TOLERANCE TO THE BEHAVIORAL EFFECTS

OF METHYLPHENIDATE

THESIS

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titles.

Thirty-one rats were trained on a differential reinforce-

ment of low rate schedule. After responding had stabilized,

animals were injected with methylphenidate, twice weekly, pre-

session. Methylphenidate produced dose-dependent increases in

response rates and decreases in reinforcements. Repetition of

these doses produced a reduced drug effect, and a third admin-

istration of the 10 mg/kg dose further reduced the drug effect.

Subsequently, the effects of daily and intermittent administra-

tion were determined for this dose. Daily methylphenidate,

pre-session, produced tolerance to the behavioral effects of

methylphenidate and cross-tolerance to the amphetamines.

Twice-weekly methylphenidate, pre-session, produced partial

tolerance to methylphenidate and partial cross-tolerance to

the amphetamines. Thus, periodic exposure to the behaviorally

disruptive effects of a drug of the amphetamine class reduces

the effects of subsequent exposure.

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CHAPTER I

INTRODUCTION

The amphetamines are potent central nervous system stim-
ulants which have been instrumental in studying the inter-
relationships between drugs, catecholamines, and behavior.
Drugs of the amphetamine class, which include d-amphetamine
and methylphenidate, have similar effects on behavior when
administered acutely (Browne and Segal, 1977; Harris, Snell,
and Loh, 1978). At the neurochemical level, they all appear
to increase brain catecholamine metabolism (Scheel-Kruger,
1971; Ferris, Tang, and Maxwell, 1972; Moore, 1978). The
amphetamines produce general hyperactivity, vasoconstriction,
hyperthermia, and anorexia in a variety of species, including
man (Biel, 1970). At higher doses, these drugs induce stereo-
typed behavior: continuous sniffing, biting, and gnawing in
rats, dyskinesic movements and repeated sequences of behavior
in humans (Randrup and Munkvad, 1974). The amphetamines can
precipitate paranoid psychoses in humans after chronic admini-
stration of increasingly higher doses (Griffith, Oates, and
Cavanaugh, 1968).

The amphetamines were introduced clinically because they
reduce fatigue, suppress appetite, and increase alertness.
Drugs of the amphetamine class have also been abused epidem-
ically for these central stimulant properties (Angrist and

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Sudilovsky, 1978). The amphetamines have been used therapeutically in the treatment of obesity, depression, narcolepsy, and hyperkinesis (Biel and Bopp, 1978). Methylphenidate is the most frequently prescribed drug of the amphetamine class. Methylphenidate is considered the most successful drug for controlling hyperactive behavior in children and is approved by the Food and Drug Administration for this use (Perel and Dayton, 1977). Methylphenidate decreases hyperactivity in approximately 83% of children treated with this drug for the minimal brain dysfunction syndrome (Anders and Ciaranello, 1977).

Repeated administration of drugs of the amphetamine class often results in decreased responsiveness to the effects of these drugs, so that the individual must increase the size of successive doses to produce effects of equal magnitude or duration (Kalant, LeBlanc, and Gibbins, 1971). This phenomenon of decreased responsiveness as a result of prior or repeated exposure is known as tolerance. Amphetamine has only short-term effectiveness as an appetite suppressant in the treatment of obesity because patients develop tolerance to the anorexic effects of the drug within three weeks (Strata and Zuliani, 1978). Tolerance can occur rather abruptly in children treated with methylphenidate for hyperactivity and the dose must be increased to maintain the desired effect (Perel and Dayton, 1977). Tolerance does not appear to develop to the stimulant effects of these drugs in

humans, as patients with narcolepsy can be maintained on fixed doses for years (Leake, 1958). In addition, tolerance does not develop to the psychosis-inducing effects of large doses of these drugs; in fact, chronic abusers of methamphetamine may become increasingly sensitive to the psychotomimetic effects (Ellinwood, Sudilovsky, and Nelson, 1973).

Chemical Structure of Amphetamine and Methylphenidate

The amphetamines and methylphenidate are structurally related to the catecholamines. β-phenylethylamine is the parent compound, consisting of a benzene ring and an aliphatic portion, ethylamine (Biel, 1970). The phenylethylamine molecule permits substitution at four sites (Figure 1). The basic structural requirement for direct (receptor) action of the sympathomimetics is the presence of at least one phenolic hydroxyl group (Site a), especially at the meta position, and a beta-hydroxyl group. Removal of both phenolic groups leads to compounds having a weak indirect action by releasing catecholamines, but with a longer duration of action, such as amphetamine and methylphenidate (Figure 2). In addition, removal of the phenolic and alcoholic hydroxyl groups (Sites a & b) decreases the polarity of the molecule. Thus, amphetamine and methylphenidate can pass the blood-brain barrier, producing effects on the central nervous system (Biel, 1970).

Substitution on the α or β carbon of the phenylethylamine molecule introduces an asymmetric center; the chirality of each of these carbons is significant, since several features
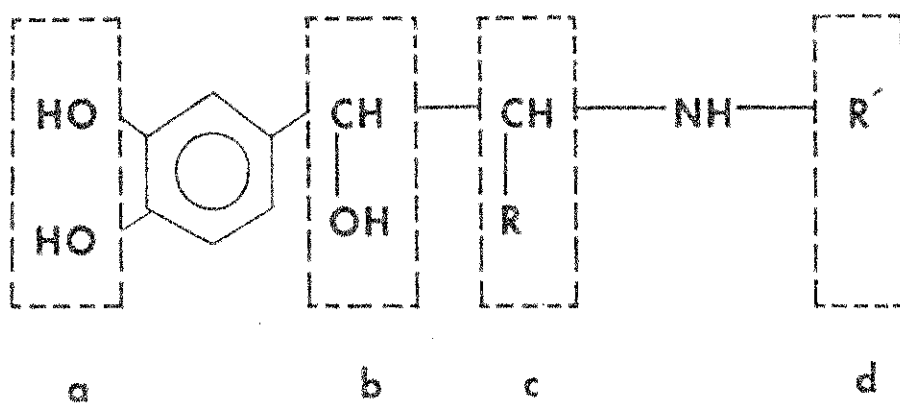
Fig. 1 — Substitution sites on the phenylethylamine molecule
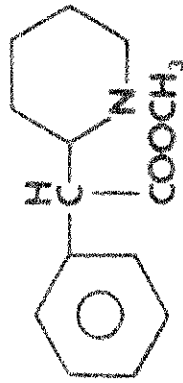
Fig. 2--Chemical Structures of Amphetamine and Methylphenidate

of catecholaminergic synapses display stereochemical selectivity (Hendley et al., 1972). Norepinephrine has an asymmetric β carbon, while amphetamine has an asymmetric α carbon; so that each can exist in two stereochemical forms. Methylphenidate is a derivative of phenylethylamine with both α and β asymmetric carbons, giving four possible isomers, and it differs from the phenylethylamine amphetamine in that the amine moiety is part of a piperidine ring (Perel and Dayton, 1977).

Pharmacokinetics and Metabolism of Amphetamine and Methylphenidate

In rats given ^{3}H-amphetamine i.p., peak concentrations in brain are observed 20 minutes after administration (Kuhn and Schanberg, 1978). The half-life of the distribution (α) phase was 30 to 54 minutes and the half-life of the elimination (β) phase was 5 to 9 hours in all tissues. In man, peak plasma concentrations occur two hours after oral administration with absorption complete in 2.5 to 4 hours (Brookes, 1977).

In rats given ^{14}C·methylphenidate·HCl intravenously, the mean half-life for the distributive (α) phase of the decay of ^{14}C concentrations in rat brain was 19 minutes and the mean half-life of the elimination (β) component was 105 minutes (Segal et al., 1976). Five minutes after i.p. administration, ^{14}C levels in plasma were about half those after i.v. administration. After 30 minutes, plasma levels of ^{14}C were approximately the same following both routes of administration and

declined in parallel. Peak ^{14}C levels in rat brain after
i.p. administration occurred after 60 minutes, compared to
20 minutes for amphetamine. No significant regional differ-
ences in the relative distribution of ^{14}C in rat brain were
observed during a two-hour period after i.v. administration
of ^{14}C·methylphenidate·HCl (Segal et al., 1976). In rats,
after either i.p. or oral administration of ^{14}C·methylpheni-
date·HCl, 50 to 60% of the ^{14}C was eliminated in urine and 30
to 40% in feces (Faraj et al., 1974).

In man, methylphenidate is essentially completely and
quickly absorbed after oral administration. The plasma half-
life of methylphenidate in man after i.v. administration is
about 1 to 2 hours (Faraj et al., 1974), which is consider-
ably shorter than the half-life of amphetamine in man (Axelrod,
1970). Some accumulation of methylphenidate was observed in
a brief multiple dose study (Faraj et al., 1974).

The major metabolic pathways of amphetamine include:
(1) aromatic hydroxylation to p-hydroxyamphetamine, with sub-
sequent β-hydroxylation to p-hydroxynorephedrine, (2) alipha-
tic hydroxylation to norephedrine, (3) oxidative deamination
to a ketone with subsequent oxidation to yield benzoic acid.
Both p-hydroxyamphetamine and norephedrine are active metabo-
lites. Amphetamine is incompletely metabolized by man; about
38% of the dose is excreted unchanged (Smith and Dring, 1970).
In man, oxidative deamination is the major metabolic pathway
(23% of the dose); hydroxylated metabolites are present in

lower amounts (about 5% of the dose). In rats, p-hydroxy-
lation is the major metabolic pathway (60% of the dose); the
rat excretes lesser amounts of unmetabolized amphetamine (14%
unchanged) than humans. Oxidative deamination products
account for only 2% of the dose (Smith and Dring, 1970).

The major route of metabolism of methylphenidate in man
is deesterification by several esterases so that about 80% of
the dose is excreted as ritalinic acid (Perel and Dayton,
1977). The remaining 20% of the dose is metabolized by the
hepatic microsomal oxidase system. In rats, microsomal oxi-
dation is a major route of metabolism of methylphenidate.
In vivo metabolism of methylphenidate in rats is similar to
that of other phenylethylamine derivatives where hydroxyla-
tion is a major pathway.

Catecholamine Neurotransmitters

Amphetamine and methylphenidate exhibit a wide range of
pharmacological activity; their profound effects on behavior
are thought to be mediated by catecholamines in the brain
(Moore, 1978). These central catecholamines, primarily dopa-
mine and norepinephrine, act as neurotransmitters; they have
been demonstrated to be important in the mediation of behav-
ior (Seiden, MacPhail, and Emmett-Oglesby, 1975). Brain
catecholamines are synthesized primarily within central
nervous system (CNS) neurons; peripherally synthesized cate-
cholamines cross the blood-brain barrier only to a limited
extent (Iversen, 1967).

The pathways involved in the biosynthesis and metabolism of the catecholamines are shown in Figures 3 and 4. Catecholamines are derived from the amino acid tyrosine, which is actively taken up by catecholaminergic neurons. The first step in catecholamine biosynthesis is the conversion of tyrosine to dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (Nagatsu, Levitt, and Udenfriend, 1964). Tyrosine hydroxylase is found in synaptosomal fractions. The enzyme is an iron-containing, mixed-function oxidase which requires tetrahydrobiopterin as its electron-donating cofactor. Tyrosine hydroxylase has been demonstrated to be the rate-limiting step in the biosynthetic pathway of the catecholamines (Levitt et al., 1965). Thus, catecholamine synthesis can be blocked quickly by administration of inhibitors of tyrosine hydroxylase. α-methyl-para-tyrosine is a potent competitive inhibitor of the substrate tyrosine (Spector, Sjoerdsma, and Udenfriend, 1965); it produces a rapid decline in brain catecholamine concentrations and is used experimentally to study the effects of various drugs on the turnover of catecholamines.

DOPA is decarboxylated to dopamine by the soluble enzyme aromatic-L-amino acid decarboxylase. This enzyme acts on all naturally occurring aromatic-L-amino acids and requires pyridoxal phosphate as a cofactor (Nagatsu, 1973). DOPA decarboxylase has the highest activity of the enzymes in the biosynthetic pathway (Cooper, Bloom, and Roth, 1978). In dopaminergic neurons, dopamine can be stored, metabolized by

Fig. 3.--Biosynthesis of the catecholamines. The enzyme tyrosine hydroxylase catalyzes the conversion of tyrosine (Tyr) to DOPA, which is decarboxylated by the enzyme DOPA-decarboxylase. The product, dopamine (DA), is converted to norepinephrine (NE) by dopamine-β-hydroxylase.

TYR

DOPA

DA

NE
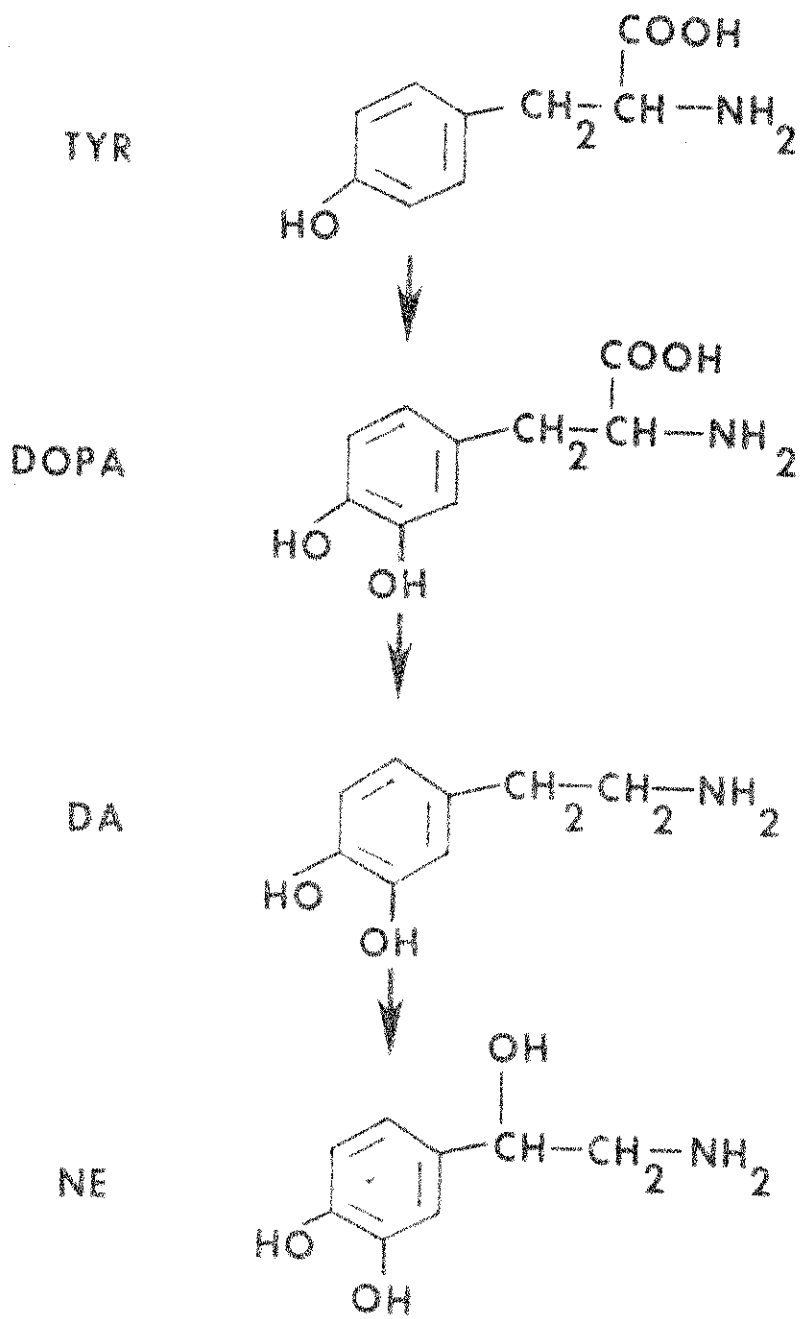
Fig. 4.--Metabolic pathway of the catecholamines. Dopamine (a) is methylated by the enzyme catechol-O-methyl-transferase (COMT) to 3-methoxytyramine (b), which is then deaminated to homovanillic acid (d) by monoamine oxidase (MAO). Alternatively, dopamine is oxidatively deaminated by MAO to 3,4-dihydroxyphenylacetic acid (c), which is then methylated by COMT to homovanillic acid (d). Dopamine can be hydroxylated in noradrenergic neurons to norepinephrine (e), which subsequently can be methylated by COMT to normeta-nephrine (g) and then deaminated by MAO to vanilmandelic acid (h), or deaminated to 3,4-dihydroxymandelic acid (f) and then methylated to vanilmandelic acid.
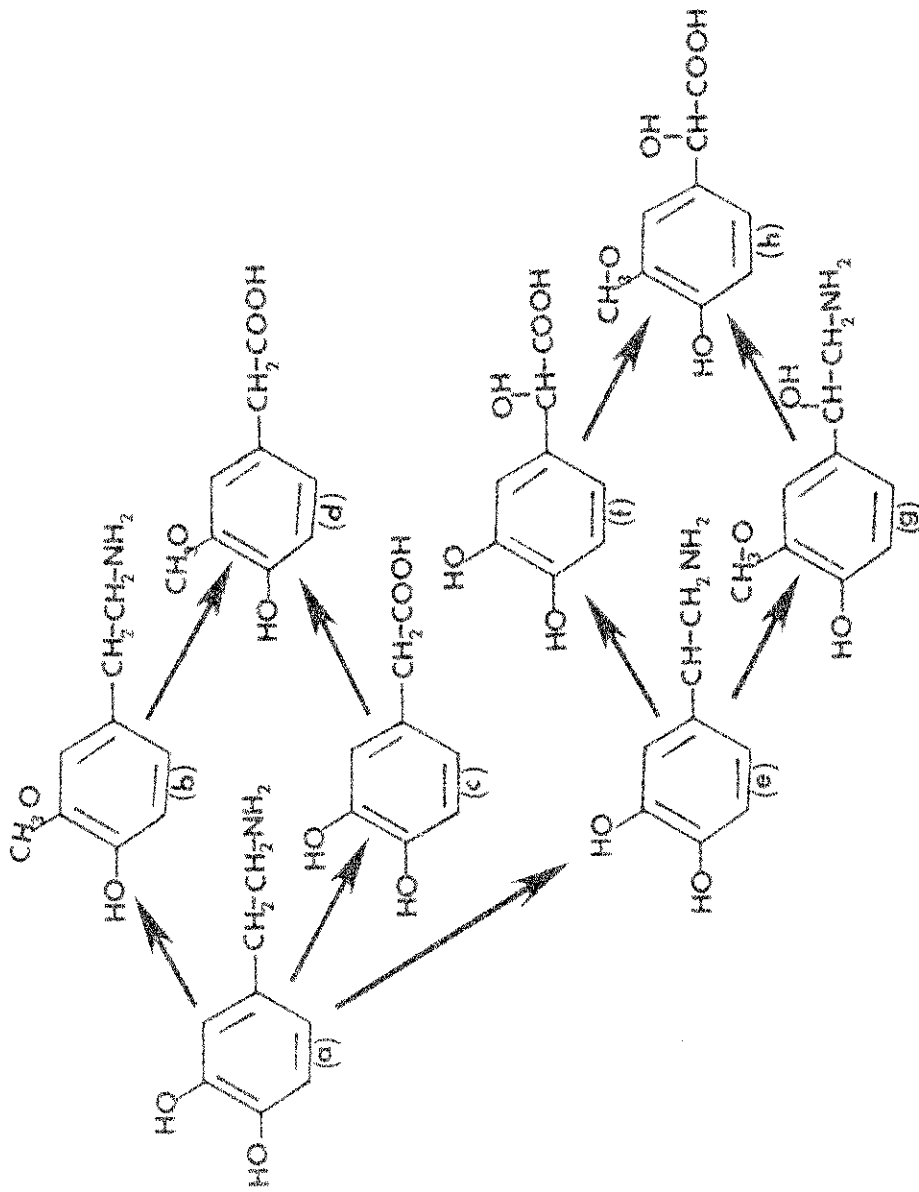
intraneuronal monoamine oxidase (MAO), or released. In noradrenergic neurons, dopamine is taken up into storage granules, where it is converted to norepinephrine. The reaction is catalyzed by dopamine-β-hydroxylase. Like tyrosine hydroxylase, dopamine-β-hydroxylase is a mixed function oxidase which utilizes ascorbic acid as its cofactor (Goldstein, 1972). Dopamine-β-hydroxylase contains copper and can be inhibited most effectively by compounds which chelate copper, such as disulfiram. After synthesis, intraneuronal catecholamines are inactivated by mitochondrial MAO. MAO is an oxidative deaminase which converts the catecholamine to its corresponding aldehyde.

