves representing the test day activities on day 8 of mice in the Chronic Saline-Post Testing-8 (solid line) and the Chronic Drug-Post Testing-8 (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of ten to twelve mice per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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Summary of Experiments IX and X

The result of the first experiment in this section demonstrated that tolerance to the activity-increasing action of d-amphetamine does not develop when the drug is administered immediately following the activity test sessions for seven days. Since it was shown in Experiment V (see p.47) that daily testing without d-amphetamine induced the development of tolerance in seven days, it would have been expected that tolerance would have developed in the subjects of the present experiment since they were also tested daily for seven days. It therefore appears that the administration of d-amphetamine after rather than before the test sessions somehow antagonized the development of tolerance that would have occurred following seven days of activity testing.

The second experiment shows, however, that even when saline is administered immediately after the test sessions for seven days, tolerance does not develop. Possible explanations for these results will be examined in the Discussion and Conclusions section of this thesis. In addition, it was found that the post-test session injections of d-amphetamine affected the peak response of the subjects; however, it is not possible to determine the nature of this effect from the data.

TABLE IV

FIGURE	TWO-WAY ANALYSIS OF VARIANCE	T-TEST
12	Main effect of dose significant at $p < .005$; interaction between dose and treatment significant at $p < .01$.	Peak effects at 2.5 mg/kg (CDT-4) and 2.5 mg/kg (CDP-8).
13	Main effect of dose significant at $p < .01$; interaction between dose and treatment significant at $p < .05$.	Peak effects between 2.5 and 5.0 mg/kg (CSP-8) and at 2.5 mg/kg (CDP-8).

The development of tolerance in different species

Preliminary experiments had demonstrated that chronic administration of d-amphetamine affected the activity of rats differently than it affected the activity of mice. The experiments in this section attempt to examine the nature of these differences and their effects on the development of tolerance to the activity-enhancing action of d-amphetamine in the rat.

In all the dose-response curves presented in this section, the abscissas represent the dose of d-amphetamine and the ordinates represent activity counts. Each point on the graphs represents the mean thirty minute activity count of a group of five to six rats per dose of d-amphetamine.

Experiment XI

This experiment was conducted to determine whether tolerance to the activity-enhancing effect of d-amphetamine occurs in the rat when the drug is administered without testing for seven days.

One group of rats received daily injections of d-amphetamine for seven pre-test days (Chronic Drug-No Testing-8). A second group of rats served as controls and were injected with saline daily for seven pre-test days (Chronic Saline-No Testing-8). Dose-response curves representing the test day activities were determined for both groups of rats on day 8, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both

dose-response curves is represented by the same subjects.

Figure 14 shows that the dose-response curve of the Chronic Drug-No Testing-8 group of rats is shifted to the left of the dose-response curve of the Chronic Saline-No Testing-8 group of rats (see Table V); additional two-way analyses of variance computed for the curves on either side of the intersection showed significant treatment effects on both sides at $p < .05$, thus demonstrating that the curves are shifted with respect to one another. This result shows that tolerance not only fails to develop in the rat following seven days of d-amphetamine administration, but it appears that this treatment leads to an increase in sensitivity to the drug's effect on activity.

Experiment XII

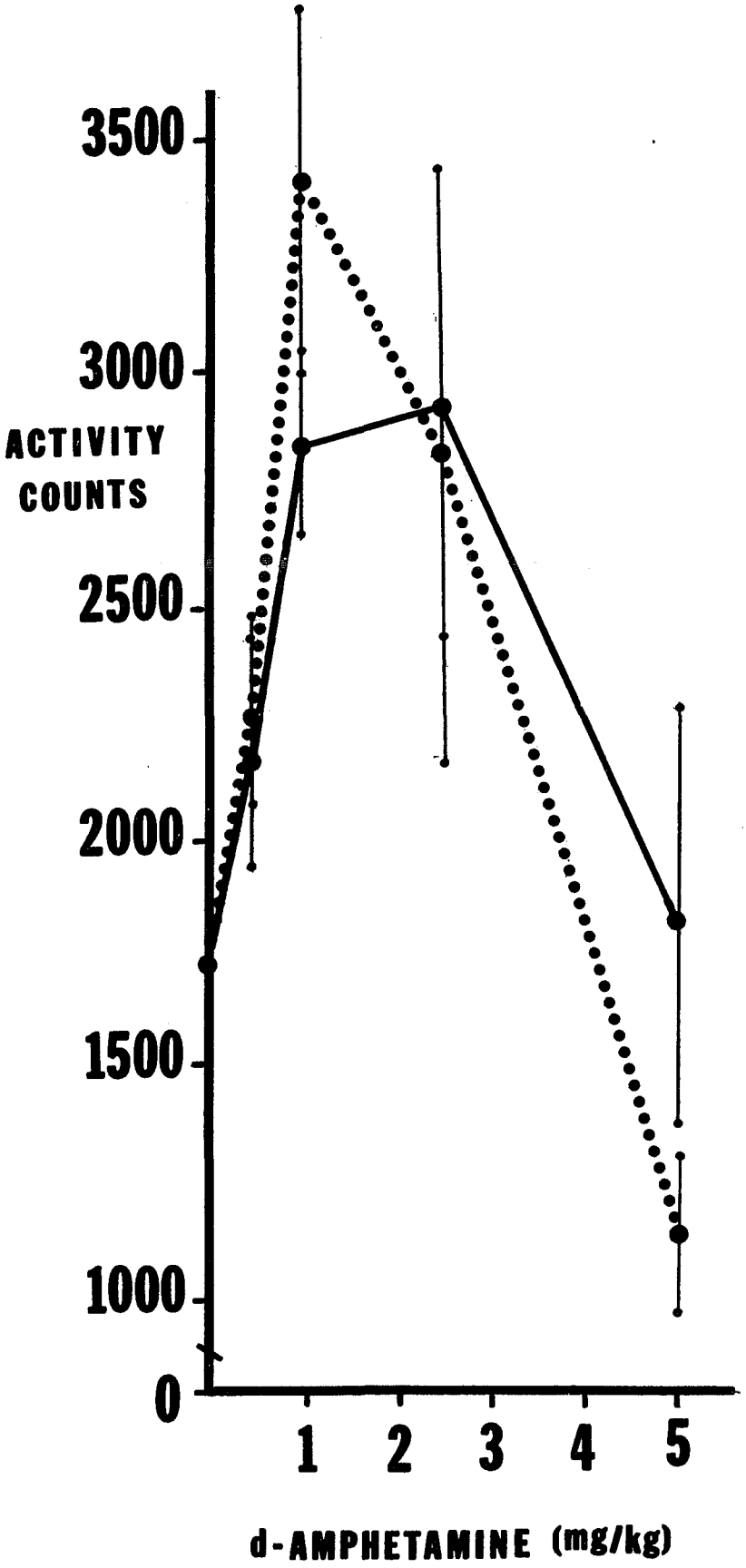
In Experiment XI it was demonstrated that tolerance failed to develop in seven days when chronic drug administration was the sole treatment variable. The present experiment attempts to determine whether rats tested with d-amphetamine for seven days show any evidence of tolerance development on day 8.

One group of rats was tested in the activity boxes daily with d-amphetamine for seven pre-test days (Chronic Drug-With Testing-8). The second group of rats was tested in the activity boxes daily with saline for seven pre-test days (Chronic Saline-With Testing-8) Dose-response curves representing the test day activities were determined for both

Figure 14: Dose-response curves representing the test day activities on day 8 of rats in the Chronic Saline-No Testing-8 (solid line) and the Chronic Drug-No Testing-8 (dotted line) groups.

