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THE EFFECT OF RESERPINE ON MACROMOLECULAR SYNTHESIS AND THE REPLETION OF BIOGENIC AMINES

Paul V. Scott

A dissertation presented to the Faculty of the Graduate School of Yale University in candidacy for the Degree of Doctor of Philosophy

From the Department of Pharmacology

The Graduate School, 1971

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Reserpine has been shown to inhibit the synthesis of RNA and protein in brain, heart, and liver. Doses of 2.5 mg/kg are required to observe this effect. A dose of 1.25 mg/kg of this alkaloid, while substantially depleting norepinephrine in brain and heart, has no apparent effect on macromolecular synthesis. The rates of repletion of norepinephrine after reserpine also appear dose dependent.

Reserpine produced no change in the rate of incorporation of precursor into RNA or protein in mouse liver slices or the rate of incorporation of amino acids with proteins in a cell-free system from mouse liver; there was no change in the state of aggregation of polysomes. Ribonucleic acid polymerase, from E. coli, however, was inhibited 50% by 1×10^{-4}M reserpine.

The postulate was advanced that the delay in biogenic amine repletion was due to the effect of reserpine on macromolecular synthesis, inhibition of RNA or protein synthesis leading to a delay in de novo synthesis of new amine binding sites and thereby introducing a lag into amine repletion of reserpine.

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I would like to thank Alan C. Sartorelli for his unstinting advice and encouragement.

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DEDICATION

Til Birgitta och Pär

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INTRODUCTION

The root of Rauwolfia serpentina was extensively employed in Indian folk medicine as a palliative in snake and scorpion bite, as a sedative, and as a tonic (1, 2). There may have been some recognition of the special properties of the active ingredients, as it was referred to as "insanity specific" in one dialect (2). A major source of confusion in the research done in the East was the number of alkaloids present in extracts of this root which have biological activity of diverse nature (3, 4), leading to some conflict in results obtained using different extracts of the same root. One of the most attractive pharmacologic principles for Western medicine was the hypotensive effect brought about by extracts of R. serpentina, and a concerted research effort led to the purification of a compound, reserpine, which was highly active in this respect (5).

In order to introduce the subject, a brief consideration will be given to the chemistry of alkaloids possessing reserpine-like activity; an outline of the way in which norepinephrine, an endogenous compound profoundly affected by this drug, is synthesized; and a more extensive discussion of the way in which norepinephrine is bound *in situ*, as this has been shown to be the site at which reserpine acts.

Since reserpine has been shown to interfere with the

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process of amine storage, it is important to know that this drug does not also alter the rates at which the cell can make norepinephrine, as we will subsequently be equating the synthesis of new storage sites with the amount of norepinephrine present. The biosynthesis of norepinephrine is thought to occur as is shown in Figure 1. On a theoretical basis it is possible to postulate that reserpine would act to inhibit the influx of dopamine into the granule (within which the conversion to norepinephrine takes place in whole or part), and one would anticipate, therefore, inhibition of the conversion of labeled dopamine to norepinephrine in the presence of reserpine (6). It also has been reported (7) that reserpine inhibits the conversion of tyrosine to dopa in vitro, but this may not have much bearing on the long-term effects of this drug.

A recent report indicated that the rate of synthesis of norepinephrine was increased after reserpine (8); these authors attributed this increase to "induction" of the enzyme tyrosine hydroxylase, but it does not seem clear from this work that reserpine directly affects the rate of synthesis or degradation of this enzyme protein, and the use of the word induction thus seems unwarranted.

The structure of reserpine and some active congeners is shown in Figure 2. The total synthesis of this alkaloid was carried out by Woodward (10), and the chemistry of reserpine and the Rauwolfia alkaloids has been reviewed in detail elsewhere (5). The derivatives shown in Figure 2 are the only compounds known to exhibit the full range of pharmacologic effects exerted by reserpine in doses comparable

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Legend to Figure 1

PATHWAY FOR THE BIOSYNTHESIS OF NOREPINEPHRINE

The heavy arrows represent the major pathway of norepinephrine biosynthesis. The light and dashed arrows represent possible alternative routes.