Norepinephrine or dopamine are released from catechoaminergic neurons during periods of neuronal stimulation and diffuse across the synaptic cleft to interact with specific receptors on the postsynaptic membrane. The neurotransmitter is subsequently inactivated by diffusion into the circulation, metabolism by catechol-O-methyltransferase (COMT), or active reuptake. The primary mechanism for terminating the actions of catecholamines appears to be reuptake of the transmitter into the presynaptic neuron. The reuptake process is highly dependent on sodium and temperature and can be blocked by inhibition of Na^+,K^+-activated ATPase. Also, this transport mechanism can be saturated by high concentrations of catecholamines (Cooper, Bloom, and Roth, 1978).

Neurochemical Effects of Acute Administration of Amphetamine

Amphetamine and methylphenidate are presumed to act by facilitating transmission at catecholaminergic synapses in the central nervous system. The mechanisms of action of amphetamine-like drugs have been postulated to include (1) direct stimulation of catecholamine receptors, (2) inhibition of monoamine oxidase, (3) blockade of reuptake of neurogenically released catecholamines, and (4) release of catecholamines (Moore, 1978). A schematic model of a central catecholaminergic neuron indicates the possible sites at which these drugs have been proposed to act (Figure 5). This section will evaluate the evidence for these proposed mechanisms and for the relative roles of dopamine and norepinephrine in the behavioral effects of these drugs.

Early theories suggested that amphetamines produced their central stimulant effects by inhibiting monoamine oxidase (Mann and Quastel, 1940). With the discovery of potent MAO inhibitors in the 1950's, this mechanism seemed less likely, since the effects of amphetamine were distinctly different from those of the most specific MAO inhibitors. Although some MAO inhibitors (tranylcypromine and pheniprazine) have amphetamine-like properties, others (iproniazid and pargyline) do not (Poschel and Ninteman, 1964; Stein, 1964). Some amphetamine-like drugs inhibit MAO; however, their inhibitory effects are weak compared to the more specific and potent MAO inhibitors. In addition, amphetamines can produce their

Fig. 5.--Schematic diagram of a catecholaminergic synapse depicting sites (indicated by numbers 1 to 4) at which amphetamine has been postulated to act. Norepinephrine or dopamine is stored in amine vesicles until the arrival of a nerve action potential, when the amines are released and diffuse across the synaptic cleft to activate receptors on postsynaptic neurons. Amphetamine has been proposed to [1] mimic the actions of norepinephrine or dopamine at postsynaptic receptor sites, [2] increase the concentrations of these amines at the receptor by inhibiting monoamine oxidase (MAO), [3] block reuptake of the released amine into the presynaptic nerve terminal, or [4] cause release of norepinephrine or dopamine.

Presynaptic Nerve Ending

MAO

② ③ Reuptake Release ④

① Receptor Stimulation

Postsynaptic Receptor
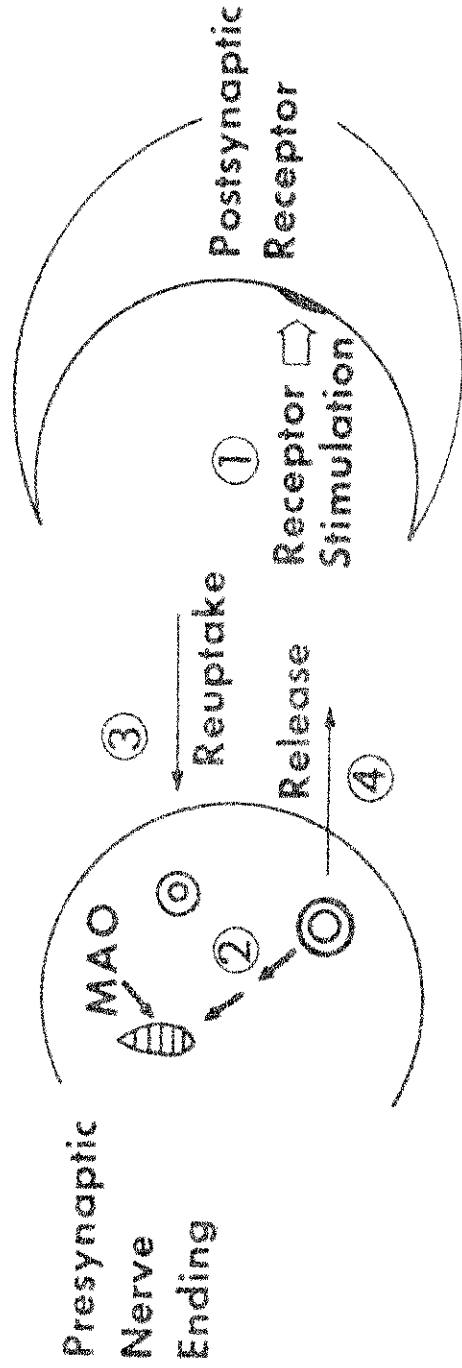
stimulatory effects when MAO is already inhibited (Moore, 1978). Thus, inhibition of MAO is not a primary mechanism of action of the amphetamines.

After the demonstration that the central effects of amphetamine persisted after monoamine depletion by reserpine, amphetamine was proposed to act directly on catecholamine receptors (Van Rossum, 1962; Smith, 1963). The first observations that the effects of amphetamine might be indirect and dependent upon brain catecholamines were those of Stein (1964), who proposed that amphetamine exerted its stimulatory effects by releasing norepinephrine from nerve terminals in the brain. This hypothesis stemmed from Stein's observations that the facilitating effects of amphetamine on self-stimulation in rats were enhanced by MAO inhibitors and diminished by reserpine, which depletes brain catecholamines by interfering with storage in granules. Subsequently, the development of the specific and potent tyrosine hydroxylase inhibitor, α-methyl tyrosine (Spector et al., 1965) led to the demonstration that inhibition of catecholamine synthesis by pretreatment with α-methyl tyrosine suppressed the behavioral effects produced by d-amphetamine (Weissman and Koe, 1965). These results suggested strongly that amphetamine acted indirectly by releasing catecholamines from the presynaptic terminals.

Glowinski and Axelrod (1965) proposed that amphetamine exerted its behavioral effects by blocking the reuptake of impulse-released catecholamines. However, several

experimental findings weaken this hypothesis. Although some tricyclic antidepressants which block reuptake increase the effects of amphetamines, those effects are also increased by iprindole, which does not block reuptake (Freeman and Sulser, 1972). In fact, the tricyclic antidepressants may enhance the central stimulant effects of amphetamine by increasing brain concentrations of amphetamine, because the tricyclics decrease hepatic metabolism of amphetamine (Lewander, 1969; Freeman and Sulser, 1972).

Experimental techniques which reduce catecholamine levels in the brain have been used successfully to investigate the neurochemical effects of amphetamine. Two procedures used in experiments studying concomitantly the neurochemical and behavioral effects of amphetamine are: (1) blocking catecholamine synthesis with α-methyl tyrosine to estimate catecholamine turnover after administration of amphetamine and (2) depleting catecholamines stored in granules with reserpine to examine the effects of amphetamine on stored, as opposed to newly synthesized, catecholamines. Considerable evidence obtained from these two techniques supports the suggestion that amphetamines exert their central effects by release of catecholamines. Pretreatment with α-methyl tyrosine, but not pretreatment with reserpine, blocks the behavioral effects of amphetamine (Weissman, Koe, and Tenen, 1966; Rech and Stolk, 1970). These findings suggest that the anti-amphetamine effects of α-methyl tyrosine result from the inhibition of catecholamine synthesis.

Several lines of evidence suggest that the primary mechanism of amphetamine is through release of dopamine rather than norepinephrine. (1) Although α- and β-adrenergic receptor blockers do not alter the central stimulatory effects of the amphetamines (Moore, 1978), neuroleptics with specific dopamine receptor blocking properties abolish the behavioral effects of d-amphetamine (Van Rossum, 1967). (2) Low doses of d-amphetamine increase turnover of dopamine in striatum but not norepinephrine in the telencephalon (Costa, Groppetti, Naimzada, 1972). In addition, d-amphetamine produces a dose-related increase in dopamine turnover (Gerhards, Carenzi, and Costa, 1974; Carenzi et al., 1975). Furthermore, a dose of amphetamine that increases motor activity increases the rate of turnover of brain dopamine preferentially (Papeschi, 1975). (3) Selective depletion of dopamine with 6-hydroxydopamine lesions decreases the effects of amphetamine (Hollister, Breese, and Cooper, 1974), while selective depletion of norepinephrine or destruction of CNS noradrenergic neurons does not block the effects of amphetamine (Creese and Iverson, 1975).

In vitro studies have found that d-amphetamine increases the concentration of ^{3}H-norepinephrine in chopped rat brain slices when norepinephrine reuptake was blocked by desipramine or cocaine (Azzaro, Ziance, and Rutledge, 1974). Thus, d-amphetamine apparently increases the release of norepinephrine in vitro (Ferris et al., 1972; Ziance, Azzaro, and

Rutledge, 1972). In these studies, inhibition of uptake was probably not the primary mechanism, but it did help increase synaptic concentrations of norepinephrine after release of norepinephrine (Heikkila et al., 1975). In experiments where the influence of reuptake of amphetamine-induced efflux of ^{3}H-catecholamines was minimized, d-amphetamine was shown to be more effective in releasing dopamine (Raiteri et al., 1975).

From studies of in vivo release of exogenously administered radioactive catecholamines, d-amphetamine injected systemically or administered by cerebroventricular perfusion in cats increased the efflux of ^{3}H-norepinephrine and ^{3}H-dopamine (Carr and Moore, 1970). Subsequent experiments showed that the origin of ^{3}H-catecholamines in these studies was from dopamine terminals in the caudate nucleus (Von Voigtlander and Moore, 1973). In addition, lesions of the nigrostriatal pathway decreased efflux of ^{3}H-dopamine evoked by amphetamine, indicating that the change in concentrations of exogenously administered catecholamines after amphetamine administration was partially dependent upon ongoing activity of the nigrostriatal pathway (Von Voigtlander and Moore, 1973). These results suggest that the probable primary mechanism of d-amphetamine is through facilitating release of dopamine.

Studies of the in vivo release of endogenous catecholamines provided further support for the hypothesis that the

primary mechanism of action of amphetamine is release of dopamine. When ^{3}H-tyrosine of high specific activity was used, amphetamine increased the efflux of endogenously synthesized ^{3}H-dopamine from the superfused caudate nucleus of the cat (Besson et al., 1971). Amphetamine increased efflux of endogenously synthesized ^{3}H-dopamine from the superfused monkey caudate nucleus (Gauchy et al., 1974) and increased efflux of ^{3}H-norepinephrine and ^{3}H-dopamine from the posterior hypothalamus of cats (Philippu, Glowinski, and Besson, 1974). When the cerebral ventricles of cats were perfused with cerebrospinal fluid containing ^{3}H-tyrosine, amphetamine was demonstrated to increase efflux of ^{3}H-dopamine. This effect declined over time despite the continued presence of the drug (Chiueh and Moore, 1975a). α-methyl tyrosine added to the cerebrospinal fluid accelerated this decline in the efflux of ^{3}H-dopamine. Pretreatment with reserpine depleted endogenous and labeled dopamine but did not alter amphetamine-induced efflux of newly synthesized dopamine. These results suggest that amphetamine initially releases dopamine from a storage pool, but that continued release of dopamine is dependent upon ongoing amine synthesis.

Like amphetamine, methylphenidate added to perfusing cerebrospinal fluid increases the efflux of ^{3}H-norepinephrine in cats (Moore, Carr, and Dominic, 1970). Methylphenidate added to ^{3}H-tyrosine caused an increase in the efflux of ^{3}H-dopamine (Chiueh and Moore, 1975b). Unlike amphetamine,

however, the effects of methylphenidate on endogenously synthesized ^{3}H-dopamine in cats were blocked by pretreatment with reserpine (Chiueh and Moore, 1975b), while the effects of methylphenidate on locomotor activity in mice were not significantly altered by α-methyl tyrosine (Dominic and Moore, 1969). These results suggest that methylphenidate, like amphetamine, appears to increase release of catecholamines; these effects of both drugs on brain catecholamines probably account for their nearly indistinguishable effects on behavior.

Mechanisms of Tolerance

Chronic administration of amphetamine results in the development of tolerance to some of the behavioral effects of the drug. Tolerance is defined as decreased responsiveness to a drug which is acquired after prior or repeated exposure to that drug or to one similar to it in pharmacological activity (Goldstein, Aronow, and Kalman, 1974). Tolerance is characterized by the necessity of increasing the size of successive doses to produce effects of equal magnitude or duration or by an inability of subsequent administration of the same dose of a drug to be as effective as the preceeding dose.

Tolerance may be the result of conditions which produce (1) a decrease in the effective concentration of the drug at the site of action, or (2) a reduction in the normal reactivity of the receptor which makes it less sensitive to the same

concentration of drug. Tolerance developed by the first mechanism is usually called dispositional or metabolic tolerance; tolerance developed by the second mechanism is referred to as functional tolerance or cellular tolerance (Kalant et al., 1971; Levine, 1973). Alternatively, Goldstein et al. (1974) distinguish between tolerance developed by indirect mechanisms and tolerance developed by direct mechanisms. The distinction of Goldstein et al. between direct and indirect mechanisms is prefered to the traditional definitions of metabolic and functional tolerance because it avoids the confusion encountered in the literature on tolerance to the behavioral effects of drugs.

Tolerance develops through indirect mechanisms in two ways. First, tolerance may occur when drug absorption is reduced, when the rate of drug elimination is increased, when movement of drug across biological membranes is diminished, or when the amount of non-receptor binding of the drug is increased (Goldstein et al., 1974). Second, tolerance may occur when the biological effect of a drug is increasingly antagonized through homeostatic mechanisms even though the receptor maintains its sensitivity to the drug (Goldstein et al., 1974).