Each point on the graphs represents the mean thirty minute activity of a group of five to six rats per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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groups on day 8, when all subjects were tested in the activity boxes with d-amphetamine. The zero drug dose in both dose-response curves is represented by the same subjects.

Figure 15 shows that the dose-response curve representing the Chronic Saline-With Testing-8 group of rats is shifted to the right of the dose-response curve representing the Chronic Drug-With Testing-8 group of rats (see Table V). This result shows that with or without testing, chronic drug administration appears to increase the rat's sensitivity to the effect of d-amphetamine on activity.

Summary of Experiments XI and XII

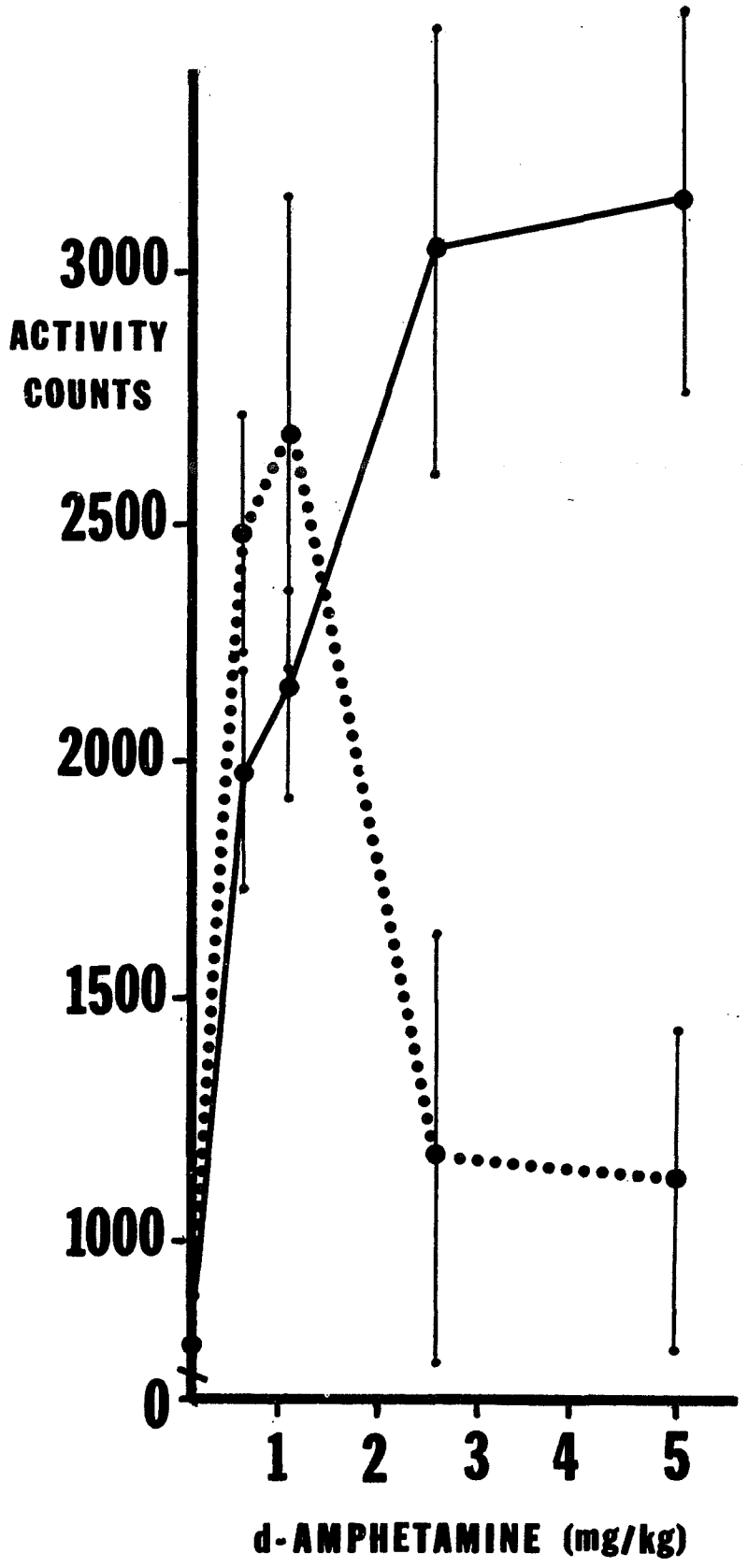
If tolerance is defined by a shifting of the dose-response curve to the right, then a shifting of the dose-response curve to the left indicates that an increased sensitivity to the drug has occurred. Experiment XI showed that rats which were administered d-amphetamine for seven days without activity testing became more sensitive to the drug's effects when tested with d-amphetamine on the test day. This result differs from that obtained with the analagous group of mice, in which chronic administration of d-amphetamine without concurrent activity testing caused the mice to become less sensitive or tolerant to the effects of the drug on the test day (see Experiment I, p.31).

This effect of heightened sensitivity to d-amphetamine in the rat following chronic drug administration was further demonstrated in Experiment XII, in which the dose-response

Figure 15: Dose-response curves representing the test day activities on day 8 of rats in the Chronic Saline-With Testing-8 (solid line) and the Chronic Drug-With Testing-8 (dotted line) groups.

Each point on the graph represents the mean thirty minute activity of a group of five to six rats per dose of d-amphetamine.

Vertical lines indicate the standard errors of the mean.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curve representing the test day activities of rats tested with d-amphetamine for seven days is shifted to the left of a control group. Although the data do not reveal whether the subjects which were tested with saline for seven days were tolerant on day 8, the comparison of the two groups indicates that rats tested with drug were more sensitive to the drug's effect on activity than were the saline-treated rats. This result also differs from that obtained with the corresponding group of mice, in which mice which were tested both with and without drug had developed the same degree of tolerance (see Experiment V, p.46). The possible mechanism of this apparent increase in sensitivity to d-amphetamine in the rat will be discussed in the Discussion and Conclusions section of this thesis.

TABLE V

FIGURE	TWO-WAY ANALYSIS OF VARIANCE	T-TEST
14	Main effect of dose significant at $p < .05$; interaction between dose and treatment significant at $p < .05$.	Peak effects between 1.0 and 2.5 mg/kg (CSN-8) and (CDN-8).
15	Main effect of dose significant at $p < .005$; interaction between dose and treatment significant at $p < .05$.	Peak effects at 1.0 mg/kg (CDT-8) and greater than 1.0 mg/kg (CST-8).

DISCUSSION AND CONCLUSIONS

The following discussion will attempt to correlate the present findings with those reported in the literature, and to arrive at conclusions about their possible significance. The following topics will be discussed: the nature of the locomotor response to amphetamine, tolerance (including classical theories of tolerance and behavioral tolerance), the temporal relationship between drug administration and activity testing, species differences and the metabolism of d-amphetamine with regard to tolerance development and finally, implications. The latter section attempts to extrapolate the results of the present study to those of human studies.

The locomotor response to amphetamine

The amphetamine dose-response curve for locomotor activity takes the shape of an inverted-U; this has been demonstrated in mice (Davis *et al.*, 1974; Glick and Milloy, 1973; Rethy *et al.*, 1971; Smith, 1963, 1965; Stromberg and Svensson, 1975) and in rats (Adler, 1961; Lu *et al.*, 1972; Taylor and Snyder, 1971). The inverted-U function is not specific to the activity-enhancing effect of amphetamine, and has been shown to occur with amphetamine-induced

effects on operant behavior (DeOliveira and Graeff, 1972; Kelleher and Morse, 1968). It is also not restricted to amphetamine; for example, the inverted-U dose-response curve occurs with eating behavior and locomotor activity in rats following the administration of delta-9-THC (Glick and Milloy, 1972a, 1972b), with phenobarbital-induced locomotor activity in rats (Crowley, 1972), with apomorphine-induced locomotor activity in mice (Thornburg and Moore, 1975) and with operant behavior in monkeys following the administration of either morphine or cocaine (Woods and Schuster, 1968).