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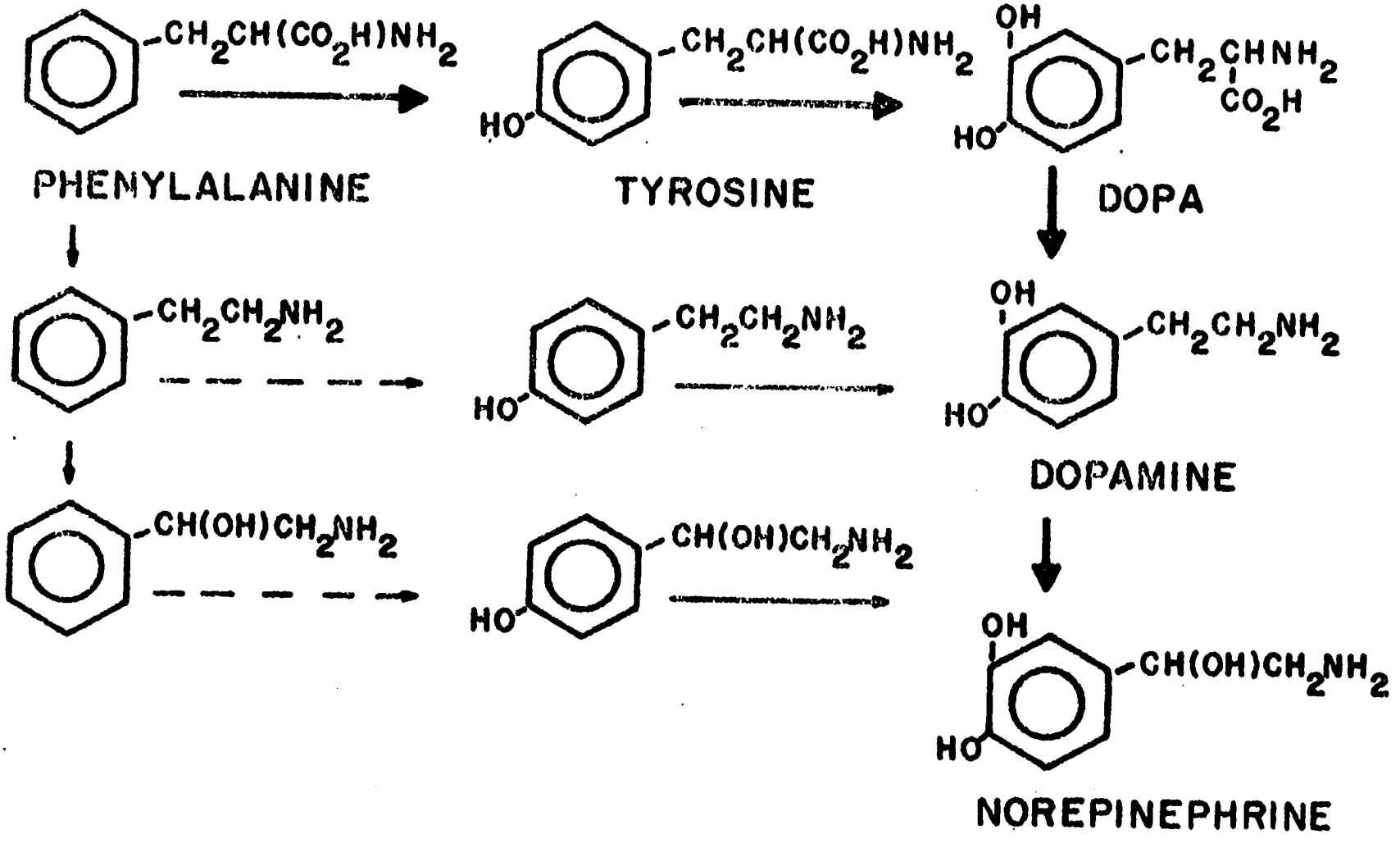

with those employed for this alkaloid.

The initial observations on the mechanism of action of reserpine were made in 1955 by Brodie and his co-workers (11, 12), who showed that rabbit brain 5-hydroxytryptophan levels were markedly reduced after a single dose of this alkaloid. Subsequently, this finding was enlarged by a number of laboratories to include the observation that reserpine depleted all of the so-called "biogenic amines"--NE, for example (13, 14). There are many speculations as to the significance of this reserpine-induced depletion of amines in terms of the post treatment behavior, and this literature has recently been reviewed (15).

A number of generalizations can be made about the depletion of amines by reserpine. These include the following (16, 17): (a) biogenic amines (as exemplified by norepinephrine) are depleted from all tissues in which they are normally present; (b) there exists a differential sensitivity to reserpine-induced depletion from tissue to tissue (i.e., intestine is more resistant to this alkaloid than is either heart or brain); and (c) the rate of repletion is also dependent on the tissue in which this rate is determined.

The most obvious way that a drug could act to produce a decrease in the amount of a given molecule present in a tissue would be by inhibiting the synthesis or transport of the compound in question or both; these mechanisms do not appear to be suitable explanations for the mode of action of reserpine, as (a) after dosage with reserpine, a large increase

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Figure 2

THE STRUCTURE OF RESERPINE AND SOME CONGENERS

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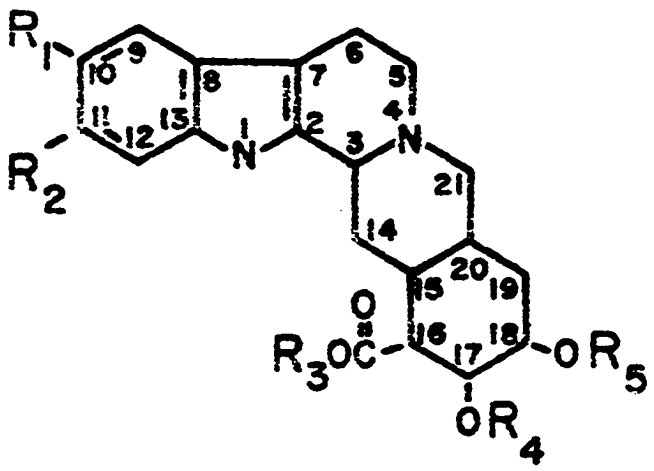
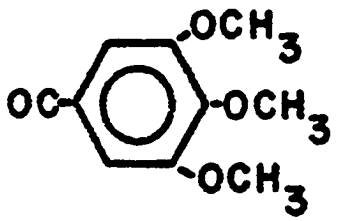
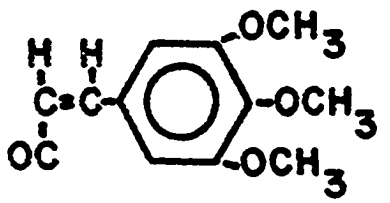
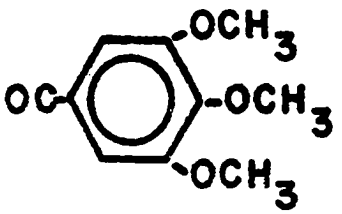


	R_1	R_2	R_3	R_4	R_5
RESERPINE	H	OCH_3	CH_3	CH_3	
RESCINNAMINE	H	OCH_3	CH_3	CH_3	
DESERPIDINE	H	H	CH_3	CH_3	

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in the excretion of deaminated metabolites of biogenic amine occurs (18, 19) with the rate of excretion of the deaminated metabolites being roughly proportional to the rate of depletion; (b) the conversion of tyrosine to L-DOPA (see Figure 1, page 2), which is the apparent rate-limiting step in the biosynthesis of norepinephrine, is not inhibited in the presence of reserpine in a way which would lead to such profound results in those observed for amines after this alkaloid (20). Indeed, there is a report described above which indicates an increased rate of synthesis of L-DOPA from tyrosine after reserpine (8); and (c) short times (5-10 minutes) after administration of ^{3}H-NE, control and reserpine pretreated tissues show roughly equal uptake of amine, but with longer periods of exposure the reserpine treated tissue contains only small amounts of NE, indicating that transport of norepinephrine into the neuron is unimpaired (21). These results show that the synthesis and transport of norepinephrine do not appear to be sites of action of reserpine, and, in contrast to normal animals, reserpinization leads to a state in which the amines are rapidly catabolized.