Experimental studies with amphetamine suggest that tolerance by indirect mechanisms involving altered absorption, distribution and elimination would not account for most of the observed tolerance to the behavioral effects of the drug.

Increased rates of drug metabolism should have a negligible effect on those drug effects that are measured within minutes of drug administration (Kalant et al., 1971). As predicted, chronic administration of amphetamine does not result in an increase in the rate of amphetamine metabolism (Ellison et al., 1971). Further, tolerance to amphetamine is not due to an increased rate of excretion or to reduced concentrations of the drug in the brain (Magour, Coper, and Fähndrich, 1974). Siegel et al. (1968) found no differences in tissue distribution of amphetamine between tolerant and non-tolerant cats. Kuhn and Schanberg (1978) have reported that chronic administration of increasingly higher doses of amphetamine (10 mg/kg to 30 mg/kg twice daily) to rats results in altered distribution of amphetamine and its metabolites. However, they found that chronic administration of lower doses (5 mg/kg twice daily) did not significantly alter elimination of amphetamine and its metabolites compared to rats treated chronically with saline. The authors acknowledged that these findings can not account for tolerance to the anorexia or the disruption of reinforced behavior produced by amphetamine. In addition, the doses of amphetamine used in these experiments are twenty to forty fold greater than those used in behavioral studies and, thus, highly toxic behaviorally.

Tolerance develops through direct mechanisms when the sensitivity of the receptor changes. Ascribing tolerance to direct mechanisms requires the demonstration of similar

tissue concentrations of drug or active metabolite in toler-
ant and non-tolerant animals. Tolerance which develops selec-
tively to specific drug effects is more likely the result of
direct mechanisms.

A direct mechanism for tolerance to amphetamine has been
proposed by Brodie, Cho, and Gessa (1970) and Lewander (1971).
This mechanism assumes that p-hydroxynorephedrine, the hydrox-
ylation metabolite of amphetamine, acts as a false neurotrans-
mitter, displacing a portion of the norepinephrine stored in
noradrenergic neurons; thus, more amphetamine is required to
release a sufficient quantity of norepinephrine to produce a
behavioral response. This hypothesis appeared to be supported
by observations that p-hydroxynorephedrine caused a concentra-
tion-related release of ^{3}H-norepinephrine into chopped
cerebral cortex in vitro (Wenger and Rugledge, 1974). However,
this proposed mechanism of tolerance to amphetamine has not
been demonstrated to occur in brain in vivo. This mechanism
presumes that norepinephrine mediation is most significant
in the mechanism of action of the amphetamines when the exper-
imental evidence favors release of dopamine as the primary
mechanism.

Tolerance to amphetamine as a result of accumulation of
the metabolite p-hydroxynorephedrine would be difficult to
generalize to a species such as man, where metabolism by
deamination, and not by hydroxylation, predominates. In
addition, p-hydroxynorephedrine has not been shown to

accumulate significantly in the brain following peripheral or intraventricular administration of amphetamine or p-hydroxy-amphetamine, the p-hydroxynorephedrine precursor (Freeman and Sulser, 1974). Furthermore, p-hydroxynorephedrine-mediated tolerance could not account for tolerance to the behavioral effects of l-amphetamine or methylphenidate, since neither of these drugs appears to be metabolized to a false transmitter (Browne and Segal, 1977). The observations by Pearl and Seiden (1976) that no significant radioactivity in the brain occurred 24 hours after chronic administration of radiolabeled methylphenidate provide additional evidence that methylphenidate is not metabolized to a false neurotransmitter which is stored in catecholaminergic neurons. Thus, these results are all incompatible with the assumption that p-hydroxynorephedrine plays a major role in the mechanism of tolerance to amphetamine. Central mechanisms for tolerance to the amphetamines could include refractoriness of cate-cholamine receptors, induction of neurotransmitter synthesis, or greater availability of functionally antagonistic neuro-transmitters, for example, increased release of 5-hydroxy-tryptamine (Sparber and Tilson, 1972). However, the mechanism of tolerance to the behavioral effects of amphetamine and methylphenidate is still unknown.

The literature on tolerance to the behavioral effects of the amphetamines is often confusing because the terms metabolic tolerance, physiological tolerance, learned

tolerance, and <u>behavioral</u> tolerance are used without clarifying whether these terms imply different mechanisms of tolerance or simply describe different experimental procedures (Corfield-Sumner and Stolerman, 1978). Tolerance developing by either direct or indirect mechanisms, as discussed earlier in this section, can be manifested behaviorally in the same way. Thus, the distinction drawn in some studies between physiological and learned tolerance is not useful. The demonstration that an environmental or behavioral variable can augment the development of tolerance does not preclude the mediation of this tolerance through physiochemical mechanisms (Corfield-Sumner and Stolerman, 1978).

Some investigators use the term <u>learned</u> tolerance to describe the development of tolerance to the behavioral effects of a drug when the subjects are allowed to perform the behavior repeatedly while under the influence of the drug. However, this definition does not specify whether the drug effect on behavior decreases over time as a result of a shift in the behavioral baseline which is unrelated to drug administration or whether the behavioral disruption produced by the drug has decreased as a function of changes in the variables maintaining that behavior which have occurred as a result of repeated exposure to the drug. The term <u>behavioral</u> tolerance, or tolerance to the behavioral effects of a drug, does not imply a mechanism or specify the controlling variables, and, as a purely descriptive term, is preferable to learned tolerance.

Analysis of Drug Effects on Behavior

Drug effects on behavior are studied by establishing experimental control over a behavior, administering a drug, and measuring changes from the control performance produced by the drug. Drug effects are usually studied on behaviors which occur frequently and consistently and which can be maintained and observed over a long period of time. The desired behaviors or responses to study are those which can be performed easily and repeatedly and which can be defined discretely by the experimenter. Responses typically studied include lever pressing by rats and monkeys and disk pecking by pigeons (Ferster and Skinner, 1957).

The techniques of operant conditioning encompass a systematic framework of principles and procedures which are highly useful for studying drug effects on behavior. The analysis of operant behavior provides precise measures for controlling and quantifying behavior which are objective and can generate similar response patterns across species. Operant behavior is behavior controlled by its consequences. If the consequences, or environmental events, immediately following a behavior or response increase the frequency of that response, then this consequence or event is defined as a reinforcer. When every response is followed by a reinforcer, the organism is said to be on a continuous reinforcement schedule. However, when each response is reinforced, responding will decrease over time due to satiation. Responding can

be maintained over a long period of time by scheduling rein-
forcers to occur intermittently. There are a variety of
schedules of reinforcement presentation which produce a range
of response rates; these various schedules of reinforcement
generate different temporal patterning of responses.

Four general classes of environmental variables can
influence the effects of drugs on behavior:

(1) stimulus variables--type of stimulus, duration,
   intensity, and complexity;

(2) response variables--physical form or topography,
   duration, species-specific or shaped by exper-
   imenter;

(3) antecedent variables--organism's behavioral history
   and past experience with the drug, current
   deprivation state;

(4) consequence variables--type of reinforcer, magnitude,
   schedule of reinforcement.

In behavioral pharmacology, drug effects are frequently
explained by actions of drugs on one or more of the above var-
iables. Patterns of responding maintained by various schedules
of reinforcement have provided sensitive behavioral baselines
against which drug effects are measured. One schedule used
frequently in studies of the behavioral effects of drugs is
the differential reinforcement of low rate (DRL) schedule.
The DRL schedule specifies that only those responses sepa-
rated in time by a specific interval will be reinforced. A

response made before this time interval has elapsed causes the interval to restart. The pattern of responding generated by DRL schedules has been described as timing behavior (Sidman, 1955) and these very low rates of spaced responses can be disrupted by a number of drugs. Several drugs, including the amphetamines and methylphenidate, increase rates of DRL responding (Sidman, 1955; Schuster and Zimmerman, 1961; Pearl and Seiden, 1976), thus usually decreasing the number of reinforcers obtained.

Behavior is not only a dependent variable in behavioral pharmacology; it is also a determinant of drug effects. In a series of experiments which provided the framework of behavioral pharmacology, Dews (1955) studied the effects of various doses of pentobarbital on fixed-ratio (every 50th response was reinforced) and fixed-interval (first response after 15 minutes was reinforced) performance in pigeons. For both schedules of reinforcement, the response rate was increased by low doses of pentobarbital and decreased by intermediate and high doses. However, at one dose (1 mg) performance was differentially affected by the two schedule conditions. At this dose, relative to their saline control values, responding on the FR schedule was increased while responding on the FI schedule was decreased. Thus, the same dose of a drug was shown to increase or decrease operant responding depending upon the schedule of reinforcement maintaining the behavior.

After showing that the effects of pentobarbital were dependent upon the schedule of reinforcement which maintained responding, Dews (1958) studied the effects of methamphetamine on fixed-interval, variable-interval, and fixed-ratio performance in pigeons. When the drug effects were again observed to be dependent upon the schedule of reinforcement, Dews offered a general interpretation of the results which has come to be known as the rate-dependency theory. According to this interpretation, drug effects are primarily determined by the patterns of responding maintained by different schedules of reinforcement, with different rate-dependency functions for different classes of drugs (Kelleher and Morse, 1968). The demonstration that the effects of drugs on operant behavior depend critically upon the baseline rate of responding has forced the recognition that drugs do not create behavior; rather, they modify existing behavior, particularly the temporal pattern of behavior.

Characteristics of Tolerance to Amphetamine and Methylphenidate

The behavioral effects of acute administration of d-amphetamine and methylphenidate are qualitatively similar. Both drugs produce anorexia (MacPhail and Gollub, 1974; Pearl and Seiden, 1976), increased general motor activity (Browne and Segal, 1977), a pattern of stereotyped behavior which includes repetitive sniffing, grooming, and gnawing (Randrup and Munkvad, 1974; Browne and Segal, 1977), and changes in the rates of schedule-controlled responding (Dews, 1958;

Kelleher and Morse, 1968; Harris et al., 1978). The inten-
sity and temporal pattern of these effects are dose-dependent.

Repeated administration of amphetamine or methylpheni-
date results in the development of tolerance to many of the
behavioral effects of both drugs, including the anorexia
(Magour et al., 1974; Pearl and Seiden, 1976) and the dis-
ruption of operant behavior controlled by fixed-ratio (FR)
and differential reinforcement of low rate (DRL) schedules
of reinforcement (Schuster, Dockens, and Woods, 1966; Tilson
and Sparber, 1973; Pearl and Seiden, 1976). Moreover, Pearl
and Seiden (1976) demonstrated cross-tolerance between d-
amphetamine and methylphenidate to the effects of these drugs
on milk consumption and DRL performance. Tolerance does not
develop to drug-induced stereotyped behavior (Lewander, 1971),
increased general motor activity (Schuster and Zimmerman,
1961; Magour et al., 1974), or increases in responding to
discriminated and free operant avoidance (Schuster et al.,
1966; Barrett, Leith, and Ray, 1974). Indeed, repeated
administration of d- and l-amphetamine and methylphenidate to
rats produces a progressive decrease in the latency and
increase in the intensity of stereotyped behavior (Browne and
Segal, 1977). In addition, although animals become tolerant
to the effects of amphetamine on total food or water intake
during a testing session, the changes in the pattern of food
or water consumption produced by amphetamine are only partially
affected by chronic administration (Ghosh and Parvathy, 1973;

MacPhail and Seiden, 1976). Thus, tolerance to the effects of amphetamine or methylphenidate appears to be selective.

Several investigators have measured the duration of tolerance after cessation of drug administration. Schuster et al. (1966) studied the effects of continued daily administration of 1.0 mg/kg of d-amphetamine on the operant performance of rats on a multiple FI(30 second)DRL(30 second) schedule in which the fixed interval and DRL components alternated throughout the session. Following this chronic drug regime, the animals' performance on the multiple schedule was measured for 26 to 32 days. Dose-response measurements obtained one month after cessation of daily administration showed that for both the FI and DRL schedule components, animals who developed tolerance during the chronic administration phase remained resistant to the effects of amphetamine one month after the cessation of chronic administration of the drug. Fischman and Schuster (1977) observed that tolerance to the response-suppressant effects of d-methylamphetamine on a DRL schedule presisted for at least three months after cessation of a three- to six-month chronic drug regime. MacPhail and Seiden (1976) reported that tolerance developed to the adipsia produced by amphetamine after 28 days of daily administration of 1.6 mg of the drug; this tolerance to the decrease in water consumption persisted for 25 to 57 days after discontinuation of the daily injections. However, these authors report that tolerance to the effects of 1.6 mg

of amphetamine on the temporal pattern of water consumption within the testing session was maintained after cessation of daily drug administration. They suggest that amphetamine may have acquired stimulus control over water intake.

How complete is tolerance to the behavioral effects of amphetamine or methylphenidate? That is, to what degree does the behavior return to its predrug baseline? The results presented in several studies suggest that tolerance to the anorexic effects of these drugs is complete; food or water consumption return to saline control values (Carlton and Wolgin, 1971; Magour et al., 1974; MacPhail and Seiden, 1976; Pearl and Seiden, 1976). Tilson and Sparber (1973) reported complete tolerance to the rate-decreasing effects of d- and l-amphetamine on FR30 performance after chronic administration. Campbell and Seiden (1973) found complete tolerance to the rate-increasing effects of d-amphetamine on DRL responding. Pearl and Seiden (1976) reported that response rates for DRL performance returned to approximately 100% saline control values after chronic administration of d-amphetamine or methylphenidate and that the number of reinforcements earned returned to approximately 80% saline control levels. Schuster et al. (1966) present data which indicate that after 30 days of administration of 1 mg/kg d-amphetamine, response rates on the DRL component were within 110% saline control values and that the reinforcers obtained increased from 31-47% saline control to 67-97% saline control.

Variables Controlling the Development of Tolerance

Some of the controlling variables that determine whether
tolerance develops to an amphetamine-induced effect have been
identified. Carlton and Wolgin (1971) investigated the devel-
opment of tolerance to the anorexigenic effects of amphetamine.
Rats were first given pre-test injections of amphetamine (2 or
3 mg/kg) to determine the extent of the decrease in milk con-
sumption measured against saline controls, and then admini-
stered amphetamine daily before the feeding sessions until
tolerance developed (approximately seven days). A second
group of rats was pre-tested with amphetamine to measure the
anorexigenic effects and then was administered amphetamine
after each feeding session; these animals did not show toler-
ance when the drug was subsequently administered prior to the
feeding sessions. The authors concluded that the development
of tolerance is contingent upon the temporal relationship
between the administration of amphetamine and feeding sessions.

Campbell and Seiden (1973) extended the findings of
Carlton and Wolgin to operant performance. They studied the
relationship between the development of tolerance to the dis-
ruptive effects of amphetamine on DRL performance with admini-
stration of the drug both before and after behavioral testing
sessions. Amphetamine (1.5 mg/kg) given before the session
initially disrupted behavior; partial tolerance developed to
these effects over a 27-day course of administration. Post-
session administration of amphetamine had no effect on DRL

performance. When the post-session treated rats were given amphetamine before the testing session, behavioral disruption occurred, indicating that tolerance did not develop to amphetamine given after behavioral testing sessions.

These results confirm the description by Schuster et al. (1966) of the critical role of decreased reinforcement frequency in determining whether tolerance will develop to d-amphetamine. Schuster et al. (1966) proposed that tolerance develops when the drug interferes with the completion of response requirements for reinforcement, thus decreasing the total reinforcement density. They predict that for those behaviors where acute administration of amphetamine decreases reinforcement frequency, such as for behavior maintained by DRL schedules, tolerance will develop. For those behaviors where amphetamine administered acutely facilitates performance, such as for behavior maintained by avoidance schedules where acute administration of amphetamine decreases the number of electric shock presentations, tolerance will not develop to drug-induced changes in response rates.

A variable in the development of tolerance which has not been explored is the role of frequency of drug administration. In most experiments dealing with tolerance, drugs are administered at least once daily. Although it has been suggested that intermittent administration will delay or avoid the development of tolerance to d-amphetamine (Winsberg et al., 1972), no systematic study has evaluated the relationship

between the interval of amphetamine administration and the development of tolerance. Recently, Smith and McKearney (1977) demonstrated in pigeons that the rate-increasing effects of d-amphetamine administered twice weekly diminished with repeated injections; however, they did not describe these results in terms of tolerance following intermittent drug administration. Similar results have not yet been reported for intermittent administration of amphetamine or methylphenidate to rats.

The present experiments asked the following questions: (1) Is daily administration a necessary condition for the development of tolerance to the behavioral effects of methylphenidate? (2) Will daily or intermittent administration of methylphenidate produce cross-tolerance to other amphetamine-type drugs? (3) Will tolerance occur after daily post-session administration of methylphenidate? (4) Will acute administration of behaviorally active doses of methylphenidate alter brain catecholamine metabolism?

CHAPTER II

MATERIALS AND METHODS

Subjects

The experiments used male, Sprague-Dawley rats (Holtzman Co.), 60 days old at the beginning of each experiment. The animals were housed two per cage in a temperature monitored room (23°C). Each animal was handled daily. All animals had unrestricted access to food (Purina Rat Chow) in their home cages. The rats' body weights were maintained at 300 ± 3g throughout the experiment by adjusting the duration of their daily access to water.