It is difficult to interpret the exact nature of the inverted-U function with respect to the amphetamine effect on locomotor activity. With low doses of amphetamine, locomotor activity is enhanced; higher doses of amphetamine produce stereotypy and a subsequent decrease in activity. Kelleher and Morse (1968) have stated that the depressant effects of a drug must be interpreted cautiously, since a large enough dose of any drug will abolish responding. One of the problems at hand, then, is to determine whether the decreased activity (and/or stereotypy which accompanies it) following the administration of large doses of amphetamine is a different behavioral manifestation of the mechanism responsible for the enhanced activity elicited by lower doses of amphetamine, or whether it is a completely different phenomenon altogether. In other words, is the inverted-U function a continuous graded curve representing a single action of amphetamine or is it a summation of two discrete curves

representing opposing actions of the drug?

An important factor to be taken into account here is that the shape of the amphetamine inverted-U curve and its position on the ordinate is determined by the predrug baseline rate of activity. At the same doses, amphetamine will increase the responding of animals with normally low baseline rates and depress the responding of animals with normally higher baseline rates (Clark and Steele, 1966). These "rate-dependent" effects have been shown to occur with different species of animals in various testing situations (Kelleher and Morse, 1968). That locomotor activity is also subject to rate-dependent effects was demonstrated by Glick and Milloy (1973). Mice were habituated to activity boxes for a week and on day 8, received single doses of d-amphetamine which covered the entire dose-response range. The results showed that at the doses near the peak of the dose-response curve, d-amphetamine depressed the activities of the most active mice and enhanced the activities of the least active mice.

Although the data presented in this thesis cannot further elucidate the nature of the amphetamine dose-response curve, evidence from the rate-dependence studies has shown that the shifting of the inverted-U curve depends on the baseline rate of activity. This suggests that the inverted-U curve represents a single action of amphetamine rather than a summation of two opposing actions. In addition, if two mechanisms were assumed to be responsible for the

inverted-U nature of the amphetamine dose-response curve, then each of the contributing curves would have to undergo a similar shift, which seems unlikely.

Tolerance

As discussed in the Introduction, the development of tolerance to the activity-enhancing effect of amphetamine has been widely disputed. This thesis has attempted to resolve the question of whether or not such a tolerance can occur by examining dose-response curves covering the entire range of doses for this particular behavior. The activities of the subjects were examined and compared after acute and chronic treatment. Tolerance was assumed to have occurred if a dose-response curve was shifted to the right.

Pharmacologists have traditionally defined tolerance as the diminished response elicited by a drug following repeated administration. However, tolerance may be more broadly defined in terms of a shifting of the dose-response curve to the right, regardless of what shifts the curve. Inherent in such a definition is the assumption that all variables which shift the dose-response curve to the right may indicate possible mechanisms of tolerance development. For example, in the presence of a competitive antagonist, the dose-response curve for an agonist drug is shifted to the right without a change in the slope or the maximum response. The competitive antagonist acts by altering the effective affinity of the agonist drug for the receptor

(Goldstein et al., 1974). Since more of the agonist drug is required to overcome the block and reinstate the original response, competitive antagonism could be considered as a form of tolerance development, within the above definition.

The present experiments have shown that behavioral variables can also shift the dose-response curve to the right. Following seven daily injections of d-amphetamine, tolerance to the activity-increasing action of the drug occurred in mice when they were tested with d-amphetamine on day 8. Similarly, mice which received seven daily test sessions without d-amphetamine showed evidence of tolerance development (dose-response curve shifted to the right) when tested with the drug on day 8.

In addition to the present work there is other evidence that behavioral variables can significantly affect an organism's sensitivity to the effects of a psychoactive drug. Rats tested in activity boxes prior to receiving delta-9-THC showed facilitation of tolerance when tested with the drug (Glick and Milloy, 1972b). It has been reported that rats which had had experience with a Y-maze were less affected by d-amphetamine when tested in the Y-maze than were inexperienced rats (Steinberg et al., 1961). The response of rats to an amphetamine-barbiturate mixture in a Y-maze was abolished when the subjects had been exposed to the apparatus prior to receiving the drug mixture (Porsott et al., 1970). The results of Rushton et al. (1963) indicated that even a single brief exposure to an unfamiliar environment

can markedly affect subsequent reactions to centrally-acting drugs. It appears from these findings that pre-drug experience in the testing situation can act to desensitize the animal to the effects of a drug, but this contingency has not previously been considered to contribute to the development of tolerance (Porsott et al., 1970).

The shift to the right of the dose-response curve for a given drug action by a behavioral manipulation can be considered to be behaviorally-induced tolerance. Although the mechanism for such an effect might be different from that for the development of tolerance following chronic drug administration, the fact that behavioral variables can change brain amine levels and interact with acute drug effects suggests that behavioral and drug-induced tolerance might result from similar mechanisms. Moore (1963) found that d-amphetamine caused a greater depletion of norepinephrine in aggregated mice than in isolated mice, suggesting that the behavioral interaction of the mice potentiated the norepinephrine-depleting effect of d-amphetamine. That behavior per se can induce a drug-like change in brain chemistry has been shown by Lewy and Seiden (1972), who reported that operant behavior increased the metabolism of norepinephrine in the rat brain. Hurwitz et al. (1971) found that disulfiram further decreased norepinephrine levels in rats performing a behavioral task than it did in trained, nonperforming rats and in untrained control rats. Glick et al. (1973) demonstrated that two days of food deprivation produced a

significant depletion of hypothalamic norepinephrine and interacted with the neurochemical effects of d-amphetamine.

Classical theories of tolerance

Most of the classical theories of tolerance have been formulated for morphine-like drugs or other central nervous system depressants; the theoretical models which attempt to account for the development of tolerance to depressant drugs presuppose the subsequent development of physical dependence, although the mechanisms are not necessarily identical. At present, the existence of physical dependence can be ascertained only by the abstinence syndrome which manifests itself upon withdrawal of the drug. In the case of drugs which act by depressing the function of the central nervous system, the withdrawal signs appear as a rebound hyperexcitability. It has been generally assumed that physical dependence occurs only with the central depressant drugs (Seevers and Deneau, 1963); however, if physical dependence could develop following the chronic administration of a central stimulant drug, the abstinence syndrome would be expected to manifest itself as a rebound depression of function. There is evidence to demonstrate the presence of withdrawal symptoms following the chronic administration of amphetamine. Oswald and Thacore (1963) found that after the withdrawal of amphetamine from amphetamine addicts, the amount of time spent in REM (rapid-eye-movement) sleep decreased, and increased when the drug was reintroduced. The addicts were described as "listless"

and "sleepy" without the drug (Oswald and Thacore, 1963). Thus it appears very likely that physical dependence can develop to amphetamine, and this will be assumed when existing theories of tolerance and physical dependence are modified to account for the effects elicited by amphetamine.

All the models of tolerance and physical dependence to be discussed are variants of the homeostatic theory, in which tolerance has been viewed as a compensatory reaction on the part of the organism to reestablish the pre-drug state. An attempt will be made where possible to fit the results of the present experiments to these models.