Other studies with reserpine have led to the conclusion that biogenic amines are stored in tissues when the amines are not functioning (17, 22), and that reserpine is acting at the level of the binding site for the amines (i.e., the granule) rather than having a simple direct effect on the amines themselves. The types of evidence which lead to that conclusion include the finding that the relationship between time and the specific activity of norepinephrine after infu-

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sion of radioactive norepinephrine into a tissue or animal is expressed by biphasic decay kinetics, one phase which is fast and a second slower rate which appears to represent the mixing of the amine with endogenous material (the approximate half times are about 5 and 20 minutes in the perfused rat heart (23, 24). Centrifugation in a sucrose gradient of homogenates of adrenergically innervated tissue labeled with ^{3}H-norepinephrine reveals a peak of radioactivity which migrates with the microsomal region of the gradient; this has been taken as an indication that norepinephrine is present in a stored form in granules present in this fraction (25). Both uptake and release of norepinephrine follow a pattern which indicates that the most labile fraction of norepinephrine observed in perfused rat heart is the amine which is being perfused (26), as the specific activity of the amine released on stimulation from heart after cessation of perfusion of radioactive norepinephrine has a higher specific activity than would be expected if the perfused material mixed totally with the endogenous pool.

A number of studies have been directed at an understanding of the properties of the amine-storage granule *in situ*, but there have been many other studies on the properties of the binding site *in vitro*, as well. This information can be classified under three headings for convenience: (a) the site and rate of synthesis of the granule, (b) the chemical composition of the granule, and (c) models of the biogenic amine binding mechanism of the granule.

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The evidence obtained by Hillarp and his co-workers (27, 28) using histochemical fluorescence to localize and quantitate the norepinephrine in amine containing tissues has led to a broader understanding of the ways in which amines are employed in neural tissues. Since the development of this technique, it has become accepted (20) that (a) amines are concentrated in "varicosities" which are thought to occur in synaptic regions; (b) transport (uptake) and storage are separate functions, as transport occurs at the cell membrane and the binding occurs at the granular level; and (c) reserpine affects storage and not extra-intracellular transport. Among the observations which support this point of view are the findings that a proximo-distal gradient of fluorescence exists at appropriate time after reserpine (29). Furthermore, contralateral ligations at different levels of the sciatic nerve of the cat show reaccumulation of amine first above the highest ligation after reserpine (29). These points are enlarged upon by some observations of directional flow in the axon in which it was shown that the area between two ligations on the same nerve did not have a net increase in the amount of norepinephrine present with time, whereas the area just above the top ligation increased its content of this amine (30). These results have been interpreted to mean that the amine binding site or granule is synthesized in the nerve cell body and is transported down the axon by some special process which is appreciably faster than that enjoyed by most large molecules (30, 31). A point which the Swedish workers have not considered in their dis-

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cussions is that the rate of reappearance of granules is probably dependent upon the gene frequency for granular components, the rate of synthesis of both RNA and protein, and the rate of transport of the newly formed granule down the axon from the nerve cell body.

Other evidence on the site of formation of granules is that the fluorescence produced by the biogenic amines after reserpine treatment appears first in the perinuclear zone and then subsequently moves down the axon towards the axon terminal (32, 33, 34). The fluorescence observed around the nucleus which migrates down the axon is depleted by a second dose of reserpine (35), indicating that the amines are bound in granules.

It is also possible to paint the nerve cell body with a solution of reserpine (35). The rate of depletion of the amines in various parts of the axon can then be determined. The first area depleted is the nucleus, while the area above an axonal ligation is depleted only slowly. From these results, it was postulated (35) that depletion in the area of the ligation was due to migration, from the nucleus to the site of the ligation, of granules which had been exposed to reserpine in the nerve cell body. The means by which they were depleted at the ligation was suggested to be indirect, since the granules from the nerve cell body area that had been treated would arrive at the site of the ligation faster than a solution of reserpine (35).

Another line of evidence (35) indicating that reserpine

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acts irreversibly on the amine storage granule was obtained in an experiment in which two ligations with an interval of a few centimeters were made on the sciatic nerve of the cat. The cats were then treated with tetrabenazine, a drug like reserpine which depletes stores of biogenic amines. This agent differs from reserpine in that the amine depletion is rapidly reversible. The fluorescence of amines following tetrabenazine administration declined above, between, and below the axonal ligations, and upon reversal, the fluorescence reappeared above, between, and below the ligated areas. When reserpine was used to decrease amines, fluorescence appeared only above the top ligation, and only after a period of time corresponding to the interval required for the migration of new granules from the nerve cell body to the top ligation (35). These results were interpreted to mean that: (a) the biogenic amine storage granules remain intact and functional for some time after contact with the nerve cell body is lost, as shown by the tetrabenazine experiment, and (b) that reserpine causes irreversible damage to the granules which renders them incapable of any capacity to bind biogenic amines after exposure to the drug, as shown by the finding that granules below and between axonal ligations were unable to bind amines.

A more speculative argument for the requirement of the nerve cell body in the synthesis of new granules after exposure to reserpine comes from the observation (36) that colchicine treated nerve cell body regions had sharply increased perinuclear fluorescence and greatly diminished fluorescence

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in the areas of axonal ligation. Colchicine is thought to disrupt the neurotubular (37) apparatus which is required to transport the newly synthesized granules from nucleus to synapse.