Behavioral Apparatus

Eight experimental chambers (30 x 46 x 36 cm) constructed of wire mesh screen mounted on plexiglass (Rayfield Equipment Co., Chicago) were enclosed in sound and light attenuating 3/4 inch plywood boxes. A 2.5 W bulb mounted on the ceiling provided houselight for the chambers. Exhaust fans ventilated the chambers and masked extraneous sounds. Each chamber contained a single-response lever, 10.5 cm above the mesh floor, mounted on the wall, to the left of a small opening permitting access to a 0.01 ml capacity dipper. Operation of the lever (15 g in the vertical direction) closed a micro-switch and activated the dipper. Rayfield digital logic

modules programmed the experimental events and recorded the data.

Behavioral Procedure

Thirty-one rats were used in the behavioral experiment. Each animal was trained to drink water from a dipper in the experimental chamber. Then, successive approximations to a lever-press response were followed immediately by water presentation. When the lever-press response was established, each animal received three days of training on a continuous reinforcement schedule (30 minute sessions). This initial training phase of the experiment was followed by 74 sessions of training in Phase 1, 36 sessions in Phase 2, 77 sessions in Phase 3, and 53 sessions in Phase 4 (Table 1). Sessions in Phases 1 through 4 lasted 45 minutes. All animals were tested five days a week; two days a week the animals remained in their home cages with restricted access to water.

Phase 1: Behavioral Training.--After lever-press training, all animals were placed directly on a differential reinforcement of low rate (DRL) 18 second schedule of reinforcement. A state diagram of the DRL 18 second schedule is given in Figure 6 using the notational system of Snapper, Knapp, and Kushner (1970) to specify the behavioral conditions that hold for each step of the procedure. The circles represent the "states" or conditions that the animal can be in at any given time during the session, and the arrows represent an

Table 1

Treatment Conditions for Behavioral Experiment

Phase	Condition	# of Sessions
1	DRL Training	74
2	Methylphenidate Dose-Response Measurements	36
3	Chronic Methylphenidate Administration	77
	Daily MP Pre-Session (N = 7)	
	Daily MP Post-Session (N = 8)	
	Twice Weekly MP Pre-Session (N = 16)	
4	Cross-Tolerance Testing	53

Fig. 6.--State diagram of the DRL 18 second schedule.
The circles represent the experimental conditions in which
the animal can be; the arrows represent the events necessary
to move the animal from one state to another. In the dia-
gram, "R" means that the animal made a lever press response,
and "R̄" means that the animal did not make a lever response.
If the animal responds during the 18 second interval, the
clock resets and the animal returns to "Start." After the
termination of the 18 second interval, a lever response will
be followed by water presentation.
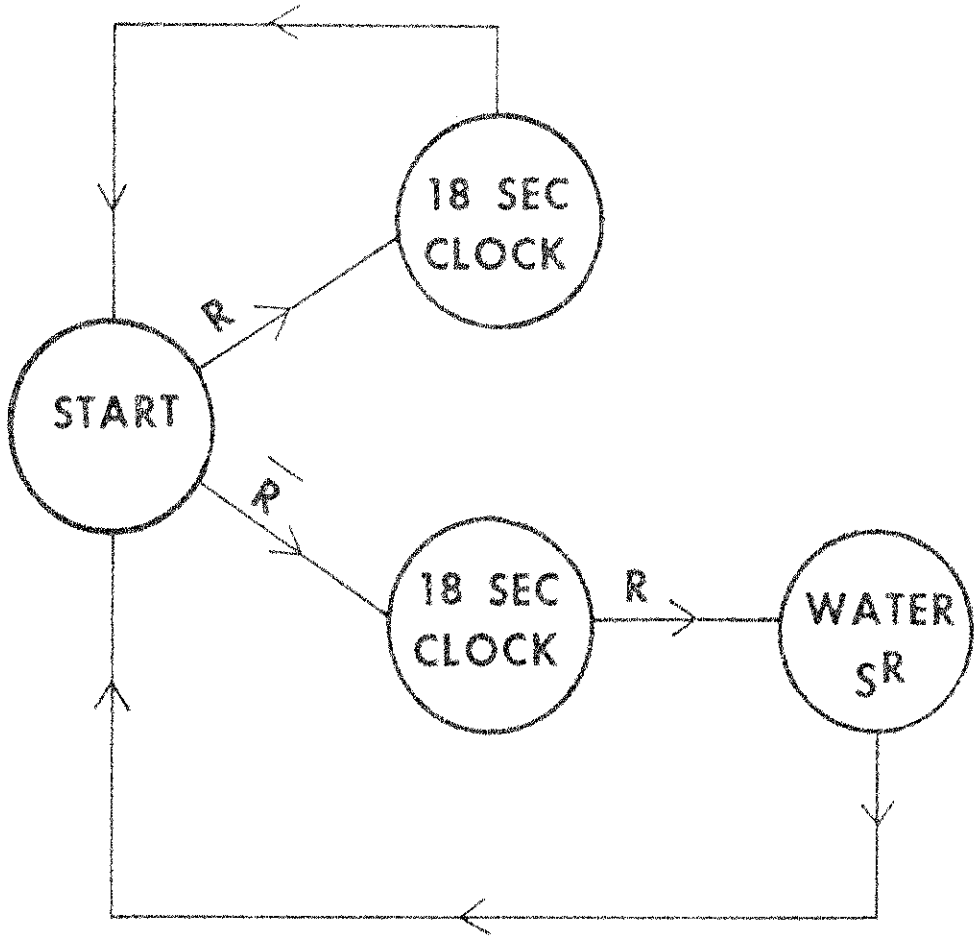
instantaneous transition from one state or condition to the next (Clark et al., 1974). Under this schedule, a lever response made at least 18 seconds after the preceding lever response was followed by water reinforcement. The DRL 18 second schedule requires that the animal make a timing discrimination by waiting at least 18 seconds between responses. Thus, the schedule characteristically generates very low, stable rates of spaced responding in a testing session. All rats received 34 days of training on the DRL 18 second schedule, followed by 40 additional training sessions in which saline was injected 20 minutes before the session. Drug testing began when the baseline for all animals was considered stable, that is, when the response and reinforcement measures no longer showed systematic changes from session to session.

Phase 2: Methylphenidate Dose-Effect Curves.--Dose-response measurements were obtained for six doses of methylphenidate. Methylphenidate or saline was injected on Tuesdays and Fridays, 20 minutes before the session. A repetition of the 10 mg/kg dose of methylphenidate did not produce a rate-increasing effect equal in magnitude to that of the first administration of that dose. Therefore, dose-response measurements for all animals were repeated for five doses, excluding the lowest dose (1.25 mg/kg). A third administration of the 5 and 10 mg/kg doses of methylphenidate was then given to all animals.

Phase 3: Chronic Methylphenidate Administration.--The rats were separated into three groups which were matched as closely as possible for their mean response to the third 10 mg/kg methylphenidate test dose. One group (N=7) received daily methylphenidate 10 mg/kg, 20 minutes before the session, five days a week. A second group (N=8) received daily methylphenidate 10 mg/kg immediately after each experimental session, five days a week. A third group (N=16) received 10 mg/kg of methylphenidate, 20 minutes pre-session, on the other three experimental days. Each group was maintained on this regimen for 77 sessions. On the 78th day of daily administration all three groups were given methylphenidate 10 mg/kg, 20 minutes pre-session.

Phase 4: Cross-Tolerance Tests.--The procedure in Phase 4 was identical to that in Phase 3 except that on alternate Fridays cross-tolerance tests were conducted. Test doses of methylphenidate (10 mg/kg), d-amphetamine (1.25 mg/kg), l-amphetamine (2.5 mg/kg), methamphetamine (0.6 mg/kg), and saline were given to all rats, 20 minutes pre-session. The experiment ended after 240 sessions.

Drugs

All drugs were dissolved in 0.9% saline solution and injected i.p. in a volume of 1 ml/kg body weight. All doses refer to the weight of the salt. Methylphenidate hydrochloride was a gift of CIBA-Geigy. l-amphetamine sulfate was a

gift of Smith Kline and French Laboratories. d-amphetamine sulfate and methamphetamine hydrochloride were obtained from Sigma Chemical.

Catecholamine Assay

Changes in whole brain catecholamine concentrations after administration of methylphenidate were measured following synthesis inhibition with α-methyl tyrosine (Figure 7). Twenty rats, weight approximately 300 g, were injected with α-methyl-para-tyrosine methyl ester (Sigma Chemical), 150 mg/kg of the free base. Twenty minutes later, the rats were injected with either saline or methylphenidate 10 mg/kg. Two hours after the second injection, each rat was decapitated. The brain was removed immediately and stored in liquid nitrogen until assayed. The tissue samples were frozen within five minutes after death because the catecholamine content of the brain decreases rapidly post-mortem at room temperature (Nagatsu, 1973). Catecholamines were extracted and then purified by cation-exchange chromatography (Bertler, Carlsson, and Rosengren, 1958). Norepinephrine and dopamine concentrations of each brain were measured fluorimetrically according to the method of Laverty and Taylor (1968).

Catecholamine Extraction.--The extraction and purification of norepinephrine and dopamine were conducted at acid pH because catecholamines are highly unstable at alkaline pH (Nagatsu, 1973). The catecholamines were extracted from the

Whole Brain

|←——Store -80°C
10 ml 0.4N perchloric acid——✕——Homogenize at 4°C
|←——Centrifuge at 4°C

Supernatant

0.1 ml 2% ascorbic acid——→
0.25 ml 4% EDTA——→
|←——Adjust pH to 6.5 with
    5N K_2CO_3
|←——Centrifuge at 4°C

Dowex 50W-X4
(200-400 mesh, Na^+ & K^+ form, buffered at pH 6.5 with
0.1M phosphate buffer)

5 X 70 mm column

60 ml H_2O——✕——Washed
3.5 ml 1N HCl——✕——Washed
8 ml 1N HCl——✕——Eluted
|——→Eluate (Norepinephrine)
8 ml 2N HCl——✕——Eluted
|——→Eluate (Dopamine)

Eluate

|←——Lyophilize

Dried Residue

1 ml 0.01N HCl——✕——Reconstitute

Catecholamine Samples

0.5 ml 0.1M PO_4 buffer
0.02N I_2 in NaI 5% w/v——✕—— Iodine oxidation
alkaline sulfite solution——✕—— Tautomerization
glacial acetic acid——✕—— Acidification
|←——Read on Fluorometer
    380/480 nm for norepinephrine
    320/375 nm for dopamine
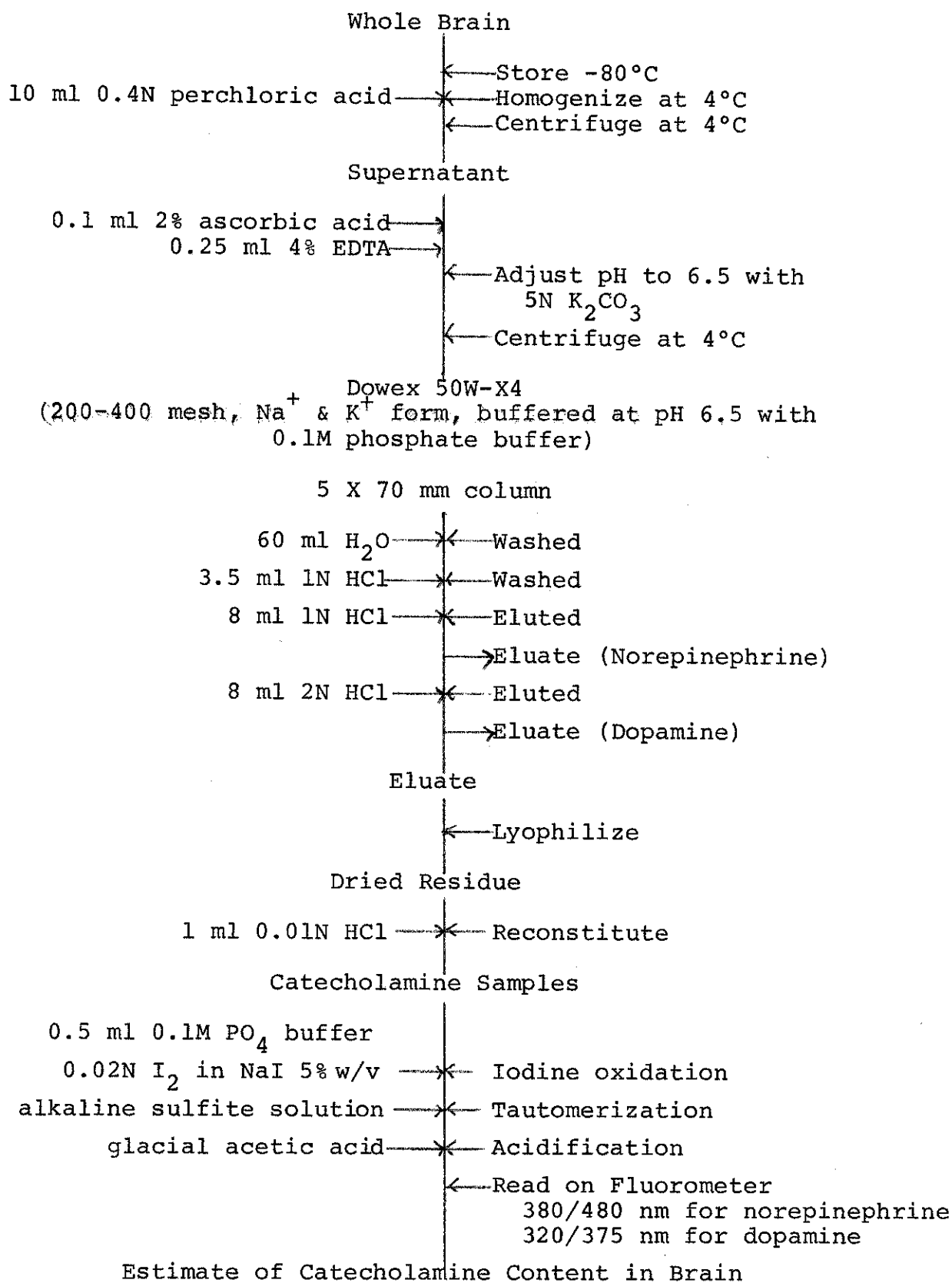
Estimate of Catecholamine Content in Brain

Fig. 7.--Flow chart of catecholamine assay.

whole brain tissue samples and proteins were precipitated
with perchloric acid. The perchloric acid was subsequently
removed by neutralizing the sample with potassium carbonate
(K_2CO_3) to pH 6.5, at 4°C, and then centrifuging (30,000 g)
at 4°C to remove the potassium perchlorate ($KClO_4$) precipi-
tate. This allows the catecholamines to be purified by ion-
exchange chromatography without prior isolation on alumina
(Nagatsu, 1973).

Each whole brain sample was weighed and homogenized for
30 seconds with a Polytron Kinematic GmbH (Brinkmann Instru-
ments, Inc., Westbury, New York) in 6 ml 0.4N perchloric acid.
The polytron was rinsed with 4 ml 0.4N perchloric acid and
the 4 ml rinse was added to the sample. The combined 10 ml
homogenate was centrifuged at 30,000 g for 20 minutes at 4°C
to remove the proteins. The supernatant was decanted and
frozen.

Catecholamine Purification.--A strong cation-exchange
column was used to purify dopamine and norepinephrine. The
Dowex resin (AG 50W-X4, 200-400 mesh, Bio-Rad Laboratories,
Richmond, California) was prepared by washing with 2N HCl,
then rinsing with H_2O to neutral pH. This procedure was
repeated using 2N NaOH and rinsing with H_2O to neutrality.
The resin was loaded under water into 7 cm glass columns,
5 mm in diameter. 1 cm glass wool was packed into the tip
of each column; 5 cm of resin was then added.

The columns were assembled on a syringe apparatus and rinsed with 20 ml 0.1 M PO_4 buffer with 0.1% EDTA at pH 6.5, followed by 5 ml H_2O. The tissue samples were thawed and prepared for the columns: 0.1 ml of freshly prepared 2% ascorbic acid and 0.25 ml of 4% EDTA were added to each sample. The samples were then neutralized to pH 6.5 with 5N K_2CO_3 at 0°C and centrifuged at 30,000 g for 20 minutes at 4°C. The nautralized samples were applied to the Dowex columns at a slow rate (approximately 0.5 ml/minute) and washed with 60 ml H_2O. Column recovery standards for NE and DA were treated similarly.

Preliminary studies measured the elution curve for NE and DA on these columns using 50 μg each of norepinephrine and dopamine standards taken to 10 ml, eluting using the native fluorescence method. The results indicated that good separations of norepinephrine and dopamine are obtained by eluting first with 12.5 ml 1N NCl and then with 8 ml 2N HCl. Based on the results of these elution curves of catecholamine standards, the norepinephrine in the tissue samples was eluted with 12.5 ml 1N NCl; the first 3.5 ml were discarded. Dopamine was then eluted with 8 ml 2N NCl. The eluate was collected and stored at -80°C. The eluate from the columns was evaporated to dryness under a vacuum and reconstituted with 1 ml 0.01N HCl.