Collier (1965, 1966) proposed a theory to account for the development of tolerance and physical dependence which is based on certain assumptions about drug-receptor interactions. These assumptions are as follows:

a) Two kinds of receptors, termed "active" and "silent" exist. Interaction of a drug with an "active" receptor yields a pharmacological response, while interaction of a drug with a "silent" receptor fails to produce a pharmacological response.

b) The intensity of the pharmacological response varies directly with the number of active receptors available for combination with the drug molecules. However, the intensity of the pharmacological response varies inversely with the number of silent receptors since these act by combining with drug molecules

which would otherwise interact with the active receptors.

c) The administration of a drug can change the number of either kind of receptor, either for the drug or for an endogenous chemical with which the drug interacts.

d) The action of a drug may consist of depriving a cell of an endogenous chemical, either by occupying a receptor for that chemical or by limiting the amount of that chemical produced or released. A drug may also produce an excess of an endogenous substance, either by releasing it from storage or by inhibiting its destruction.

e) In the presence of an excess or deficiency of a chemical substance, the biological system is distorted, and the number of receptors of either type for that substance changes in a direction to decrease the distortion.

According to this model, tolerance could arise either by a drug-induced decrease in the number of active receptors or by a drug-induced increase in the number of silent receptors. In the former situation, the number of active receptors could be decreased by chemical inactivation or destruction of the molecule on which these receptors are located. Chronic administration of drug would thus result in a decreased pharmacological response, i.e. tolerance, since fewer active receptors would be available to interact with the

drug molecules. However, in this case, an increase in the dose of drug (increased number of drug molecules) would not reinstate the original response if the total number of active receptors was decreased. Since the definition of tolerance states that an increase in the dose of drug with repeated administration produces an effect originally elicited by a smaller dose of drug, decreasing the number of active receptors cannot be accepted as a working model to account for the development of tolerance, in those cases where the maximal response in the tolerant animal is similar to that in the non-tolerant animal.

If tolerance were to arise by an increase in the number of silent receptors, then these receptors would act by taking up part of an original dose of drug without giving a pharmacological response. This would result in tolerance since the same dose of drug would produce a lesser response with time. An increased dose of drug would reinstate the original response since the number of active receptors is unchanged; a subsequent rise in the number of silent receptors would compensate for the greater amount of drug. Collier (1966) does not limit the site of silent receptors to the active site for the drug. He thus considers the induction of metabolizing enzymes by barbiturates and the resulting dispositional tolerance to be an example of silent receptor increase.

Physical dependence is proposed to occur in either of three ways:

a) Chronic administration of a drug which acts by decreasing the concentration of an excitatory transmitter results in an increase in the number of active receptors for the transmitter. Following withdrawal of the drug, the supply of transmitter increases at a faster rate than the extra receptors are removed. This leads to abstinence symptoms of hyperexcitability.

b) Chronic administration of a drug which acts by increasing the concentration of an inhibitory transmitter results in a decrease in the number of active receptors for that transmitter. Following withdrawal of the drug, the amount of inhibitory transmitter decreases at a faster rate than the supply of receptors can increase.

c) The drug acts by occupying the receptor for an excitatory transmitter, causing the active receptors for that transmitter to increase. Following withdrawal of the drug, the transmitter receptors are vacated at a faster rate than the surplus receptors are removed, leading to abstinence symptoms of hyperexcitability.

Each of these three models necessitates that the drug be of the central depressant type, since in each case the abstinence symptoms are manifested by hyperexcitability.

In order to adapt Collier's model of tolerance and physical dependence to account for the actions of amphetamine,

it would have to be assumed that chronic administration of amphetamine would lead to an increase in the number of silent receptors. Since amphetamine acts by increasing the concentration of catecholamines at the receptor, a greater number of silent receptors would be required to take up the excess catecholamines and subsequently decrease the response. The occurrence of physical dependence to amphetamine, on the other hand, would be explained by noting that following withdrawal of the drug, the silent receptors would combine with pre-drug levels of catecholamines, thus preventing the normal pre-drug response and leading to abstinence symptoms manifested by decreased excitability.

The results of the present experiments could be interpreted within the framework of Collier's model of tolerance. It was shown that in mice, tolerance to the activity-increasing effect of d-amphetamine followed a definite time course of development, requiring between four to seven days to manifest itself, regardless of dosage. It could be assumed that the development of tolerance reflected the time involved in the formation of the silent receptors. The finding that behavior, i.e. pre-drug experience in the testing apparatus, produced tolerance to the activity-increasing effect of d-amphetamine in mice could also be interpreted within the framework of Collier's hypothesis, if it is assumed that both behavior and psychoactive drugs affect central mechanisms in similar ways (see p.88). A behavior which would increase the amount of catecholamines at the receptor might also induce

a compensatory increase in the number of silent receptors. The four to seven days required for the development of this behaviorally-induced tolerance to d-amphetamine in mice may reflect the time necessary for the formation of these silent receptors.

Martin (1968) developed a homeostatic theory of redundancy to account for the occurrence of tolerance and dependence to morphine-like drugs. Although this model was formulated at the neuronal level, it could be adapted for the receptor, enzyme, tissue or system level. The basic assumptions underlying the redundancy theory are:

a) There exist two or more neuronal pathways for mediating a physiological function.
b) Each of these pathways differs in its susceptibility to the actions of a particular drug.

In order to exert its characteristic effect, the drug acts by interrupting one of the redundant pathways without affecting the other. A negative feedback loop sensitive to the decrease in output of the total pathway activates the unused pathway and causes it to hypertrophy. The hypertrophied pathway then completely or partially assumes the function mediated by the previously used pathway. The reestablishment of function thus leads to the occurrence of tolerance, which develops as a result of hypertrophy of the redundant pathway rather than as a result of a decreased effect on the first pathway. Following withdrawal of the drug, the primary

pathway returns to its original state of excitability; coupled with the increased functioning of the normally redundant pathway, this leads to an exaggerated response which is manifested as withdrawal hyperexcitability. Evidence for the ability of neural pathways to hypertrophy under decreased stimulation is scant, though it has been shown that chronic stimulation of the adrenal medulla in morphine-dependent animals results in increased catecholamine levels (Gunne, 1963; Maynert and Klingman, 1962). This result was interpreted as a hypertrophic response of this tissue.

To account for the development of tolerance and dependence to drugs such as amphetamine, the redundancy theory could be modified to assume that 1) the primary neural pathway is excitatory, and that the drug acts to further increase its excitability, and 2), an alternate inhibitory pathway exists which is not used under normal circumstances. Following activation of the primary pathway by the drug, a negative feedback mechanism sensitive to the level of excitation would then activate the inhibitory pathway, resulting in a decrease of excitation. More of the drug would then be necessary to elicit excitation and tolerance would result. Withdrawal of the drug would leave the inhibitory pathway functioning at a supranormal level, while the primary excitatory pathway would return to its pre-drug level of excitation. This would lead to a depression of function, which would in turn lead to the expected withdrawal response following dependence to a stimulant drug.

In terms of the present results, the time course for the development of tolerance (four to seven days in the mouse) to the activity-enhancing effect of d-amphetamine could be interpreted as the amount of time necessary for the redundant inhibitory pathway to become activated. However, if this were the case, it is difficult to explain the failure of tolerance to develop in four days when d-amphetamine was administered twice a day. It would be expected that the redundant pathway would become activated at a faster rate in order to compensate for the increased amount of drug. The four to seven days required for the behaviorally-induced tolerance to the activity-increasing effect of d-amphetamine to develop in the mouse could also be viewed as the amount of time necessary for the behavior to effect a change in the total level of activation and subsequent hypertrophy of the redundant inhibitory pathway.