From a different point of view Magus and Sartorelli (38) and, subsequently, Mueller and Shidemann (39) employed inhibitors of macromolecular synthesis to attempt to delay the repletion of amines after treatment of intact animals with reserpine in the belief that demonstration of such a delay would be suggestive evidence for the requirement of such synthesis in the process of repletion.

Although granules have been isolated from only a few sources with enough purity to warrant chemical analysis (40, 41), they generally appear to contain protein, RNA and/or free nucleotides, lipid and biogenic amines. There are indications that some or several of the enzymes involved in the biosynthesis of norepinephrine are present in the granule (42), either as part of its structure or in solution within the granule. Teleologically the presence of synthetic enzymes for norepinephrine contained in the protected environment of the storage site is quite satisfying, although the data do not yet seem conclusive except for dopamine-β-hydroxylase (42, 43).

Early studies on the amine binding properties of isolated granular fractions were occasionally candid in their concession that results obtained in such preparations had little similarity to the properties of the granules <u>in situ</u>

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(44). However, granules *in vitro* have been shown to require the presence of ATP and Mg^{2+} for uptake of amines (45). Part of the discrepancy between *in situ* and *in vitro* may derive from the fact that most of the *in vitro* studies have been carried out using granules obtained from bovine splenic nerve. Granules from this source have not been exposed to synaptic conditions, and there are indications that granules may dramatically change after exposure to the environment of the synapse (46). Thus, the properties of the granular material being studied apparently bear little relationship to the actual amine binding site as it exists at the synapse.

It is worthy of note that various groups have begun to prepare antibodies to splenic nerve granules both for enzymatic (43) and structural proteins (47), and have thus demonstrated that granules, in tissues from which they have not been purified, react immunologically as do granules from splenic nerve. This finding would appear to indicate that the chemical composition of the granules in these different sites is fairly uniform.

Models of the binding of amines have been proposed which envision electrostatic interaction between the negative charges of either RNA or its precursor nucleotides to neutralize the positive changes of the biogenic amines (48, 49). These models are speculative but are worth consideration.

In the report cited above of Magus and Sartorelli (38), a dose of 7.5 mg/kg was shown to decrease the rate of incorporation of radioactive precursors into RNA and protein while

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mildly stimulating precursor incorporation into DNA in L5178Y lymphoma cells removed from the peritoneal cavities of mice and incubated *in vitro*. These results indicated that reserpine inhibited the synthesis of certain types of macromolecules and prompted the present work which has had two goals: (a) to extend the observations on the inhibition of macromolecular synthesis by reserpine in non-malignant tissues; and (b) to determine if there is a relationship between the effect of reserpine on depletion of biogenic amines and the action of this drug on the synthesis of certain macromolecular species.

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METHODS

In Vivo Synthesis of RNA: The rate of incorporation of tritiated uridine, (UR-^{3}H) uniformly labeled from New England Nuclear, into a cold acid-insoluble, hot acid-soluble fraction was used as a measure of the rate of synthesis of RNA. CD1 mice of about 25 g were injected intraperitoneally with 100 µC UR-^{3}H (500 µC/mg), and the radioactive material was allowed to incorporate for twenty minutes. Animals were then killed by cervical dislocation, and various tissues were rapidly removed and frozen on dry ice covered with aluminum foil. The frozen tissues were then disrupted by homogenization in 5% trichloroacetic acid (TCA) either in an Ultra-Turrax or in a Potter-Elvehjem teflon-glass motor driven homogenizer. The samples were centrifuged for 5 min in an International PR-2 at 1500 x g at 0-4° C. The TCA insoluble material was washed 2x with 5% TCA, 2x with 95% EtOH, 1x with EtOH/Et_2O(3/1,v/v), 1x with 95% EtOH, 1x with 5% TCA, and finally suspended in a volume of 5% TCA and hydrolyzed in a boiling water bath for 15 min. The samples were again centrifuged and 0.5 ml of the supernatant was assayed for radioactivity. A fraction of the remainder of the supernatant was used for determination of the amount of DNA present (50).

In Vivo Synthesis of Proteins: The rate of incorporation of 1-^{14}C-valine (New England Nuclear) into hot TCA insoluble

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material was taken as a measure of the rate of synthesis of proteins in the various tissues assayed. Mice were injected with 5 μC of l-^{14}C-valine (7 μC/μmole), and twenty minutes was allowed for incorporation. Animals were killed by cervical dislocation, and the tissues were removed and treated in a manner identical to that used above for estimating the rate of synthesis of RNA until the EtOH extraction step following ethanol-ether; at this point samples were hydrolyzed in 5% TCA. Samples were then treated in two different ways at different times with the same apparent results. Some samples were washed 2x with absolute Et_2O, dried over NaOH *in vacuo*, suspended in 20% EtOH and plated on preweighed aluminum planchets. The planchets were then dried *in vacuo*, weighed and the amount of isotope was determined with a Nuclear-Chicago gas-flow automatic planchet counter. The counts were corrected for self-absorption and the results are expressed as cpm/mg protein. With other samples the final precipitate was dissolved in a volume of 88% formic acid and a portion counted in a Packard Tricarb liquid scintillation spectrometer in ethanolic POPOP. Another portion was employed for determination of the concentration of protein present by the Lowry method (51).