Catecholamine estimation.--After separation of dopamine and norepinephrine with cation-exchange columns, the

concentrations of these catecholamines in the brain tissue samples were estimated using a modification of the fluorimetric hydroxyindole assay of Laverty and Taylor (1968). Their procedure specifies 0.1 to 0.5 ml of sample brought to a total volume of 1.1 ml with a pH 6.5, 0.1 M phosphate buffer. The procedure used in the present experiment took 1.0 ml of sample and added 0.5 ml of phosphate buffer. Since concentrations of catecholamines in brain tissue are on the order of 500 ng per gram of tissue, a highly sensitive and specific method such as fluorometry is necessary. Although catecholamines have native fluorescence due to their phenol ring, it is nonspecific and can be used only to detect total catecholamine content. Therefore, estimates of purified catecholamines are usually made by converting these compounds to derivatives which have specific fluorescence in the visible wavelength. The Laverty and Taylor fluorimetric assay is based on the conversion of catecholamines to the highly fluorescent trihydroxyindoles under conditions which ensure maximal fluorescence and sensitivity (Figure 8).

The trihydroxyindole reaction proceeds in two steps: oxidation and subsequent rearrangement (Figure 8). The reconstituted amines were oxidized with iodine solution (0.02N I_2 in NaI 5% w/v) in a buffer solution of 0.1M PO_4 (for norepinephrine, pH 6.5; for dopamine, pH 7.0). After an optimal oxidation time (3 minutes), the oxidation was stopped and the fluorophore was stabilized with an
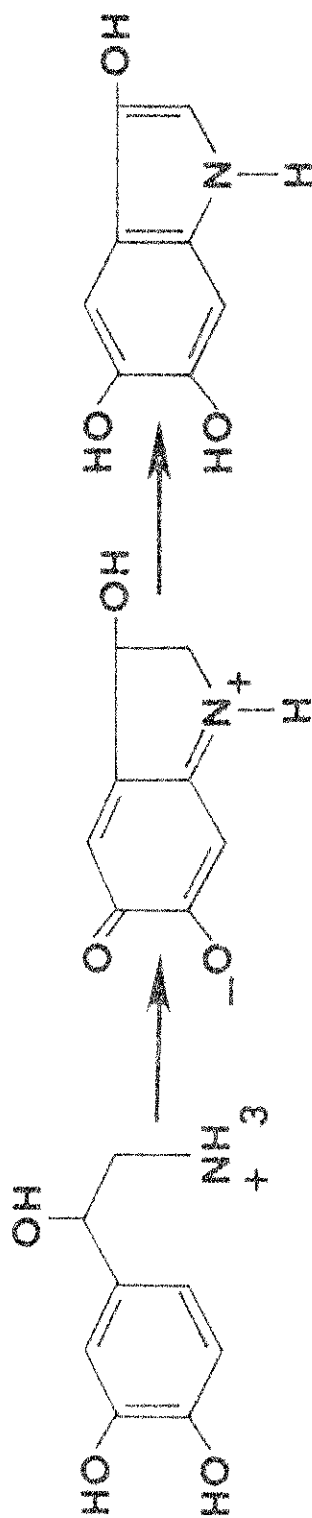
Fig. 8—Trihydroxyindole Reaction for Norepinephrine

antioxidant alkaline sulfite solution ($Na_2SO_3 \cdot 7H_2O$) 2.5% w/v,
Na_2EDTA 1% w/v in 2.5N NaOH). Since acidification of the
alkaline reaction mixture gives maximal fluorescence, the
final pH for maximal fluorescence is obtained by adding gla-
cial acetic acid. The dopamine samples were subsequently
heated at 100°C. After the specified development time (25
minutes for norepinephrine; 40 minutes for dopamine), the
samples were read on the fluorometer. The excitation/
emission wavelengths were 380/480 nm for the norepinephrine
samples and 320/375 nm for the dopamine samples. Samples
with added internal norepinephrine or dopamine standards and
a reagent blank were run in parallel with the unknown sam-
ples.

Analysis of Data

Repeated measures analysis of variance (Winer, 1971) was
used to test the results of the dose-effect and cross-
tolerance measurements. Newman-Keuls test was used to detect
differences between treatments whenever the results of an
analysis of variance were significant. Differences between
means for whole brain catecholamine levels after treatment
with α-methyl tyrosine or α-methyl tyrosine and methylpheni-
date were tested using two-sided t-tests for independent
samples (Dixon and Massey, 1969).

CHAPTER III

RESULTS

Behavioral Experiment

Phase 1: Behavioral Training.--After 74 days of train-
ing on the DRL 18 second schedule, all animals had developed
stable, low rates of responding. Mean response rate for all
31 rats was 3.30 responses per minute, and the mean propor-
tion of reinforcements earned was 0.510. Standard errors
were less than 1.5% of the means. This control performance
is comparable to baseline data obtained in earlier experi-
ments on tolerance to amphetamine-like drugs using a DRL
schedule (Schuster et al., 1966; Pearl and Seiden, 1976).
Figure 9 shows the acquisition of stable responding for a
representative animal.

Phase 2: Methylphenidate Dose-Response Measurements.--
Methylphenidate produced a dose-dependent increase in the
rate of responding (Figure 10) and a dose-dependent decrease
in the reinforcements earned (Figure 11). Peak rate increas-
ing effects occurred at 20 mg/kg (338% of saline control).
A repetition of the 10 mg/kg dose gave a less pronounced
increase in response rate (186% of saline control compared
to 293% for the first administration of that dose). There-
fore, the dose-response measurements for four additional doses

54

Fig. 9.--Acquisition of stable responding on the DRL
18 second schedule for a representative rat. Right hand
ordinate: mean rate of responding for two sessions as
responses per minute (open circles). Left hand ordinate:
percent available reinforcements earned where 100% equals
150 reinforcements in the 45 minute session (closed cir-
cles).

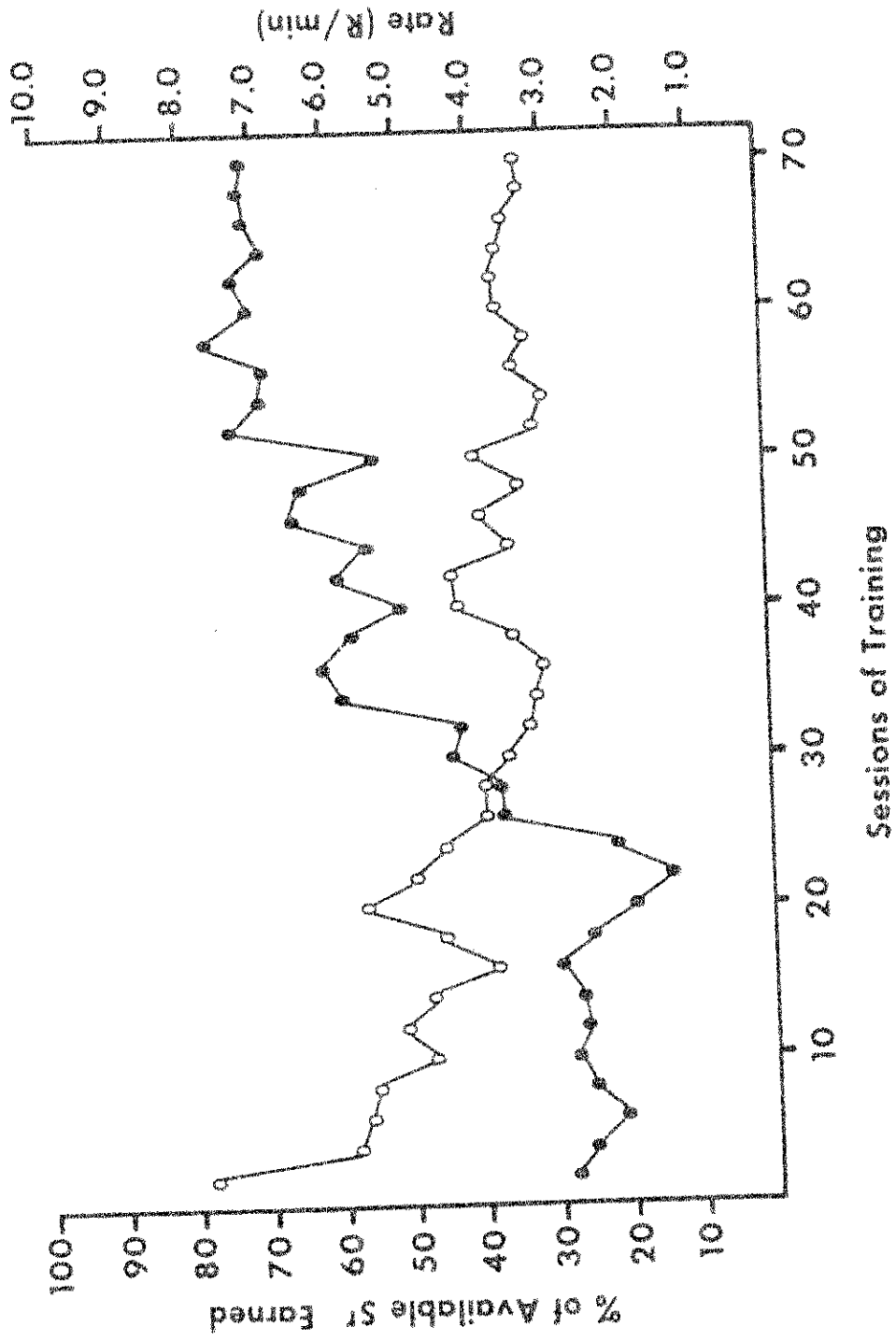Fig. 10.--The effects of methylphenidate on mean response
rate for all subjects (N = 31). Measurements for methylpheni-
date or saline were made twice weekly. Saline point (3.31 ±
0.49; mean ± standard deviation) represents the mean for
four administrations. Solid line represents the first dose-
response determination. Unjoined points represent results
for a third determination for the 5.0 and 10.0 mg/kg doses.

Fig. 11.--The effects of methylphenidate on mean percent-
age of available reinforcers earned where 100% equals 150
reinforcements in the 45 minute session (N = 31). Measure-
ments for methylphenidate or saline were made twice weekly.
Saline point (0.49 ± 0.10; mean ± standard deviation) repre-
sents the mean for four administrations. Solid line repre-
sents the first dose-response determination. Broken line
represents the second dose-response determination. Unjoined
points represent results for a third determination for the
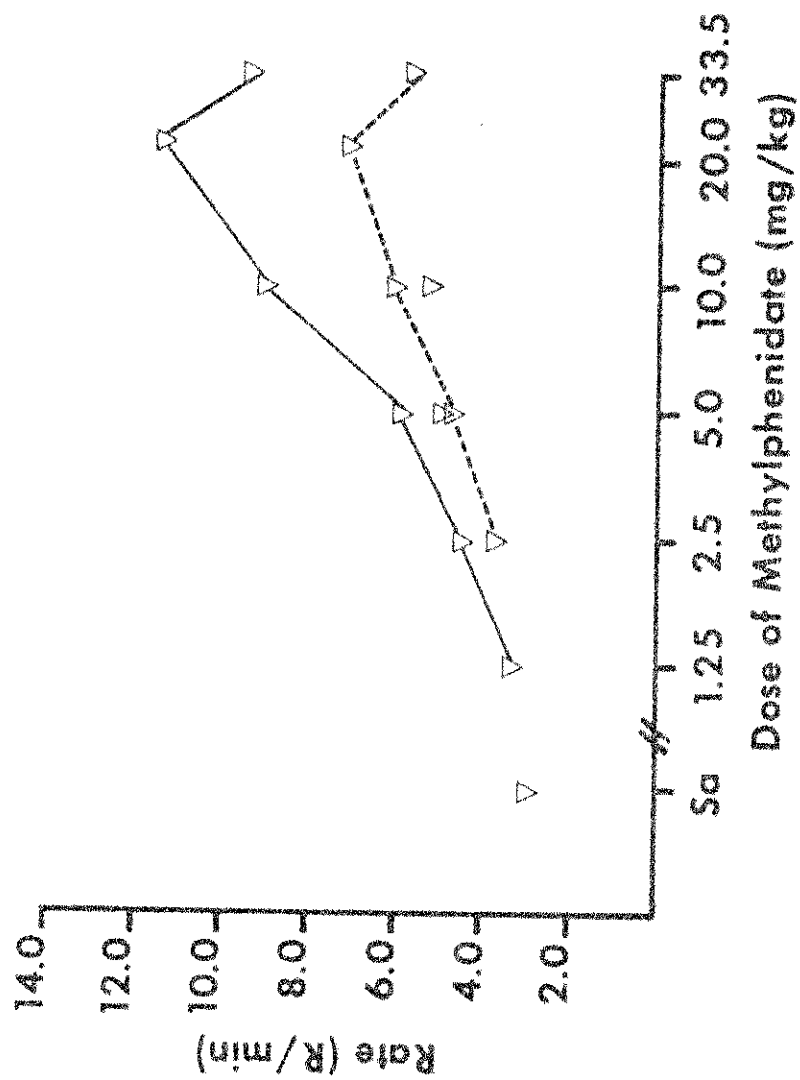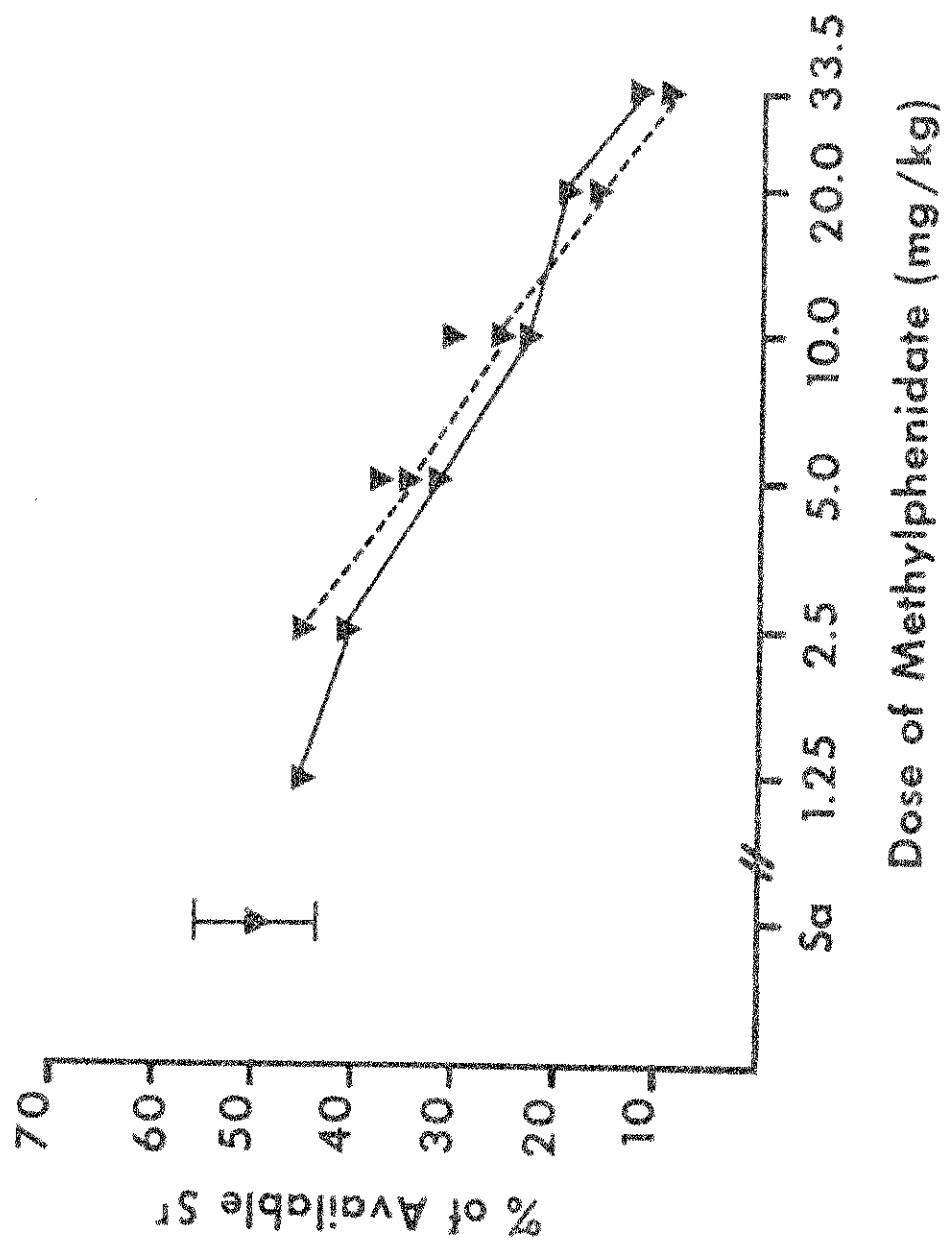5.0 and 10.0 mg/kg doses.

% of Available St vs. Dose of Methylphenidate (mg/kg)

of methylphenidate were repeated to determine if the dose-
effect curve had shifted, since tolerance is characterized by
a shift of the dose-effect curve to the right.