In contrast to the redundancy theory of Martin (1968), Jaffe and Sharpless (1968) have argued that tolerance and physical dependence result from a disuse rather than hypertrophy of nervous function. The disuse supersensitivity model postulates that tolerance and dependence result from a normal compensatory reaction to an altered pattern of nervous activity which occurs in the presence of a central depressant drug. The latent hyperexcitability which is manifested when the drug is withdrawn is viewed as a result of the disuse or depression of the nervous pathways rather than to a depressive effect by the drug on the neurons <u>per se</u>. Disuse

supersensitivity can also account for the phenomenon of cross-dependence among depressant drugs such as that between ethanol and barbiturates. Drugs which act at different sites or which occupy different receptors could produce similar withdrawal symptoms by decreasing the flow of nervous impulses along the same nervous pathway.

Pharmacological denervation supersensitivity was demonstrated in peripheral effector organs by Emmelin (1961), who decreased the cholinergic input of the salivary gland of the cat by the chronic use of ganglionic blocking drugs. He found that the sensitivity of the gland gradually increased to both acetylcholine and norepinephrine and to its response to stimulation of the intact nerve which remained. Emmelin concluded that an effector deprived for some time of a transmitter acquires a supersensitivity to stimuli.

Further experiments (Trendelenburg, 1963; Trendelenburg et al., 1962) differentiated between disuse and denervation supersensitivity in peripheral adrenergic junctions. The latter type of supersensitivity involves the impairment of uptake of the transmitter in the nerve ending and the former is produced by the disuse of the transmission apparatus per se.

Jaffe and Sharpless (1965) measured the threshold for pentylenetetrazol-induced seizures in cats after varying periods of deep barbiturate intoxication. Eighteen to twenty-two hours following the last dose of barbiturates, the seizure threshold decreased. This result was interpreted as a

manifestation of withdrawal. These authors suggested that physical dependence may be a disuse phenomenon in the central nervous system analogous to the pharmacological denervation supersensitivity in the peripheral effector organs, since both followed a similar time course of development.

Friedman and Jaffe (1969) demonstrated that cholinergic neurons participate in the central control of body temperature in the mouse; administration of pilocarpine produces a hypothermia which can be antagonized by the administration of scopolamine. Friedman et al. (1969) then showed that pilocarpine elicited an exaggerated hypothermic response in mice following the withdrawal of chronically administered scopolamine, and attributed this effect to the development of supersensitivity. Additionally, they found that the supersensitive mice were also tolerant to scopolamine since larger doses were required to antagonize the pilocarpine-induced hypothermia. Tolerance and disuse supersensitivity developed and decayed at the same rate in this experiment, and tolerance to scopolamine was observed only when supersensitivity to pilocarpine was present. However, these investigators did not eliminate the possibility that tolerance could develop in the absence of supersensitivity.

Although the development of supersensitivity usually peaks between two to three weeks (Emmelin, 1961; Friedman et al., 1969; Jaffe and Sharpless, 1968), the time course for the development of supersensitivity does not have to necessarily develop at the same rate in all neural junctions.

Ungerstedt (1971) has reported data suggesting that dopamine receptors in the striatum become supersensitive to apomorphine in two to three days following destruction of the nigro-striatal pathway by 6-hydroxydopamine.

Disuse supersensitivity has been employed mainly to explain the occurrence of tolerance and physical dependence to drugs which depress the activity of the central nervous system. If one postulates that the supersensitivity develops at the synapse where the drug is acting, it is difficult to adapt this theory to accomodate drugs like amphetamine unless amphetamine activation actually represents disinhibition (Stein, 1964b). The development of supersensitivity by pharmacological means necessitates a decrease in the concentration of transmitter at the receptor, which subsequently becomes supersensitive to a lesser amount of transmitter. Tolerance develops since a greater amount of drug is needed to further inhibit the release of transmitter and to decrease its concentration at the receptor. Amphetamine, on the other hand, acts to increase the concentration of transmitter at the receptor, and tolerance could develop only in the presence of receptor subsensitivity. Evidence for receptor subsensitivity was demonstrated by Overstreet et al. (1974). These investigators found that following the development of tolerance to DFP, an irreversible anticholinesterase agent, rats performing an operant task were less sensitive to bilateral hippocampal administration of carbachol. The mechanism of tolerance development to DFP was attributed to a

decreased sensitivity to acetylcholine of receptors in the dorsal hippocampus.

Goldstein and Goldstein (1968) proposed an enzyme expansion theory to account for the development of tolerance and physical dependence to drugs which depress the activity of the central nervous system. These authors postulate that tolerance and dependence result from a homeostatic adjustment of the regulatory mechanisms which control steady-state enzyme levels in the central nervous system. This theory is based on the following assumptions:

a) The synthesis or release of an excitatory neurotransmitter (M) is mediated by an enzyme or carrier protein (E). The steady-state level of M is regulated by its rate of synthesis, i.e. the activity of E, and the rate at which it is removed from its site of action.
b) The steady-state level of the protein E is governed by its rates of synthesis and degradation; thus the amount of E can be increased by increasing the rate of synthesis or by decreasing the rate of degradation.
c) The protein E can be inhibited by a depressant drug.
d) The level of E is regulated by the endproduct inhibition of M, the excitatory transmitter.

The immediate effect of the drug following administration is to inhibit the protein E, thereby indirectly

decreasing the amount of the excitatory transmitter M at the receptor. Due to endproduct inhibition, a smaller quantity of M would lead to an increase in the synthesis of E, and a subsequent rise in the level of M. This would lead to the development of tolerance since more drug is needed to inhibit E. Withdrawal of the drug disinhibits E so that an excessive amount of M is produced resulting in hyperexcitability. The system returns to normal as the excess amount of M represses E and the pre-drug steady-state condition prevails.

The enzyme expansion theory as formulated, can only account for the development of tolerance and physical dependence produced by drugs which depress the central nervous system by inhibition of an excitatory transmitter. However, this model could be modified to explain the development of tolerance to drugs which stimulate the central nervous system in either of two ways: 1) The drug acts to increase the concentration of the protein E rather than to inhibit it. Following administration of the drug, the amount of E (and consequently the amount of M) increases with a resulting hyperexcitability. Endproduct inhibition then acts to decrease the amount of E and tolerance develops following the subsequent decrease in the amount of M. Withdrawal of the drug would result in a decrease in the levels of both E and M and would be manifested by a depression of nervous activity. 2) If the drug acts to inhibit an inhibitory transmitter, then administration of the drug would first elicit excitation by decreasing the amount of the inhibitory transmitter via the

inhibition of E. Tolerance would occur when the endproduct inhibition would increase the synthesis of E and the amount of inhibited transmitter at the receptor. Withdrawal would be expected to take the form of increased inhibition due to the excess concentration of M at the receptor. Since amphetamine produces its stimulant effects by increasing the concentration of transmitter at the receptor, only the first hypothesis presented could be used to account for the development of tolerance to this drug.

The results of the present experiments do not appear to substantiate this concept of enzyme expansion. The administration of d-amphetamine to mice once daily for seven days resulted in the development of tolerance to its activity-enhancing effects, whereas the administration of d-amphetamine twice daily for four days failed to produce tolerance. It would be expected that the postulated protein E would be even more sensitive to the effect of a larger dose of drug and tolerance would have developed to compensate for this effect. Although behavior has been shown to directly affect catecholamine levels in the central nervous system (see p.88), there is no evidence to warrant the assumption that behavior could indirectly alter catecholamine levels by an effect on a regulatory protein.