In Vivo Determination of Norepinephrine: Tissues were dispersed with an Ultra-Turrax homogenizer in 15% TCA, and the acid insoluble material was sedimented at 10,000 x g for 10 min. The supernatant was decanted into a tube containing 0.5 ml 10% EDTA and 0.1 ml 1M Tris, pH = 8.2. This solution was

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neutralized to pH 8.2 to 8.5 with NaOH and was then rapidly poured over an alumina (British Drug House) column (as described by Roth) (52). The alumina was washed with distilled water until the effluent pH was equal to that of the distilled water. The adsorbed norepinephrine (NE) was then eluted with successive 1 ml washes of 0.2 N and 0.1 N perchloric acid. Of this total of 2 ml, 0.5 ml was taken for oxidation and 0.5 as blank. These were taken from the effluent collection tube into another tube containing 0.5 ml of 1 M phosphate buffer (pH 7.0). The final pH of this solution was approximately 6.5-7. To the samples that were to be oxidized was added 50 µl of 0.25% potassium ferricyanide, and exactly 3 min later, 0.75 ml of alkaline ascorbate (88 ml of 5 N NaOH, 2 ml of ethylene diamine, and 10 ml of a 10% ascorbic acid). Alkaline ascorbate was added first to appropriate blanks. The fluorescence of the samples was determined on the Aminco Bowman fluoro microphotometer, using a primary filter, with a peak narrow band pass of 405 nm and a secondary filter (WRATTEN-65A) with a peak narrow band pass of 495 nm. Recoveries were 90-100% and are uncorrected.

Sucrose Gradients for Polyribosome Profile: The gradients were formed by a Beckman Gradient Former, using sucrose TKM (15 mM $MgCl_2$, 20 mM KCl and 0.1 M Tris-HCl, pH 7.4) solutions varying in sugar concentration from 20 to 50%. The sample to be assayed was homogenized with an Ultra-Turrax homogenizer in 4 vols of 0.25 M sucrose TKM. The homogenates were centrifuged through this medium at about 10,000 x g for 10 min. The 10,000 g supernatant (200 µl) was layered on top of an 11 ml gradient after adding deoxycholate to a final

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concentration of 1%. The samples were then centrifuged at 280,000 x g for 70 min. The gradient was removed from the tubes by the injection of 58% sucrose into the bottom of the tube using an Isco Gradient Collector. The material was then forced through a chamber in which the absorbence at 256 mμ was determined and recorded.

Preparation and Assay of RNA and Protein Synthesis in Slices of Liver: Mouse liver was placed on a circle of filter paper which was mounted on top of a beaker inverted in ice; the paper was moistened with Krebs II (53) medium without radioactive precursor. Slices were made by drawing a Stadie blade across a surface formed by two pieces of glass coverslips glued to either side of a slide mount, with a gap between the coverslips of about 1 cm. The first slice was discarded, and the subsequent 2 or 3 slices were employed in the incubation.

The slices were incubated at 37° for various periods of time as indicated in the legends. Initiation of incorporation was brought about by the addition of the slice to Krebs II (53). Before the tissue slices were placed into the reaction beakers, they were gassed with 95% oxygen 5% CO_2, and were stoppered. Just after the tissue was added, the gas mixture was again bubbled into the medium for about 30 seconds.

At the end of the indicated interval of incubation, the beakers were transferred to an ice bath and cold 10% TCA was added to a final concentration of about 7.5%. The RNA was prepared as described for in vivo experiments, and the protein

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was isolated and assayed by the formic acid method.

The Synthesis of Protein in a Cell-free System: The synthesis of protein in a cell-free system was determined by the method of Lim and Adams (54) which closely mimics the in vivo situation. Liver was removed from the mouse and homogenized in a glass-glass Potter-Elvehjem homogenizer with ten passes of the pestle in a medium containing 0.25 M sucrose, 0.4 mM $MgCl_2$, 2.5 mM KCl, and 5 mM Tris-HCl (pH 7.4). This homogenate was centrifuged at 10,000 x g for 10 min to remove whole cells, nuclei, mitochondria, and other particulate material. The supernatant was then recentrifuged at 105,000 x g for 1 hour; the supernatant of this centrifugation is referred to as "supernatant," and the precipitate is referred to as the "microsomes." At the end of the centrifugation the top 2/3 of the supernatant was removed with a Pasteur pipette; the remaining material was discarded. The microsomes were then collected and resuspended in the grinding medium minus sucrose in a small glass/glass homogenization vessel. A portion of the supernatant and one of the microsomes were taken for estimation of the amount of protein present by the Lowry method (51) and other fractions of each were added to a solution which had a final concentration of 10 mM $MgCl_2$, 20 mM KCl, 20 mM Tris (pH 7.4), 1 mM ATP, 20 mM creatine phosphate, and a mixture of tritiated amino acids. The reaction, which was linear for about 30 min, was allowed to proceed for 25 min. It was terminated by the addition of TCA to a final concentration of 10%. The tubes

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were then incubated at 65° for 15 min and were centrifuged. The precipitate was washed 6 times with 5% TCA. These washes were sufficient to reduce the background to approximately 30 cpm in the last two washes. The final precipitate was dissolved in 88% formic acid and the extent of radioactivity incorporated was determined in a Packard Liquid Scintillation Spectrometer.

Ribonucleic Acid Polymerase from E. coli: The enzyme was isolated by the method of Chamberlin and Berg (55) as modified by Bonner (56). The enzyme preparation could be stored for extended periods of time at -10° in a solution containing 50 mM Tris-HCl (pH 8.1), 0.1 M ammonium sulfate, 0.1 mM EDTA, 1 mM $MgCl_2$, and 5 mM glutathione.

Incubation of this enzyme was carried out at 37° in 40 mM Tris, 4 to 70 mM $MgCl_2$, and a mixture of the 4 nucleoside triphosphates at 0.4 mM each in a total vol of 0.25 ml. The reaction was linear for approximately 15 min and was allowed to proceed for 10 min. It was terminated by placing the tubes in crushed ice, adding 0.5 ml of a 0.5% solution of bovine serum albumin (fraction V, Nutritional Biochemicals). The precipitate obtained with the addition of 5 ml of 5% TCA was washed 6 times with cold 5% TCA (4 washes were sufficient to reduce the radioactivity to background), and the RNA was hydrolyzed at 90-95° for 15 min. The supernatant was collected; the radioactivity of 0.5 ml was determined in a Packard Liquid Scintillation Spectrometer.