The dose-effect curve for the response rate for the
second administration showed a reduced drug effect for all
doses tested (Figure 10). A peak rate-increasing effect was
observed again for the 20 mg/kg dose, but the rate increase
was 219% of saline control compared to 338% for the first
administration of that dose. A 2 x 5 (administration order
vs. drug dose) repeated measures analysis of variance of the
results for the response rate from the two repetitions of the
2.5 through 33.5 mg/kg doses showed a significant effect for
order of administration (p < .05) and for drug dose (p < .01).

The second administration also produced a dose-dependent
decrease in the proportion of reinforcements earned (Figure
11). However, while the decrease in reinforcements earned
for the 2.5, 5.0, and 10.0 mg/kg doses was less than that
obtained for the first administration, the decrease in rein-
forcements earned for the 20.0 and 33.5 mg/kg doses was
greater than that observed for the first administration. A
2 x 5 (administration order vs. drug dose) repeated measures
analysis of variance for the reinforcement measure showed a
significant dose effect (p < .01) but showed no significant
administration order effect.

A third administration of the 10 mg/kg dose produced a
further reduction in the drug effect, with the values for

both rate of responding and reinforcements earned closer to
saline control values. Thus, three exposures to the 10 mg/kg
dose of methylphenidate produced a successive reduction in
the drug effect. Response rates obtained for the first,
second, and third administrations of the 10 mg/kg dose were
293%, 186%, and 160% of saline control, respectively (Figure
12). The reinforcements earned for the first through third
administrations were 48%, 53%, and 64% of saline control
(Figure 13). Three administrations of the 5 mg/kg dose also
produced a successive increase in reinforcements earned. The
response rate obtained for the third administration of the 5
mg/kg dose was less than that obtained for the first admini-
stration of that dose but slightly higher than that obtained
for the second administration. A 2 x 3 (drug dose vs. admini-
stration order) repeated measures analysis of variance for
the reinforcement data showed a significant effect of both
dose and order of administration for the 5 and 10 mg/kg doses.
A Newman-Keuls test applied to the means indicated that the
results for the first and third administrations were signifi-
cantly different (p < .01). A 2 x 3 (drug dose vs. admini-
stration order) repeated measures analysis of variance for
the response rate also showed a significant effect of dose
and order of administration. A Newman-Keuls test applied to
the means indicated that the first administration was signif-
icantly different from the second and from the third admini-
stration (p < .01).

Fig. 12.--Effects of three administrations of methyl-
phenidate 10 mg/kg on response rate (N = 31). Data are
expressed as percentage of the pre-drug saline control
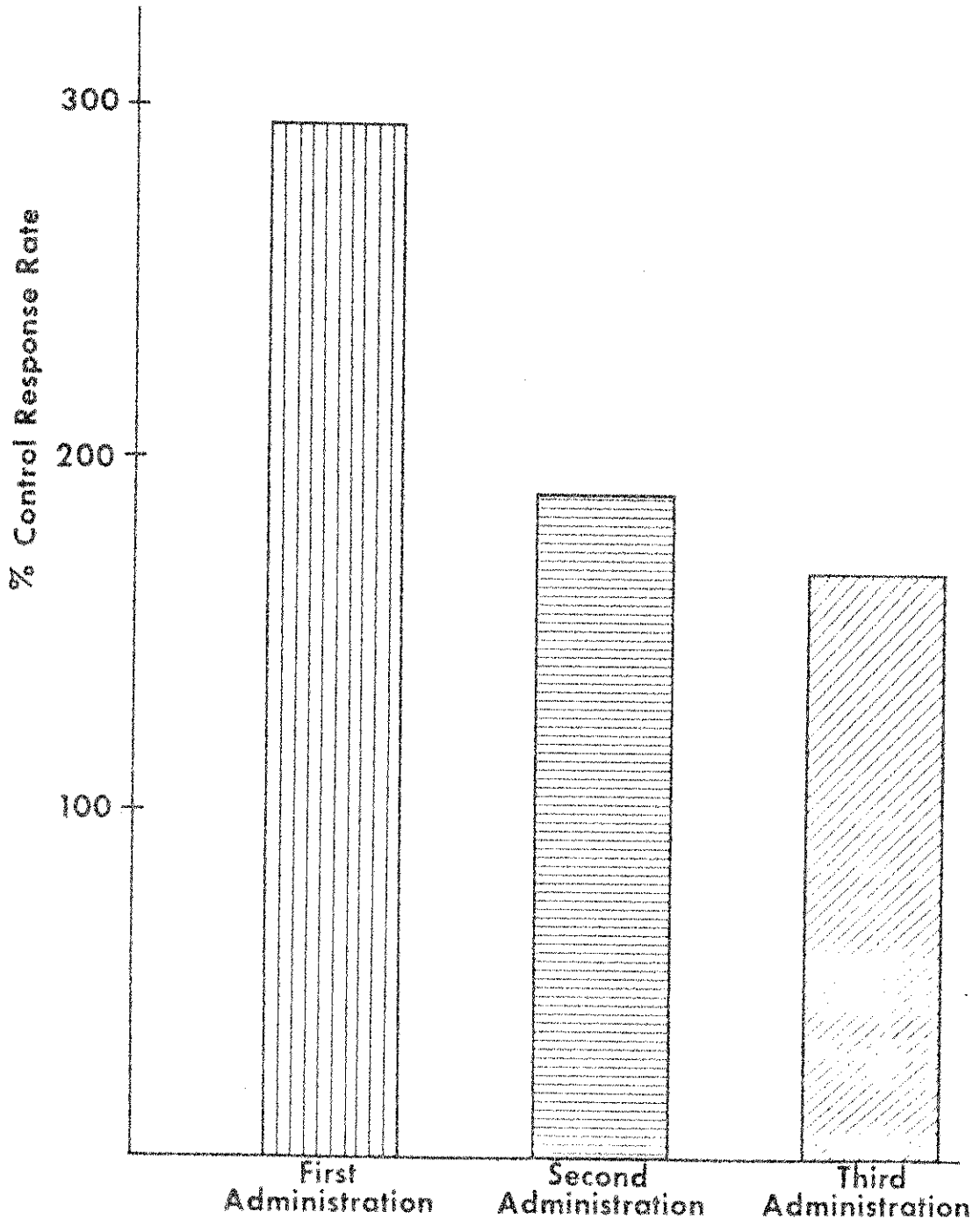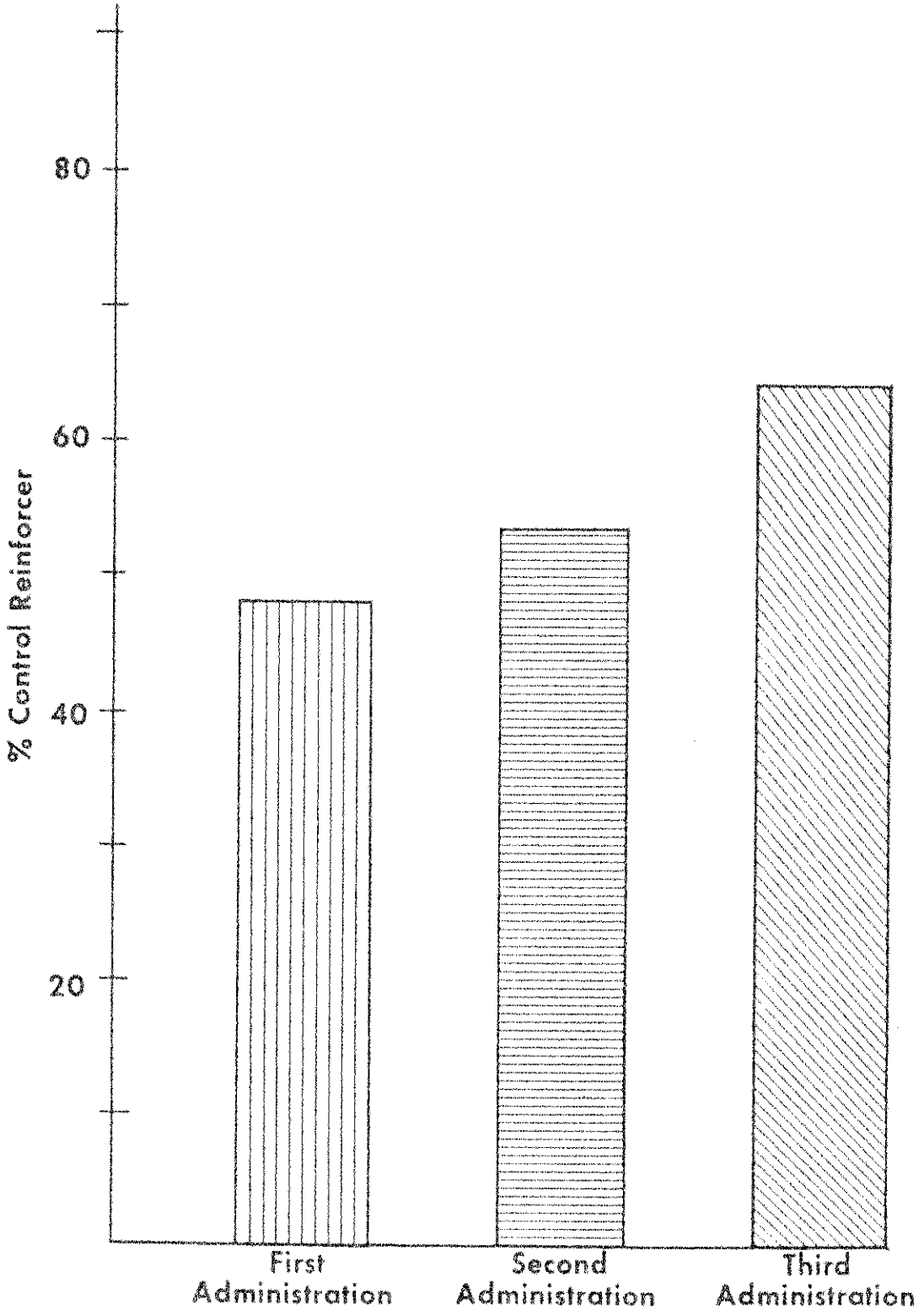response rate ($\bar{X}$ = 3.323).

Fig. 13.--Effects of three administrations of methyl-
phenidate 10 mg/kg on reinforcements earned (N = 31). Data
are expressed as percentage of the pre-drug saline control
proportion of available reinforcements earned ($\bar{x}$ = 0.494).

Although the 20 mg/kg dose produced peak rate-increasing effects for both administrations, it was not selected for chronic administration because this dose, and the 33.5 mg/kg dose, produced extremely variable response rates. In addition, these high doses appeared to dissociate lever responding from reinforcement consumption; even when responding resulted in reinforcement, water was not consumed. Faidherbe, Richelle, and Schlag (1962) have reported that cats which were trained on a multiple discrimination schedule did not consume all of their earned milk reinforcements after injections of 6 mg methylphenidate. This result may be related to the intense stereotyped behavior elicited by these doses, and, in part, may explain why the mean response rates during the second administration of these doses appear to return towards base- line while the reinforcements obtained are further reduced; the decrease in mean response rate for the second administra- tion of 20 and 33.5 mg/kg is actually a consequence of an increase in the number of rats who made no responses through- out the session. Doses of 10 mg/kg or less produced a more homogeneous effect on responding and did not dissociate responding from reinforcement. Therefore, the 10 mg/kg dose was selected for chronic administration.

Phase 3: Chronic Methylphenidate Administration.--The data obtained from the dose-response measurements suggested that intermittent administration of methylphenidate resulted in a reduced drug effect. Therefore, the effect of daily and

intermittent administration of methylphenidate was further determined for the 10 mg/kg dose. The three groups in this phase of the experiment were matched for their mean response to the third administration of the 10 mg/kg test dose of methylphenidate (Figures 14, 15, and 16), and all three groups showed the same qualitative reduction in drug effect across the three administrations of 10 mg/kg methylphenidate during dose-effect testing.

Daily post-session administration of methylphenidate produced no systematic deviation from the pre-drug saline control values for rate and reinforcement across 130 sessions of administration (Figure 14). Daily pre-session administration of methylphenidate resulted in the development of tolerance to both the rate-increasing and reinforcement-decreasing effects of methylphenidate (Figure 15). Fluctuations in data during daily pre-session administration are probably related to equipment problems occurring during one session in weeks 11 and 16 which disrupted the animals' performance for several days after each aborted session. During the cross-tolerance phase of the experiment, tolerance to the rate-increasing effects of methylphenidate continued to develop; at the end of the experiment the response rate was within 110% of the saline control value. The number of reinforcements received continued to stay at approximately 90% of the control value throughout the cross-tolerance phase of the experiment.

Fig. 14.--Effect of daily post-session administration

of methylphenidate, 10 mg/kg (N = 8). Closed symbols repre-

sent percentage of saline control reinforcements earned.

Open symbols represent percentage of saline control response

rate. The points to the left of the vertical dividing line

are data from the three administrations of the 10 mg/kg dose

of methylphenidate obtained during dose-response determina-

tions. Points to the right of the vertical line represent

the mean response rate and reinforcement values for five days

of post-session administration for weeks 1 through 18. Cross-

tolerance testing occurred during weeks 19 to 27. Post-

session testing was continued during this phase; the unjoined

symbols represent the values for either four or five post-

session administrations each week.