Behavioral tolerance

Schuster et al. (1966) proposed a theory of behavioral tolerance which states that tolerance will develop only to

those effects of a drug which will result in a loss of reward to the organism. In the experiments on which this theory was based, these investigators tested rats using only a single dose of d-amphetamine (1 mg/kg). The present study has shown that a single dose near the peak of a nonmonotonic dose-response curve cannot be employed to assess the development of tolerance. Kelleher and Morse (1968) have also noted in their review paper that a range of doses must be investigated before a behavioral action of a drug can be characterized. A similar opinion has also been stated by Kalant et al. (1971). Tolerance to the stimulant effects of d-amphetamine has also been shown to occur in this thesis, suggesting that behavioral tolerance is not limited to the disruptive effects of amphetamine as implied by Schuster et al. (1966).

Collier (1968) has attempted to account for psychic (behavioral) tolerance and dependence by applying the model of disuse supersensitivity to the reward and punishment centers in the central nervous system. The reward system in the median forebrain bundle and the punishment center in the periventricular fibers are thought to be integrated by cross-inhibition (Olds, 1962). Due to this cross-inhibition, activation of the reward system would produce an effect comparable to that obtained by inhibiting the punishment system. Such an effect would result in the reduction of drives and to the reinforcement of those behaviors leading to these effects (i.e. drug-taking behavior). Psychic dependence would thus result from a tipping of the balance between the

reward and punishment systems towards the reward system through drug administration.

According to Collier, depressant drugs would produce their characteristic effects on the central nervous system by blocking activation of the punishment system. Experiments demonstrating that meprobamate and barbiturates decrease punishment discrimination in trained rats support this concept (Geller and Seifter, 1960, 1962). Following blockade of the punishment center, post-synaptic supersensitivity would develop, necessitating an increased amount of drug to maintain the blockade. Thus a psychic or behavioral tolerance would develop. Withdrawal of the drug would lead to a rebound effect due to an intensive activation of the punishment center.

Central stimulant drugs, such as amphetamine and cocaine, would elicit their characteristic effects by blocking inhibition of the reward system. The demonstration that d-amphetamine decreases the threshold for excitation of the reward system (Stein, 1964b) is consistent with this theory. With continued drug blockade, disuse supersensitivity might be expected to develop. Such a supersensitivity would lead to tolerance during drug administration. Following withdrawal of the drug, excessive inhibition of the reward system would occur producing a rebound effect manifested by depression.

Kalant et al. (1971) suggested that the term "behaviorally-augmented tolerance" should be used in place of

"behavioral tolerance" and proposed that physiological (pharmacological) tolerance and psychological (behavioral) tolerance are functions of the same mechanism, differing only in rate of development. The results of the present study lend support to the concept of behaviorally-augmented tolerance since it has been demonstrated in this thesis that pharmacological and behavioral factors both shifted the amphetamine dose-response curve to the right, but showed no summation of tolerance development when the treatments were combined. The evidence previously presented (see p.88), that behavior per se can affect brain catecholamines, strengthens the idea that pharmacological and behavioral tolerance are functions of the same central mechanism.

The temporal relationship between drug administration and activity testing

The present study has demonstrated that tolerance to the activity-increasing effect of d-amphetamine failed to occur in mice which had received d-amphetamine injections immediately after each of seven daily activity test sessions. It was also shown that the test day activities of mice which had received either saline or d-amphetamine injections immediately following each of seven daily activity test sessions were nonsignificantly different from one another.

Since tolerance to various effects of amphetamine appears to occur only when the drug is administered before the behavioral test session, the results described in the

present study appear to raise a paradox: if mice which had been tested daily without d-amphetamine for seven days became tolerant to its activity-increasing effect when tested with the drug on day 8, why did tolerance fail to develop in mice which received d-amphetamine immediately after each of seven daily activity test sessions? Both groups of subjects had been exposed to the testing situation for the same period of time, and it would seem logical to expect that the mice which had received d-amphetamine after testing on the pretest days would have benefitted from the experience in the testing apparatus by becoming less sensitive to d-amphetamine on the test day.

That the drug and behavior must be experienced simultaneously in order for tolerance to develop was suggested by Porsott et al. (1970), on the basis of experiments testing the behavior of rats in a Y-maze. This concept has been supported by the results of Carlton and Wolgin (1971), in their study of tolerance to the anorexigenic effect of d-amphetamine. They showed that only rats which had received d-amphetamine injections before the daily feeding period became tolerant to its anorexigenic effect. Tolerance did not develop in a second group of rats which had received d-amphetamine after the feeding period for an equal amount of time. When the schedules of both groups were reversed, the second group of rats also became tolerant to d-amphetamine. Carlton and Wolgin (1971) termed this a "contingent" tolerance since the development of tolerance appeared to be contingent on

the temporal relationship between drug administration and behavioral testing. Similar results were demonstrated by Campbell and Seiden (1973), who reported that rats became tolerant to the disruptive effects of d-amphetamine in a differential reinforcement of low rate (DRL) task only when the drug was administered before the behavioral test session.

There are two possible explanations for the apparently contradictory results of the present experiments. Although both groups of mice had received an equal amount of pre-test day experience in the testing apparatus, the external variables in the previously described experiments were not the same. In the first experiment, mice were injected daily before the test sessions on each of seven days. In the second experiment, mice were injected daily immediately after the test sessions on each of seven days. On the respective test days, both groups of mice were injected before the test session. The former group of mice, having received injections before the test sessions on the pre-test days, had habituated themselves to the injection by the test day. However, the latter group of mice received the injection before the test session for the first time on the test day. It would appear that in the latter group of mice, the disruptive effect of the injection acted to enhance the sensitivity of the subjects to d-amphetamine. Sparber et al. (1973) have shown that the injection is part of an environmental contingency which can disrupt the effect of d-amphetamine on operant behavior.

A second explanation for the apparent paradox is that

the state of the central nervous system would be expected to differ before and after testing, since evidence has been previously presented to demonstrate that behavior per se affects brain catecholamines (see p. 88). Considered in this context, it would not be unusual to expect that the administration of the drug before and after testing would produce dissimilar results with respect to the development of tolerance.

Species differences

A comparison of the experimental results obtained with mice and rats in this thesis shows that each of these species reacted differently to the chronic administration of d-amphetamine; mice became more tolerant to the activity-enhancing effect of the drug while rats appeared to become more sensitive to this effect.

Tilson and Rech (1973b) reported that rats tested in activity boxes thirty minutes after injections of stimulating doses of d-amphetamine showed an enhanced response to the same dose of drug following repeated administration. These investigators suggested that the failure to observe tolerance to the activity-increasing effect of d-amphetamine in their experiment was due to conditioned activity effects. Previous reports have indicated that chronic administration of central nervous stimulants such as amphetamine can result in conditioned motor activity (Pickens and Crowder, 1967; Pickens and Dougherty, 1971). According to the classical conditioning paradigm, the pharmacological effect of the

drug can be considered the unconditioned stimulus while initially neutral stimuli associated with administration of the drug serve as conditioned stimuli (Pickens and Dougherty, 1971). In the activity experiments of Tilson and Rech (1973b) and in the present investigation, the activity-increasing action of d-amphetamine would be considered the unconditioned stimulus while the placement of the subjects in the activity boxes following the drug injection would be the conditioned stimulus. If conditioned activity occurred, then repeated pairing of the drug injection and placement of rats in activity boxes would eventually result in the elicitation of hyperactivity by the latter alone.