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Subcellular Fractionation of Liver Proteins: Mice were injected with 5 μC of ^{14}C-valine (5 μC/μmole), and this radioactive precursor was allowed to incorporate into liver proteins for 30 min. The entire liver was then removed and homogenized in 3 vol of 0.25 M sucrose TKM (0.1 M Tris-HCl pH 7.4, 15 mM $MgCl_2$, and 20 mM KCl) with 15 passes of a teflon/glass Potter-Elvehjem homogenizer. A small fraction of this homogenate was taken for determination of the specific activity of the "whole homogenate." Another fraction was mixed with 8 vol of 2.4 M sucrose TKM, and this was centrifuged at 35,000 x g for 1 hour, and the pellet so obtained was described as "nuclei." The remaining supernatant was diluted 1:1 with 0.25 M sucrose TKM and centrifuged for 1 hour at 100,000 x g. The sediment of this process is referred to as the "microsomes" and the supernatant as "supernatant." The sediments obtained for nuclei and microsomes were resuspended in water and subsequently processed as described below.

The proteins were washed free of radioactive precursor by filtering them through glass disks after adding a solution containing trichloroacetic acid and amino acids (final concentration 6% TCA 2.5% Casamino acids). Before filtration, the tubes were heated to 90-95° for 30 min in order to deactivate the RNA. The filtrates were washed 6 times with 8% TCA containing 3% Casamino acids, dried, and the amount of radioactive precursor incorporation was determined by counting the radioactivity remaining on the filter disks. A small amount of each of the resuspended precipitates was

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taken for a determination of the amount of protein present by the Lowry method (51).

Preparation of 5'-UMP from the Acid-soluble Nucleotide Pool:

The acid-soluble pool was collected and the pyrimidine nucleotides were reduced to monophosphate by treatment at 90° for 15 min. The solution was desalted with the addition of $Ba(OH)_2$ until the solution was neutral. The resulting supernatant was spotted on PEI-cellulose and developed in 1.0 N acetic acid. The spot containing the UMP was eluted with 1 N Tris (pH 8.0). The absorbance at 260 mμ and amount of radioactivity present from ^{3}H-uridine given as in Figure 3 was determined. Correction was made for non-specific ultraviolet absorbance.

Liquid Scintillation Spectrometry: Except as noted, all determinations of radioactivity were performed in a Packard Tricarb Scintillation Spectrometer using a solution containing 8 g 2,5-diphenyloxazole and 100 mg p-bis [2-(5-phenyloxazolyl)] benzene in 1 l of absolute ethanol and 2 l of toluene. The counting efficiency for tritium in aqueous solution was about 30% and for ^{14}C about 75%.

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RESULTS

To determine whether reserpine inhibited the synthesis of RNA and protein, it was necessary to measure the rate at which these molecules were synthesized under normal and treated situations. It was, therefore, necessary to insure that the rate of fixation of precursor into the polymeric molecules was linear with time and that a change in the rate of incorporation was not due to a change in the precursor pool size. Figures 3 through 6 show the incorporation of labeled precursor into RNA and protein of brain and liver of mice. It is apparent from these data that incorporation of radioactive materials was approximately linear for the particular tissues for at least 20 min except for uridine incorporation with liver; this time period was used throughout the work to be described.

Inhibition of RNA and protein synthesis by reserpine has previously been demonstrated in L5178Y ascites lymphoma tumor cells (38). It seemed of particular interest to determine if this drug acted similarly in brain and other tissues, especially those containing biogenic amines. The effect of the intravenous administration of 2.5 mg/kg of reserpine phosphate into mice at various times before injection of

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Legend to Figure 3

TIME COURSE OF THE INCORPORATION OF ^{3}H-URIDINE INTO RNA OF MOUSE BRAIN

Mice were injected intraperitoneally with 100μC of tritiated uridine (500μC/mg) in isotonic saline. At the times indicated after injection, the animals were killed, the brain rapidly removed and frozen on dry ice. The results are expressed as cpm/gm of frozen weight of brain and are the mean of from 3 to 6 determinations. Ribonucleic acid was isolated as described in the methods section for *in vivo* RNA synthesis. Vertical bars represent the standard error of the mean.