Fig. 15.--Effect of daily pre-session administration of methylphenidate, 10 mg/kg (N = 7). Closed symbols represent percentage of saline control reinforcements earned. Open symbols represent percentage of saline control response rate. The points to the left of the vertical dividing line are data from the three administrations of the 10 mg/kg dose of methylphenidate obtained during dose-response determinations. Points to the right of the vertical line represent the mean response rate and reinforcement values for five days of pre-session administration for weeks 1 through 18. Cross-tolerance testing occurred during weeks 19 to 27. Pre-session testing was continued during this phase; the unjoined symbols represent the values for either four or five pre-session administrations each week.
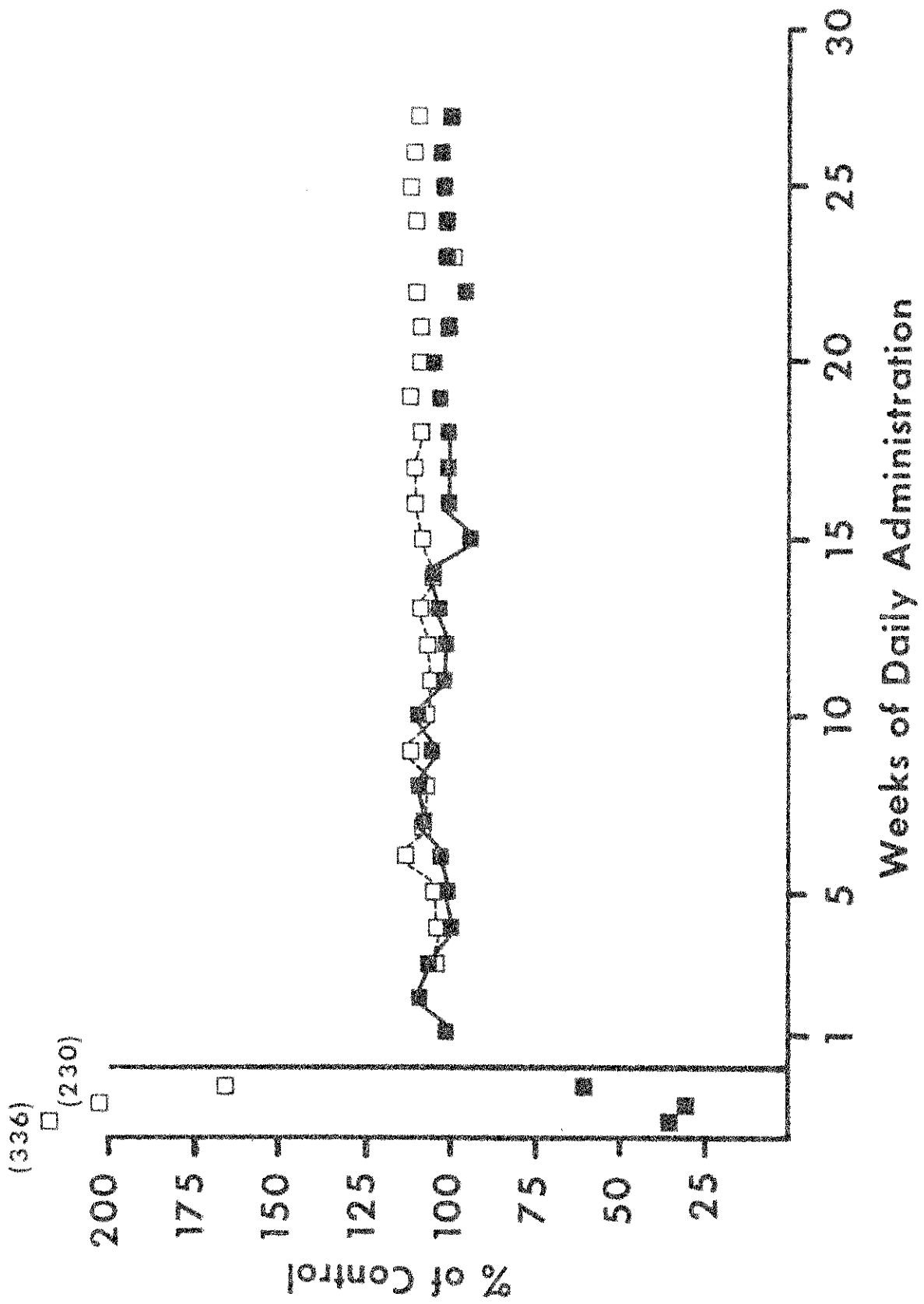
Fig. 16.--Effects of semi-weekly administration of methylphenidate, 10 mg/kg (N = 16). Closed symbols represent percentage of saline control reinforcements earned. Open symbols represent percent saline control response rate. The points to the left of the vertical dividing line are data from the three administrations of the 10 mg/kg dose of methylphenidate obtained during dose-response determinations. Points to the right of the vertical line represent the mean response rate and reinforcement values for two days of pre-session administration for weeks 1 through 18. Cross-tolerance testing occurred during weeks 19 to 27. Semi-weekly pre-session testing was continued during this phase; the unjoined symbols represent the values for either one or two pre-session administrations each week.
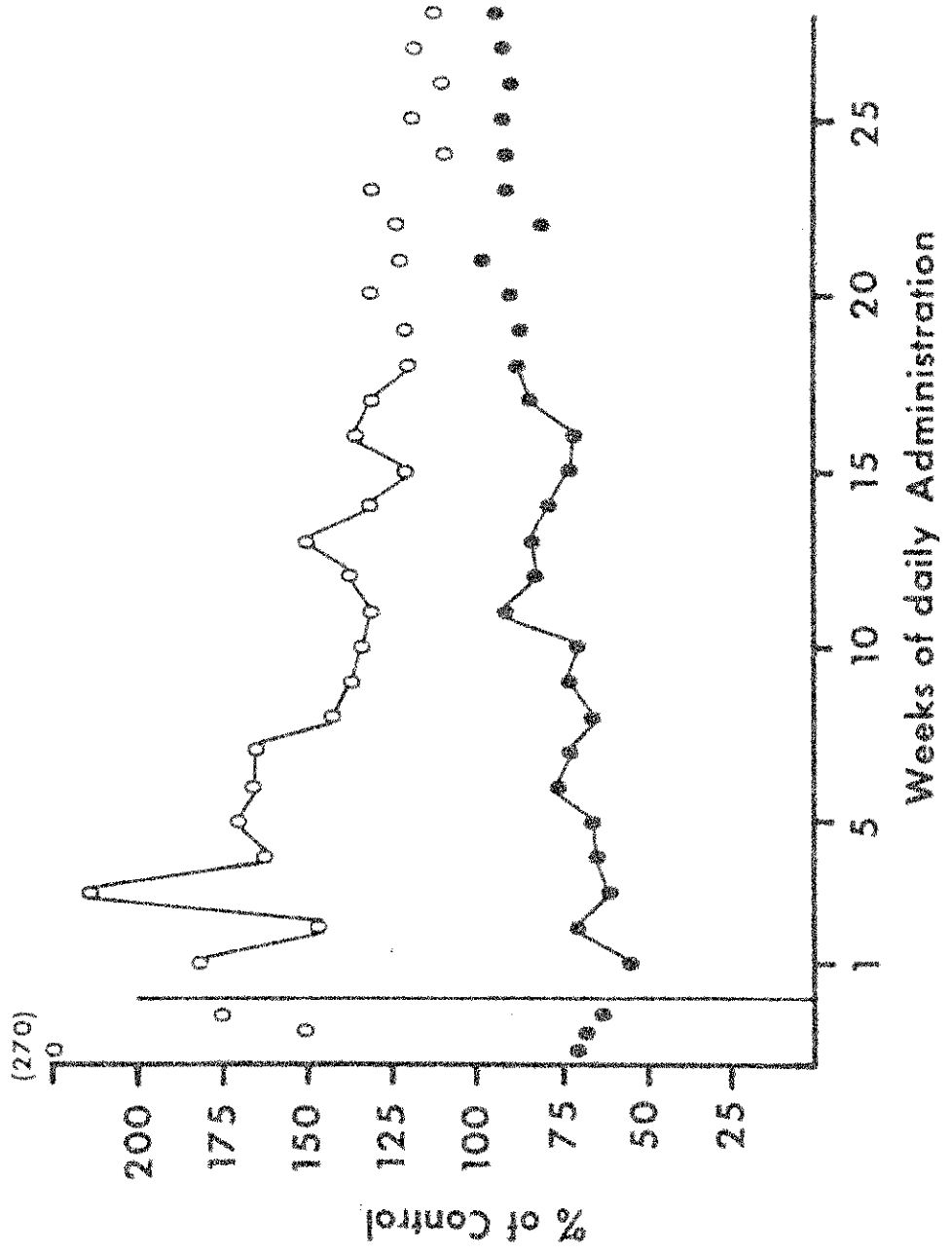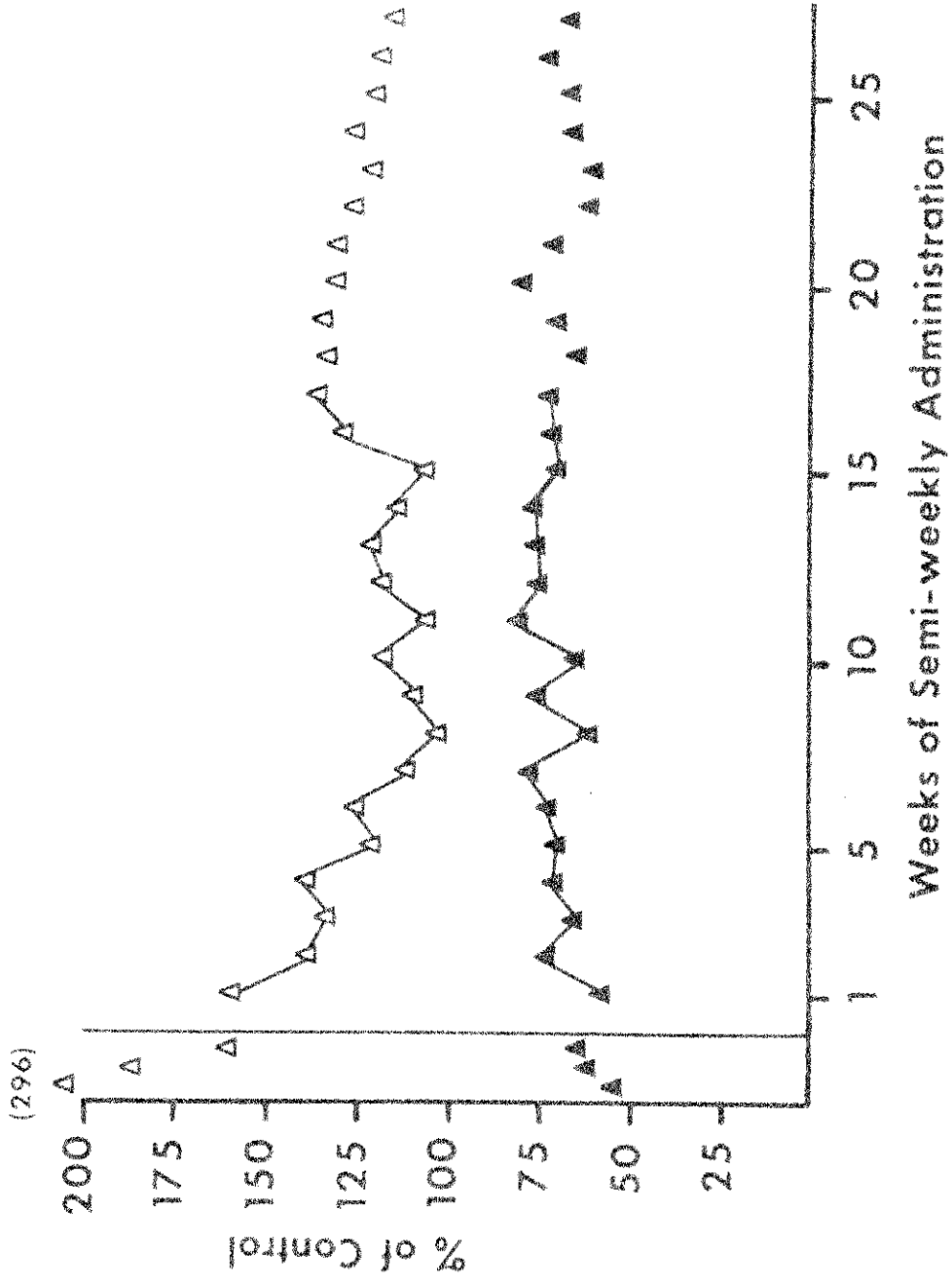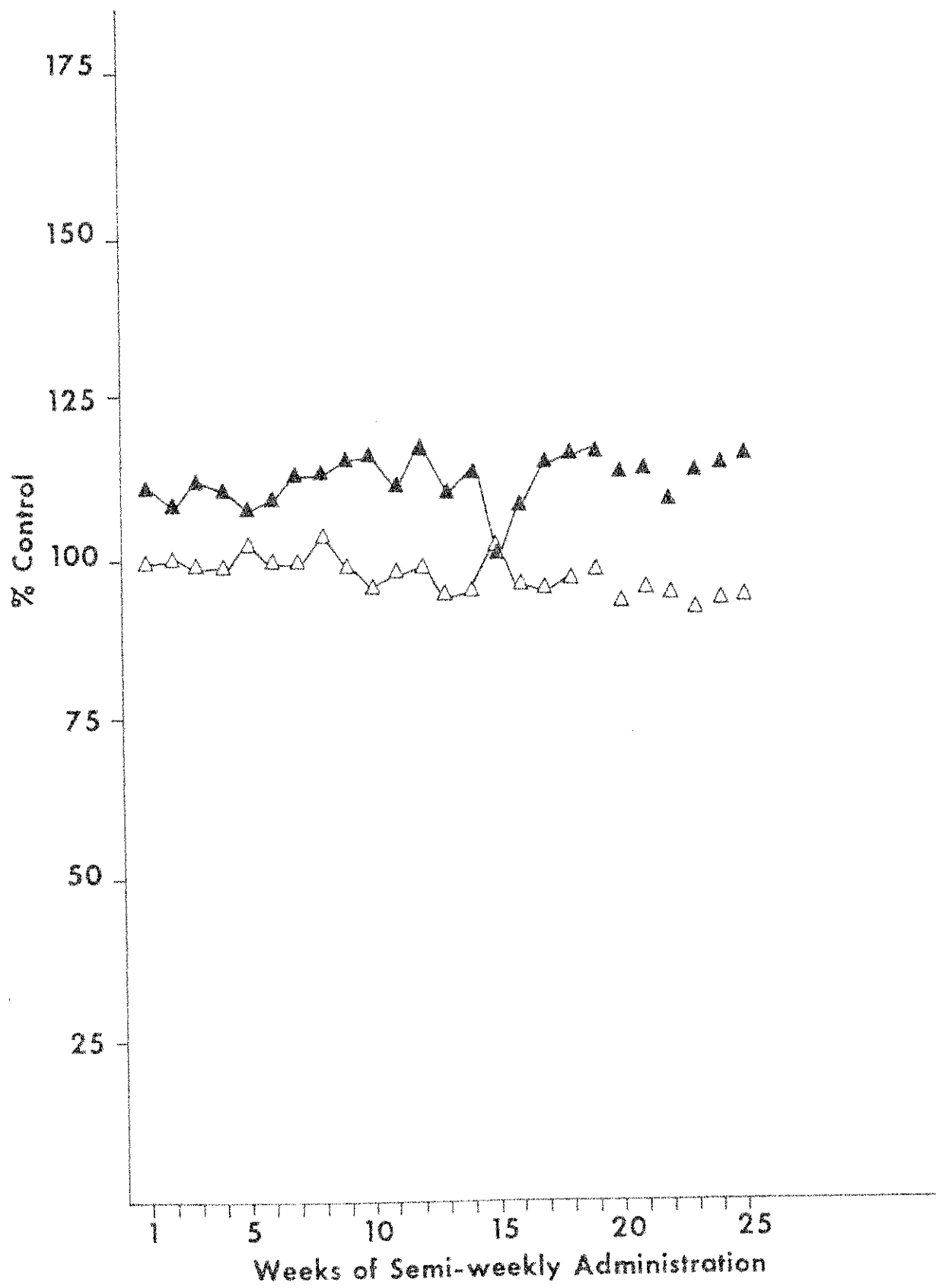
% of Control

Weeks of Semi-weekly Administration

Semi-weekly administration of methylphenidate produced a decrease in the initial rate-increasing effects of the drug after 14 weeks of intermittent administration (Figure 16). However, the increase in the proportion of reinforcements earned was not as great as that observed for the daily pre-session group. Thus, the degree of tolerance in this group appears not to be as great as that of the daily pre-session administration group. The semi-weekly group showed a sudden rate increase after 15 weeks of intermittent administration which can be accounted for by an increase in the rate of responding of four of the sixteen animals in the group. The four animals showed negligible rate-increasing effects after methylphenidate administration between weeks 8 and 15; their response rates were near their pre-drug baseline. However, beginning with the 16th week, these animals showed a significant methylphenidate-induced increase in responding and their response rates after methylphenidate administration remained high throughout the rest of the experiment.

The results for the three days of saline administration to the group receiving twice-weekly methylphenidate pre-session are presented in Figure 17. The number of reinforcements earned fluctuated around 112% of their pre-drug saline control value, and the reinforcement baseline for the saline administrations shows a slight positive slope across the 26 weeks of twice-weekly drug administration. Similarly, the response rate slowly decreased to approximately 92% of the

Fig. 17.--Saline baseline for animals receiving semi-weekly methylphenidate 10 mg/kg (N = 16). Closed symbols represent percentage of pre-drug saline control reinforcements earned. Open symbols represent percentage of the pre-drug saline control response rate. Each point represents the mean response rate and reinforcement values for three days of saline administration for weeks 1 through 18. Cross-tolerance testing occurred during weeks 19 to 27. Saline administration was continued during this phase; the unjoined symbols represent the values for three saline administrations each week.

pre-drug saline control value during the chronic drug admin-
istration phase of the experiment.

Phase 4: Cross-Tolerance Tests.--During weeks 18
through 26, test doses of methylphenidate, d-amphetamine,
l-amphetamine, methamphetamine, and saline were given to all
three groups, 20 minutes pre-session, to determine if cross-
tolerance would occur to drugs with behavioral properties
similar to methylphenidate. The doses chosen for the cross-
tolerance tests were selected because previous experiments
with rats have demonstrated that these doses are comparable
in their behavioral effects. Methylphenidate and d-ampheta-
mine have a potency ratio of about 1:8 for their rate-
increasing effects on DRL responding (Pearl and Seiden, 1976);
d-amphetamine and l-amphetamine have a potency ratio of
approximately 2:1 for their rate-increasing effects on fixed
interval responding (Tilson and Sparber, 1973); methampheta-
mine and d-amphetamine have a potency ratio of about 1:1 for
their anorexigenic effects and a potency ratio of about 3:1
for their effects on avoidance responding (Cox and Maickel,
1972).

Table 2 presents the results of the cross-tolerance tests
for the reinforcers earned for the three groups. The results
for the daily pre-session administration group were very
close to their training control value. The post-session
group showed the greatest effects for all drugs tested and

Table 2

Percentage of control reinforcers earned during cross-tolerance
tests after different chronic treatments with methylphenidate[a]

Test Treatment	Daily Treatment		
	MP Daily	MP Semi-weekly	MP Post-session Daily
saline	103.1 ± 4.4	113.4 ± 8.3	105.8 ± 12.1
methylphenidate (10 mg/kg)	97.3 ± 14.3	74.0 ± 9.7	65.1 ± 12.7[b]
d-amphetamine (1.25 mg/kg)	90.8 ± 8.4	69.4 ± 6.0	50.7 ± 9.3[b]
l-amphetamine (2.5 mg/kg)	97.3 ± 5.4	81.1 ± 6.6	71.5 ± 6.9[b]
methamphetamine (0.6 mg/kg)	98.1 ± 7.5	86.0 ± 6.5	67.0 ± 13.1[b]

[a] Data are presented as the mean ± S. E. percentage of saline control rein-
forcers for each treatment group.

[b] Differs significantly from methylphenidate daily pre-session groups by
Newman-Keuls analysis ($p < .01$).

the semi-weekly group feel between the pre-session and the post-session groups. The results of the methylphenidate pre-session test dose administered to the post-session group, following 77 sessions of post-session administration, produced a slightly reduced drug effect on reinforcements earned compared to that obtained for the third 10 mg/kg dose given during the dose-response phase of the experiment (65% saline control compared to 61%). A 3 x 4 (groups vs. drug treatment) repeated measures analysis of variance for the reinforcement data showed a significant group effect. A Newman-Keuls test applied to the means indicated that the pre- and post-session groups were significantly different ($p < .01$).

Table 3 presents the results of the cross-tolerance tests for response rate for the three groups. The results for the methylphenidate, d-amphetamine, and methamphetamine test doses follow the pattern observed in Table 2 for reinforcements earned, with the post-session group demonstrating the greatest drug effect. When administered 10 mg/kg methylphenidate pre-session, the post-session group showed a rate-increasing effect which was slightly less than that obtained for the third administration of the 10 mg/kg dose given during the dose-response phase of the experiment (159% of saline control compared to 165%). A 3 x 4 (groups vs. drug treatment) repeated measures analysis of variance for the response rate showed no significant group effect.