However, conditioned activity can be eliminated as a possible explanation for the increased sensitivity observed with the rats of the present investigation. In their conditioned activity experiments, Tilson and Rech (1973b) failed to include a group of rats which were administered d-amphetamine daily without activity testing. Such an experimental group was included in the present investigation, and showed that rats given d-amphetamine daily are also more sensitive to the effects of the drug on the test day.

Additional evidence against the argument that conditioned activity effects precluded the development of tolerance is offered by a negative report of Segal and Mandell (1974), in which they showed that a saline injection on the day following long-term amphetamine administration produced predrug levels of activity. It was also noted in this report

that Tilson and Rech (1973b) exposed rats to the activity boxes exclusively during the peak effect of the drug, thus rendering the subjects more susceptible to the effect of conditioning. Since conditioning is optimal after the administration of peak doses of drug, the intensity of the reinforcing stimulus would thus be at a maximum (Pickens and Dougherty, 1971).

That tolerance can occur to the activity-enhancing effect of amphetamine in rats has been demonstrated by Bell et al. (1974). These investigators showed that tolerance to the hyperactivating effect of methamphetamine in rats occurs in two phases. In the initial phase, which lasted for a week, tolerance development was masked by a progressive daily increase in activity such as that reported by Tilson and Rech (1973b), Segal and Mandell (1974) and the present study. In the second phase, which lasted from two to six weeks, tolerance was manifested by a decrease in activity when methamphetamine was administered once daily. Bell et al. (1974) have suggested that this cumulative effect observed in the initial phase of tolerance development might be due to a metabolic factor since the half-life of amphetamine is known to be short (see p. 17). It should be noted that in the rat, methamphetamine is partially demethylated to d-amphetamine, and subsequently metabolized as such (Caldwell et al., 1972).

Since tolerance to amphetamine has been shown to occur in the rat (Bell et al., 1974; Herman et al., 1971), then perhaps the reason for the initial increase in sensitivity

of rats to d-amphetamine in the present experiments may become more evident by examining the metabolism of d-amphetamine in the rat.

Metabolism of d-amphetamine and its relationship to tolerance development

There is much evidence to suggest that the different reaction of the mouse and rat to the activity-increasing effect of d-amphetamine following repeated administration is due to the different metabolic fates of the drug in the two species.

In the mouse, d-amphetamine is metabolized primarily via oxidative deamination. Following its conversion to phenylacetone, d-amphetamine is then oxidized to benzoic acid and excreted as hippuric acid (Benakis and Thomasset, 1970). Approximately one third of the administered dose of d-amphetamine in mice is excreted unchanged, and another third is excreted as benzoic acid and conjugates. Approximately fourteen percent of the administered dose of d-amphetamine is eliminated as p-hydroxyamphetamine, indicating that although p-hydroxylation of the aromatic nucleus contributes to the metabolism of d-amphetamine in this species, it is only a minor pathway of metabolism (Dring et al., 1970).

A heat-labile factor (Smith and Dring, 1970) in the microsomes of the rat liver inhibits the activity of the amphetamine deaminating enzyme in this species, with the result that the rat deaminates insignificant amounts of

d-amphetamine (Axelrod, 1955).

The major pathway of metabolism of d-amphetamine in the rat is para-hydroxylation of the aromatic nucleus. The resulting p-hydroxyamphetamine is then a substrate for dopamine beta-hydroxylase, which converts it to p-hydroxynorephedrine. Approximately sixty percent of the administered dose of d-amphetamine is excreted as p-hydroxyamphetamine conjugates, while thirteen percent is excreted unchanged and the remaining metabolites, p-hydroxynorephedrine and norephedrine, are excreted in minute quantities (Dring et al., 1970).

The differences in the amounts of d-amphetamine excreted unchanged by the mouse and the rat cannot be accounted for by interspecies variations in urinary pH (Smith and Dring, 1970).

It has been shown in rats that depletion of norepinephrine stores in the brain and heart by a single large dose or by successive smaller doses of d-amphetamine lasts for more than forty hours, a period of time which greatly exceeds the half-life of d-amphetamine in the body (Brodie et al., 1970). This finding has been discussed in the Introduction (see p.13) in the context of a false transmitter hypothesis that attempts to account for the occurrence of tolerance to d-amphetamine in the rat. This theory proposed that a metabolite of d-amphetamine, p-hydroxynorephedrine, accumulated in the sympathetic nerve endings of the rat, and was released by the action of d-amphetamine. However, if an active metabolite of d-amphetamine were responsible for its

central actions, then, as has been shown by the present study, d-amphetamine would increase rather than decrease the organism's sensitivity to the drug.

There is ample evidence to support the concept that active metabolites of d-amphetamine affect brain catecholamines. Taylor and Sulser (1973) have found that p-hydroxyamphetamine produced a marked, sustained depletion of norepinephrine in the rat brain. The degree of depletion paralled the brain levels of the drug. In addition, both p-hydroxyamphetamine and p-hydroxynorephedrine blocked the reuptake of radioactive norepinephrine in the brain. Taylor and Sulser (1973) also reported that d-amphetamine, p-hydroxyamphetamine and p-hydroxynorephedrine all released norepinephrine from the brain but only the metabolites significantly decreased the brain levels of norepinephrine. Breese et al. (1970) reported that beta-hydroxylation of d-amphetamine was necessary for the drug to produce its norepinephrine-depleting effects, since intracisternal injection of d-amphetamine had no effect on norepinephrine depletion. Clay et al. (1971) suggested that the metabolite in question was norephedrine, since no evidence for depletion of norepinephrine by p-hydroxynorephedrine was found. The finding that small amounts of norephedrine in the urine of rats appears following the administration of d-amphetamine supports this idea (Dring et al., 1970).

There is also indirect evidence demonstrating persistent effects of d-amphetamine on behavior. Magos (1969) found

that rats administered two injections of d-amphetamine six weeks apart showed more stereotypy after the second dose than after the first. Bauer and Duncan (1971) found that rats administered d-amphetamine twenty-four hours before training showed facilitated learning of active avoidance behavior when compared to saline-treated animals. Glick (1973) has shown that rats gradually develop an aversion to drinking solutions of d-amphetamine suggesting a cumulative effect of chronic drug treatment. Tilson and Rech (1973a) suggested that metabolic persistence was responsible for the extreme slowness of rats on a fixed-ratio schedule of responding to become tolerant to the disruptive effects of d-amphetamine. Unpublished results from this laboratory found evidence for drug persistence when examining the effect of d-amphetamine in food-deprived rats. The subjects were injected with a dose of 2 mg/kg of d-amphetamine immediately after a two hour feeding period every other day for a month. These animals showed significant decreases in food intake on the noninjection days as well as an overall weight loss when compared to saline-treated rats on a similar deprivation schedule.

On the basis of evidence presented above, it appears that in the rat, an active metabolite of d-amphetamine can affect brain catecholamines in a similar manner as does the parent compound. It would follow that this metabolite would effectively decrease the amount of d-amphetamine necessary to elicit a given effect, thereby rendering the rat more sensitive to repeated doses of the drug. Persistence of an

active metabolite might partially explain the failure of some investigators to find tolerance to certain behaviors elicited by d-amphetamine when the drug is administered repeatedly.