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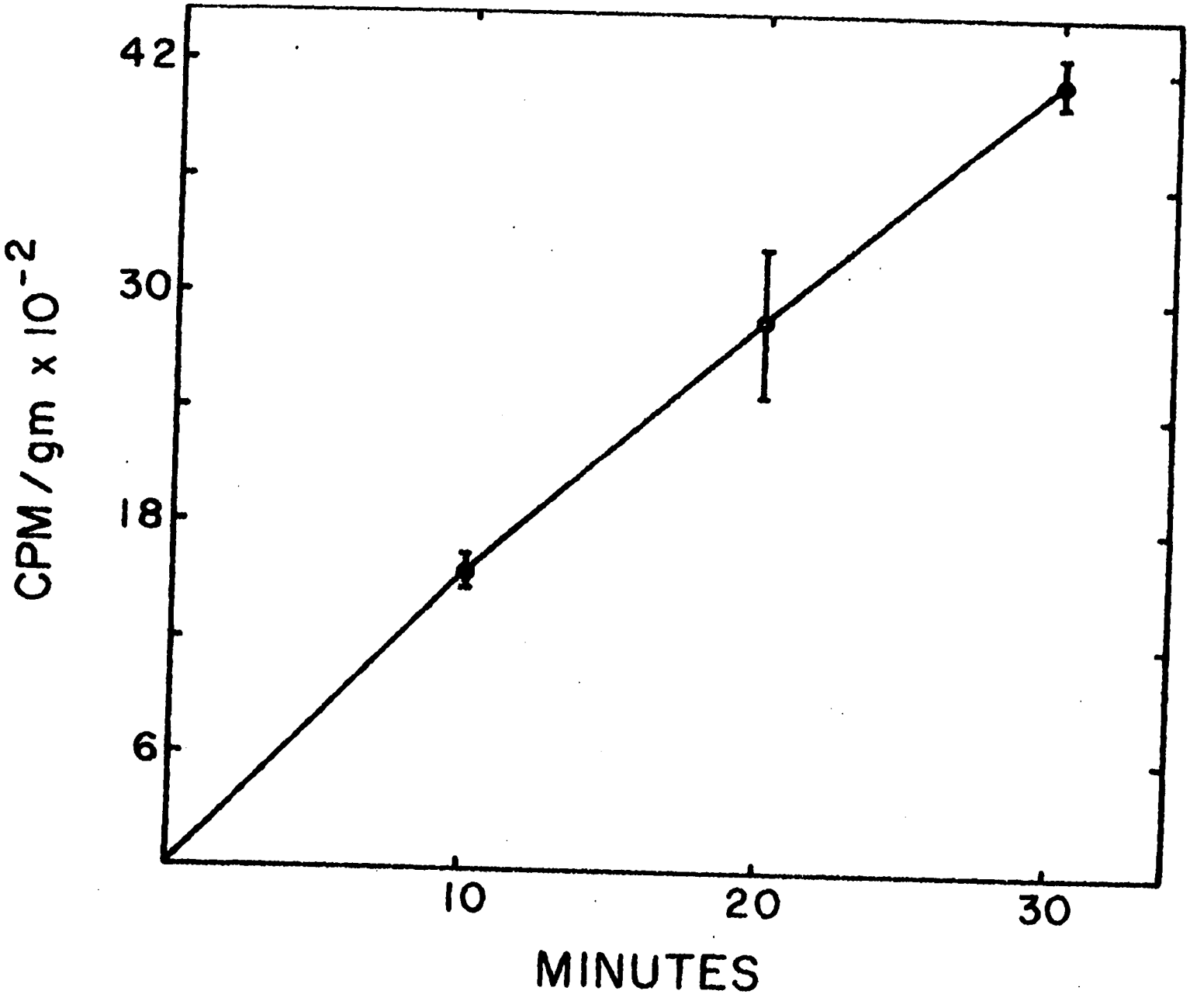

Legend to Figure 4

TIME COURSE OF THE INCORPORATION OF ^{3}H-URIDINE INTO RNA OF MOUSE LIVER

Mice were injected intraperitoneally with 100μC of tritiated uridine (500μC/mg) in isotonic saline. At the times indicated after injection, the animals were killed, the liver rapidly removed and frozen on dry ice. The results are expressed as cpm/gm of frozen weight of liver and are the mean of from 3 to 6 determinations. Ribonucleic acid was isolated as described in the methods section for in vivo RNA synthesis. Vertical bars represent the standard deviation of the mean.

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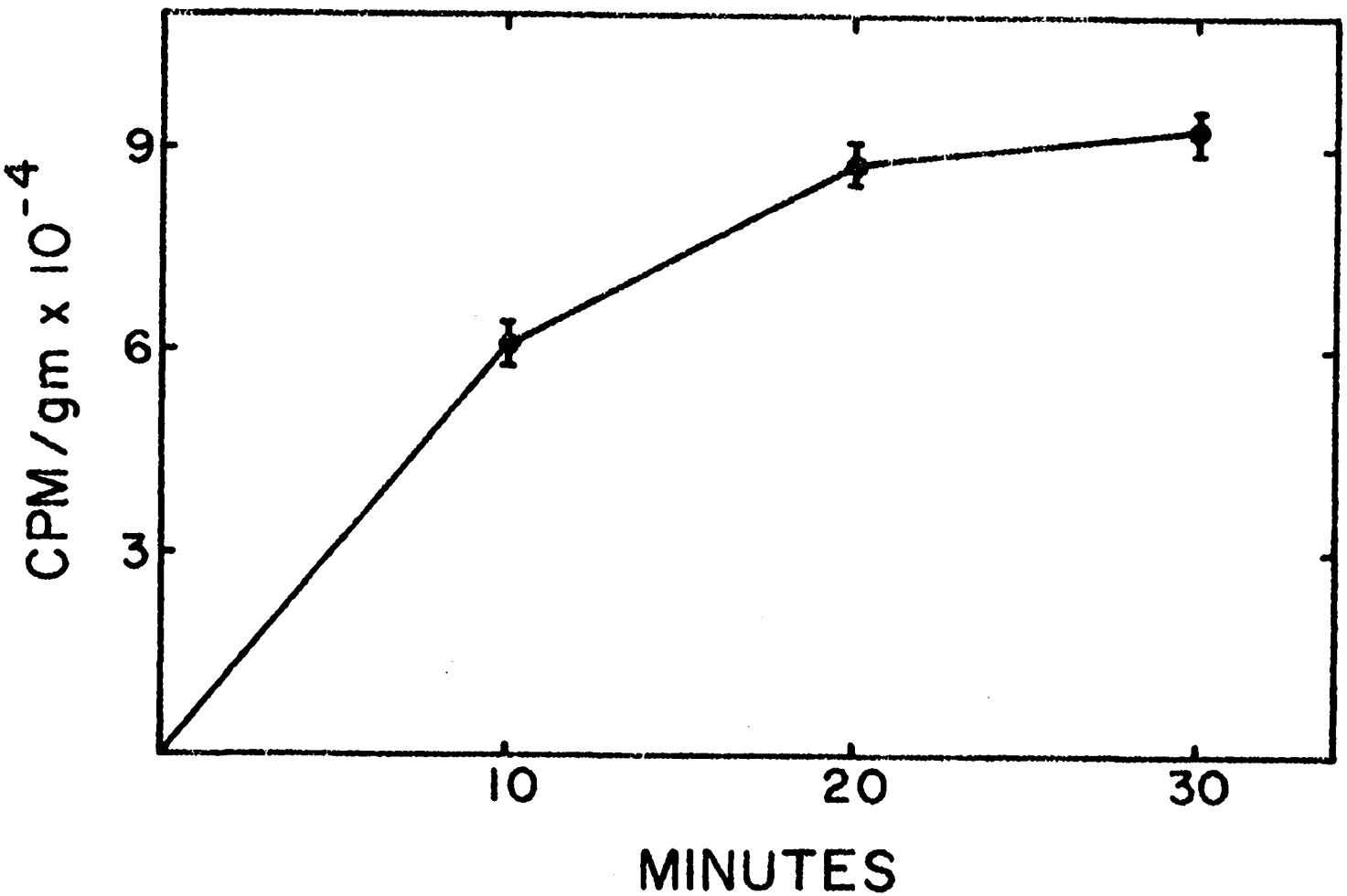