Table 3

Percentage of control response rate during cross-tolerance tests after different chronic treatments with methylphenidate[a,b]

Test Treatment	Daily Treatment		
	MP Daily	MP Semi-weekly	MP Post-session Daily
saline	93.3 ± 7.1	96.1 ± 5.6	109.0 ± 5.3
methlyphenidate (10 mg/kg)	125.9 ± 17.9	138.2 ± 24.6	158.8 ± 19.5
d-amphetamine (1.25 mg/kg)	140.0 ± 15.2	147.6 ± 9.5	153.6 ± 15.0
l-amphetamine (2.5 mg/kg)	110.6 ± 5.8	121.3 ± 7.3	118.2 ± 11.1
methamphetamine (0.6 mg/kg)	122.7 ± 15.6	123.8 ± 6.7	137.0 ± 10.8

[a] Data are presented as the mean ± S. E. percentage of saline control response rate for each treatment group.

[b] 3×4 repeated measures analysis of variance showed no significant group effect.

Neurochemistry Experiment

α-methyl-para-tyrosine was used to estimate the turnover of brain norepinephrine and dopamine after acute administration of a behaviorally active dose of methylphenidate. Methylphenidate 10 mg/kg significantly decreased (p < .01) whole brain concentrations of norepinephrine compared to α-methyl tyrosine controls (Table 4). Methylphenidate 10 mg/kg also significantly decreased (p < .05) whole brain concentrations of dopamine (Table 5). Thus, a dose of methylphenidate which produces significant increases in response rates for rats performing on a DRL schedule of reinforcement also increases the metabolism of brain norepinephrine and dopamine. In Tables 4 and 5 whole brain concentrations of norepinephrine and dopamine for ten rats are expressed as nanograms per gram of brain weight and are corrected for recovery, which averaged 59% for norepinephrine and 79% for dopamine.

The mean whole brain catecholamine concentrations for α-methyl tyrosine controls are substantially lower than those reported in the literature (Papeschi, 1975). The low catecholamine levels obtained in these experiments are probably due to the quench resulting from the large volume of sample oxidized. This assay did not follow the Laverty and Taylor (1968) procedure strictly. After eluting norepinephrine and dopamine from the columns, Laverty and Taylor take 0.5 ml aliquots of sample for the trihydroxyindole oxidation. In

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Table 4

Effects of methylphenidate (10 mg/kg) on whole brain
norepinephrine levels in rats after synthesis
inhibition with α-methyl tyrosine[a]

Treatment	Norepinephrine (ng/g)
αMT (150 mg/kg)	91.1 ± 5.1
αMT (150 mg/kg) + MP (10 mg/kg)	71.7 ± 3.0[b]

[a]Data are presented as the mean (± S. E.) value of nor-
epinephrine (nanograms per gram of whole brain) for ten rats.

[b]Differs significantly from group treated with αMT only
(p < .01)

Table 5

Effects of methylphenidate (10 mg/kg) on whole brain
dopamine levels in rats after synthesis
inhibition with α-methyl tyrosine[a]

Treatment	Dopamine (ng/g)
αMT (150 mg/kg)	323.1 ± 6.5
αMT (150 mg/kg) + MP (10 mg/kg)	293.8 ± 10.6[b]

[a]Data are presented as the mean (± S.E.) value of dopamine (nanograms per gram of whole brain) for ten rats.

[b]Differs significantly from group tested with αMT only (p < .05).

these experiments, the samples were reconstituted, after drying under a vacuum, to a volume of 1.0 ml. This large sample of amines apparently buffered the solution, which must be maintained at pH 4.8 for norepinephrine and pH 4.4 for dopamine. Because the Laverty and Taylor procedure depends upon a precise pH, a change in the final pH of even 0.1 units will substantially decrease the fluorescence intensity of the samples. In subsequent experiments in this laboratory which followed the Laverty and Taylor procedure exactly, whole brain catecholamine levels have been obtained which are equivalent to those reported in the literature with approximately 80% recovery.

CHAPTER IV

DISCUSSION

These results demonstrate that intermittent admini-
stration of methylphenidate produces tolerance. Dose-
response data show that exposure to methylphenidate on a
periodic basis reduces the rate-increasing and reinforcement-
decreasing effect of the drug over a limited range of doses.
Tolerance occurred even though at least two non-drug days
separated each dose tested.

Previous studies have presented data which also suggest
that intermittent administration produces a reduced drug
effect, but data from these studies have not been analyzed
in terms of tolerance after intermittent administration.
Smith and McKearney (1977) demonstrated in pigeons that
intermittent administration of varying doses of d-amphetamine
will result in a decreased drug effect. However, they did
not describe their results in terms of tolerance nor did
they study systematically the effect of intermittent admini-
stration of a particular dose. MacPhail and Seiden (1976)
determined the dose-response functions twice for the effect
of d-amphetamine on water intake in rats and presented the
results for each administration separately. Their second
dose-response determination showed a decreased drug effect
over the first administration for each dose tested. Schuster

et al. (1966) also presented data which indicate that the magnitude of the response-rate increase for the first exposure to a dose of 1 mg/kg dose of d-amphetamine was never observed again during repeated administration.

In addition, discontinuation of repeated administration does not assure that the initial effect observed for the amphetamines will be recovered. MacPahil and Seiden (1976) found that dose-response measurements made after discontinuation of daily administration, when tolerance was reported lost, did not recover the magnitude of drug effect observed for the first measurements made prior to daily administration.

Several experiments using d-amphetamine have presented dose-effect curves for two administrations averaged together, (e.g., Clark and Steele, 1966; Thompson and Corr, 1974; Sanger and Blackman, 1975; Stitzer and McKearney, 1977). The results of the present experiment indicate that the standard practice of averaging the results for two administrations of a particular dose may obscure reduced drug effects. In addition, the data suggest that the usual criterion of behavioral stability during dose-effect testing, the re-establishment of baseline responding between doses, may not prevent the modification of drug effect for each subsequent dose.

The results of this experiment demonstrate that daily administration of methylphenidate pre-session will result in the development of tolerance to the behaviorally disruptive effects of methylphenidate and produce cross-tolerance to

the amphetamines. This demonstration of tolerance to methyl-phenidate after daily pre-session administration is comparable to that obtained by Pearl and Seiden (1976). Intermittent administration of methylphenidate pre-session also results in the development of tolerance to the rate-increasing effects of methylphenidate, although this tolerance is not as complete as that observed after daily administration. Daily post-session administration of methylphenidate did not result in the development of tolerance.

The three groups in this experiment were matched for their mean response to the third administration of the 10 mg/kg test dose of methylphenidate (Figure 14, 15, and 16), and all three groups showed the same qualitative reduction in drug effect across the three administrations of 10 mg/kg methylphenidate during dose-effect testing. This criterion for matching the groups paralleled as closely as possible the procedure used in the Pearl and Seiden experiment, in which their animals were grouped according to their mean response to a test dose of d-amphetamine and methylphenidate. The results of this experiment are, therefore, probably not due to differences between the three groups in their initial response to the drug during dose-effect testing. Also, chronic administration of methylphenidate at this dose pro-duced no behavioral toxicities. After 130 days of methyl-phenidate administration, the post-session group showed no systematic changes from their control performance (Figure 14).

The semi-weekly group showed a slight shift from their pre-drug baseline for the three days of saline administration each week during the chronic administration phase, indicating that these animals were gradually coming under better schedule control (Figure 17). Thus, the results of the present experiment are not a consequence of any general toxic properties of methylphenidate which result in deterioration of the behavioral baseline.

Carlton and Wolgin (1971) first demonstrated that tolerance to the behavioral effects of d-amphetamine develops only when the behavior measured is performed under the influence of the drug and labeled this phenomenon, contingent tolerance. In their experiment, rats given d-amphetamine post-session for seven days did not show tolerance to the anorexic effect of amphetamine when given a test dose pre-session, while rats given d-amphetamine pre-session did develop tolerance. Campbell and Seiden (1973) demonstrated contingent tolerance to the disruptive effects of d-amphetamine on DRL performance. Post-session administration of d-amphetamine for 26 days had no effect on DRL 17.5 second performance. Administration of pre-session amphetamine to these animals produced the same disruption of performance as that observed initially for the pre-session administration group. The results of the present experiment provide further evidence for the phenomenon of contingent tolerance and extend it to the effects of chronic administration of methylphenidate. Thus, tolerance to the

behavioral effects of d-amphetamine or methylphenidate is contingent upon the relationship between the timing of administration and the behavior measured.

The contingent tolerance demonstration in the present experiment and in previous studies is consistent with the description by Schuster et al. (1966) of the controlling variables for the development of tolerance. They pointed out that if the effect of a drug is to decrease the number of reinforcements earned, then tolerance will develop to the behaviorally disruptive effects of the drug. If the drug has no effect on, or increases the number of reinforcements earned, then tolerance will not develop. In the present study, post-session administration for 77 sessions did not modify the number of reinforcements earned in the session, and results of the fourth test dose of methylphenidate 10 mg/kg showed no evidence of a further development of tolerance. In contrast, pre-session administration of methylphenidate, either daily or intermittently, did decrease the number of reinforcements earned, and tolerance did develop to the behaviorally disruptive effects

The results presented for the group receiving methylphenidate post-session demonstrated that chronic administration of methylphenidate 10 mg/kg post-session does not augment the reduced drug effect observed after three administrations of that dose during dose-response testing. The literature on the duration of tolerance to the amphetamines

(Schuster et al., 1966; MacPhail and Seiden, 1976) suggests, as these data also suggest, that the initial drug effect is never recovered. Thus, although post-session administration does not result in the development of further tolerance, the effects of prior exposure are not negated. The animals' prior history of periodic exposure to methylphenidate, which produced a progressive decrease in the response to the 10 mg/kg dose, continued to influence their response to methlyphenidate after 18 weeks during which their performance on the DRL schedule was never disrupted by pre-session exposure to methylphenidate. These observations, the results of the group receiving chronic administration of methylphenidate twice weekly, and the results of the dose-effect testing all suggest that daily administration of methylphenidate is not a necessary condition for the development of tolerance.

Traditional models of tolerance assume that tolerance is a consequence of the presence of the drug in the system. These models of tolerance would predict that, regardless of whether the animal is performing the behavior while intoxicated, tolerance, defined as a return towards baseline responding after drug administration, will develop. Hence, when tolerance occurs to the behavioral effects of a drug, the drug effect always decreases as a function of time, and this decrease in responsiveness to the drug is augmented by performing the behavior while intoxicated. Kalant et et. (1971) describe this phenomenon as "behaviorally augmented

tolerance." The results of the present experiment demonstrate that post-session administration did not result in the development of further tolerance, as Kalant et al. predict. Thus, the present results suggest that chronic administration of methylphenidate per se is not a sufficient condition for the development of tolerance.

How can we account for the observations that rats given methylphenidate 10 mg/kg post-session every day for 77 days did not develop tolerance to the behaviorally disruptive effects of the drug? Several lines of evidence suggest that catecholamines are important in the maintainance of behavior (Seiden et al., 1975). Experiments measuring the depletion of catecholamines following synthesis inhibition with α-methyl tyrosine (Schoenfeld and Seiden, 1969) or by following changes in the specific activity of norepinephrine after tritium labeling (Lewy and Seiden, 1972) have shown that operant behavior alters the metabolism of catecholamines. In addition, Emmett-Oglesby et al. (1978) found that increases in rat brain catecholamine metabolism were associated with water-reinforced responding or with periodic water presentation. Because the behavioral effects of the amphetamines are believed to be mediated through the release of catecholamines, the effects of the amphetamines on catecholamine metabolism may be modified by the animal's ongoing behavior. Thus, it may not be surprising that daily post-session administration does not produce tolerance while daily pre-session administration does.

To elucidate the neurochemistry of tolerance, the neurochemical effects of amphetamine and methylphenidate need to be determined using doses typical of those administered chronically in behavioral experiments. The neurochemical literature which was reviewed earlier is based on the effects of acute administration of extremely high, behaviorally toxic doses. Thus, the neurochemical techniques presented in this thesis provide a first step in the analysis of the neurochemistry of tolerance and offer a way of looking at the modification of metabolism of catecholamines after tolerance to methylphenidate.

Methylphenidate, d-amphetamine, l-amphetamine, and methamphetamine have qualitatively similar effects on behavior when administered acutely (Cox and Maickel, 1972; Browne and Segal, 1977; Smith and Davis, 1977). Thus, this experiment tested the hypothesis that tolerance to methylphenidate also results in tolerance to these other agents. In addition, if the intermittent administration group were not as tolerant as the daily pre-session group, as data from the semi-weekly administration group suggest, then results from cross-tolerance tests for the semi-weekly group would fall between the results for the daily pre-session group and the daily post-session group. With respect to the reinforcement measures, both of these hypotheses were clearly supported (Table 2). However, cross-tolerance test results for the rate data are more difficult to interpret. The two hypotheses are supported for

the methylphenidate and d-amphetamine test doses, but the predicted trend is not observed for l-amphetamine. This finding may be a consequence of the negligible rate-increase produced by this dose of l-amphetamine.

The demonstration that animals tolerant to methylphenidate are cross-tolerant to amphetamines suggests that similar neurochemical mechanisms underly the behavioral disruption (Kalant et al., 1971), and further suggests that an organism tolerant to the behaviorally disruptive effects of any of these drugs is tolerant to the effects of all of them. Previous studies of tolerance partially support this hypothesis. Pearl and Seiden (1976) found cross-tolerance between d-amphetamine and methylphenidate for DRL responding. In addition, cross-tolerance among the amphetamines has been demonstrated for the anorexic effects. Pearl and Seiden (1976) found cross-tolerance between d-amphetamine, methylphenidate, and l-amphetamine for milk consumption. Kandel, Doyle, and Fischman (1975) demonstrated cross-tolerance between d-amphetamine and methamphetamine to the suppression of milk consumption. On the other hand, Tilson and Sparber (1973) have reported no cross-tolerance between d- and l-amphetamine for rate-decreasing effects on fixed ratio responding. Thus, the generality of the hypothesis suggested by the present experiment, that prior exposure to a drug of the amphetamine class will result in a reduced drug effect upon subsequent exposure, may be limited to those schedule

controlled behaviors for which acute administration of the amphetamines produces an increase in the control rate of responding.

APPENDIX A

REPEATED MEASURES ANALYSIS OF VARIANCE TABLES

Effect of Order of Administration or Dose of Methylphenidate on Response Rate

Source	df	SS	MS	F	p
Between Subjects	34	5703.887	190.130	3.269	0.000
Within Subjects	9	1568.386			
Order of Admin.	1	372.407	372.407	6.402	0.012
Dose of MP	4	1045.912	261.478	4.495	0.002
Interaction	4	150.066	37.517	0.645	0.630
Residual	270	15705.628	58.169		
Total	309	22977.901			

Effect of Order of Administration or Dose of Methylphenidate on Reinforcements Earned

Source	df	SS	MS	F	p
Between Subjects	30	2.180	0.073	5.717	0.000
Within Subjects	9	3.771			
Order of Admin.	1	0.003	0.003	0.207	0.649
Dose of MP	4	3.678	0.920	72.350	0.000
Interaction	4	0.090	0.022	1.767	0.136
Residual	270	3.432	0.013		
Total	309	9.383			

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Effect of Order of Administration or Dose of Methylphenidate on Reinforcements Earned

Source	df	SS	MS	F	p
Between Subjects	30	1.761	0.059	5.214	0.000
Within Subjects	5	0.367			
Order of Admin.	1	0.243	0.243	21.630	0.000
Dose of MP	2	0.116	0.058	5.170	0.008
Interaction	2	0.007	0.003	0.308	0.735
Residual	150	1.688	0.011		
Total	185	3.816			

Effect of Order of Administration or Dose of Methylphenidate on Response Rate

Source	df	SS	MS	F	p
Between Subjects	30	309.872	30.129	1.796	0.012
Within Subjects	5	463.387			
Order of Admin.	1	155.029	155.029	9.243	0.003
Dose of MP	2	207.035	103.518	6.172	0.003
Interaction	2	101.322	50.661	3.020	0.052
Residual	150	2515.888	16.773		
Total	185	3883.147			

Effect of Chronic Administration of Methylphenidate or
Cross-Tolerance Test Drug on Percentage Control
Reinforcements Earned

Source	df	SS	MS	F	p
Between Subjects	30				
Group	2	1.907	0.953	5.644	0.009
Error	28	4.729	0.169		
Within Subjects	93				
Drug	3	0.318	0.106	1.969	0.125
Interaction	6	0.083	0.014	0.257	0.955
Error	84	4.525	0.054		

Effect of Chronic Administration of Methylphenidate or
Cross-Tolerance Test Drug on Percentage
Control Response Rate

Source	df	SS	MS	F	p
Between Subjects	30				
Group	2	0.519	0.259	0.511	0.605
Error	28	14.211	0.508		
Within Subjects	93				
Drug	3	1.533	0.511	3.301	0.024
Interaction	6	0.228	0.038	0.245	0.960
Error	84	13.000	0.155		

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