Since para-hydroxylation plays a minimal role in the metabolism of d-amphetamine in the mouse, daily administration of the drug would not be expected to produce the cumulative effect seen in the rat unless the frequency of administration were such that a sufficient amount of metabolite accumulated to elicit such an effect. In the present investigation, it was shown that mice which received d-amphetamine twice a day, with four hours between injections, became more sensitive rather than tolerant to the drug's effect on activity with repeated administration. Since d-amphetamine is eliminated in the mouse in two to six hours (Benakis and Thomasset, 1970), drug persistence could well account for this increased sensitivity to d-amphetamine following frequent and repeated administration.

Implications

The concept of behavior acting as a drug in the central nervous system has several implications when extended to drug-behavior interactions in man.

The present investigation has shown that familiarity with the testing environment (activity box) decreases the sensitivity of mice to the activity-increasing effect of d-amphetamine. It could be predicted from this result that an individual self-administering a psychoactive drug in a

foreign or unfamiliar environment would be more sensitive to the effects of the drug than would an individual self-administering the same drug in a more familiar environment. The enormous influence of setting on the individual's reaction to a psychoactive drug was noted by Weil et al. (1968) in their study of the psychological effects of marijuana on human volunteers. Kelleher and Morse (1968) have also discussed several experiments demonstrating that environmental variables can modify the magnitude or type of effect of a psychoactive drug on an individual.

Behaviorally-induced changes in brain catecholamine levels and metabolism may be related to the catecholamine hypothesis of affective disorders. This theory states that some depressions are associated with relative or absolute catecholamine deficiencies, particularly of norepinephrine, at functionally important receptor sites in the brain. Conversely, mania is thought to result from an excess of the relative or absolute amounts of brain catecholamines (Schildkraut, 1965).

The changes in brain catecholamine metabolism in the affective disorders are not necessarily of primary etiological importance; it has been suggested that the importance of psychological factors be further researched (Schildkraut, 1965). The available experimental studies with animal models suggest that environmental interactions can affect brain catecholamines. Large decreases in brain stem norepinephrine were shown to occur in the rat after shock and exposure to cold (Maynert and Levi, 1964), and it was concluded by these

investigators that stress could release brain norepinephrine. Gordon et al. (1966) demonstrated that increased synthesis of brain norepinephrine occurred in the rat after exercise and exposure to cold. It was reported by Bliss et al. (1968) that rats stressed by foot shock showed large decreases in endogenous brain norepinephrine and that stress increased the synthesis of norepinephrine. Dopamine turnover was also reported to be increased, but the dopamine levels remained constant. Thierry et al (1968) essentially confirmed these results by showing that stress due to foot shock increased norepinephrine turnover in central norepinephrine neurons of the rat, and attributed these changes to central processes involving pain and anxiety. These authors, however, concluded that dopamine was not involved in the reaction to stress, since there was no change in the turnover of striatal dopamine. The increased normetanephrine levels found in this study imply that stress acts on the same functional pool of norepinephrine as does d-amphetamine. It is conceivable that environmental factors could act similarly in man, and serve to predispose an individual to psychosis.

The paranoid schizophrenia induced by the chronic administration of high doses of amphetamine may be clinically indistinguishable from a naturally-occurring psychosis (Connell, 1958). The amphetamine-induced paranoid symptoms occur without a concommitant impairment of consciousness and intellectual function, and the drug is not thought to exacerbate latent personality disorders (Griffith et al., 1970).

There are certain similarities between the effects of chronic high doses of amphetamine in animals and man. For example, animal stereotypy has been used as a model of human psychosis (Angrist et al., 1971). The amphetamine abuser displays automatic repetitive behavior which consists of jerking movements in the face, arms and legs (Rylander, 1972). Pimozide, a drug that specifically blocks dopamine in the central nervous system (Andén, 1970), has been shown to block the amphetamine-induced enhancement of locomotor activity in animals (see p.6) and blocks the amphetamine-induced euphoria in man (Gunne, Angaard and Jonsson, 1972).

In the previous section, it was noted that metabolites of d-amphetamine may be responsible for the action of the drug in rats, when hyperactivity was involved. Similarly, metabolites play an important role in the manifestation of the amphetamine-induced psychosis in man. Angaard et al. (1973) found that the metabolism of single small doses of amphetamine was nonsignificantly different in psychotic amphetamine abusers and drug naive volunteers. No relationship between the plasma level of amphetamine and the intensity of psychosis was observed, suggesting that the accumulation of metabolites may be responsible for the development of the psychosis. Gunne, Jonsson and Angaard (1972) showed that p-hydroxyamphetamine is a major metabolite of amphetamine in man, and that the presence or absence of amphetamine and its metabolites in the blood stream correlates with the appearance and disappearance of psychotic symptoms.

Animal studies have also shown that environmental factors act to potentiate amphetamine-induced changes in brain catecholamines. It has been reported by Hohn and Lasagna (1960), Swinyard et al. (1961), Wang et al. (1969) and Weiss et al. (1961) that aggregation increased the toxicity of amphetamine in mice. Moore (1963) reported that the norepinephrine-depleting effect of d-amphetamine was enhanced in aggregated mice as compared to isolated mice. The enhanced toxicity found in the aggregated mice was correlated with the increased release of brain norepinephrine, but aggregation per se did not deplete brain norepinephrine. These results were confirmed by Mennear and Rudzik (1966). Glick et al. (1973) showed that food deprivation antagonized the d-amphetamine-induced depletion of hypothalamic norepinephrine and d-amphetamine-induced increase in striatal dopamine. These studies indicate that social and environmental factors may play a role in the etiology of psychosis in man.

SUMMARY

This thesis has explored the problem of tolerance development to the activity-enhancing effect of d-amphetamine in the mouse and rat. The criterion employed to ascertain the existence of tolerance was the shifting of the dose-response curve to the right.

It was demonstrated that tolerance occurred in mice which were tested with d-amphetamine in activity boxes following seven days of daily drug injections. The number of drug injections was found to be important since mice tested with d-amphetamine after three days of daily amphetamine injections did not show evidence of tolerance development. The interval between drug injections was also shown to be crucial; when d-amphetamine was administered twice daily for three and a half days with each pair of injections spaced four hours apart, tolerance failed to develop.

Tolerance to amphetamine-induced hyperactivity also occurred in mice following seven days of testing with the drug. However, tolerance did not occur in mice after three days of testing with d-amphetamine. The interval between treatments (drug injections plus activity testing) was also an important factor since tolerance failed to develop in mice when the treatments were administered four hours apart for three and a half days.

Behavioral variables were shown to significantly affect

tolerance development in the mouse. Seven days of pre-drug experience in the testing apparatus shifted the dose-response curve to the right when the subjects were tested with the drug. It was also shown that following only three days of pre-drug activity testing, tolerance failed to develop. The effect of pre-drug experience on the subjects' sensitivity to d-amphetamine may be related to pharmacological tolerance in terms of a common effect on brain catecholamines.

The administration of d-amphetamine or saline immediately after the test sessions for seven days failed to produce tolerance in spite of the fact that tolerance occurred following seven days of testing with both drug and saline injections administered before the test sessions. The disruptive effect of the injection per se was suggested as interfering with the manifestation of tolerance.

In contrast to the results obtained with mice, rats which received d-amphetamine for seven days, either with or without concurrent activity testing, were found to be more sensitive to the activity-enhancing effect of the drug. This heightened sensitivity was explained by the presence of a persistent metabolite of d-amphetamine. Rats tested for seven days without drug were less sensitive to d-amphetamine than were rats tested with drug for seven days, but the presence of tolerance in the former group of rats could not be ascertained.

Implications of the results were discussed in terms of the catecholamine hypothesis of affective disorders and amphetamine-induced psychosis in man.

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