Legend to Figure 5

TIME COURSE OF THE INCORPORATION OF ^{14}C-VALINE INTO THE PROTEIN OF MOUSE BRAIN

Mice were injected intraperitoneally with 5μC of D,L-^{14}C-valine in isotonic saline (85μC/mg). At the times indicated after injection, the animals were killed, the brain rapidly removed and frozen on dry ice. The results are expressed as cpm/mg of residual protein. Protein was isolated as described in the methods section for <u>in vivo</u> protein synthesis I. Vertical bars represent the standard error of the mean.

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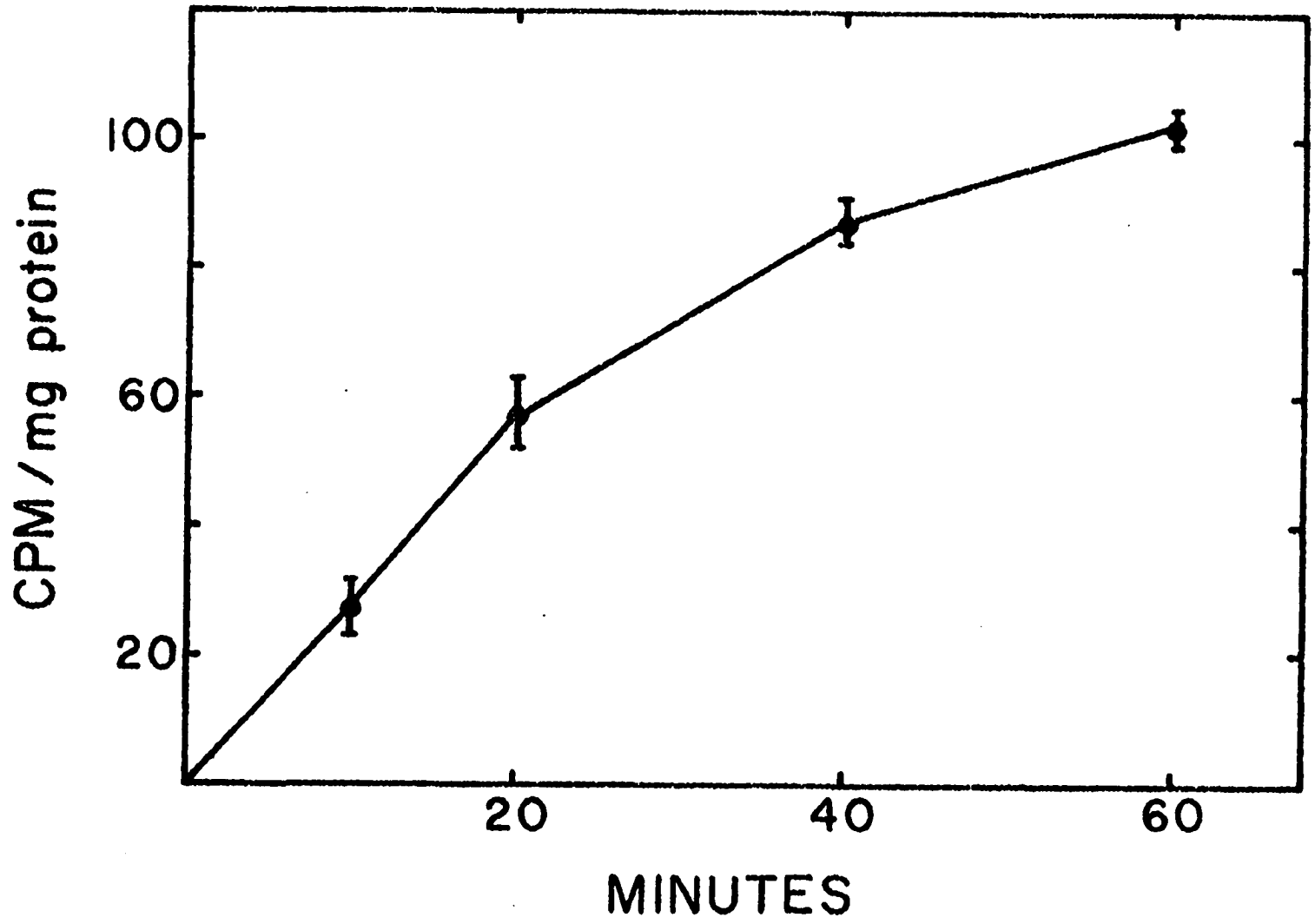

Legend to Figure 6

TIME COURSE OF THE INCORPORATION OF ^{14}C-VALINE INTO THE PROTEIN OF MOUSE LIVER

Mice were injected intraperitoneally with 5μC of D,L-^{14}C-valine in isotonic saline (85μC/mg). At the times indicated after injection, the animals were killed, the liver rapidly removed and frozen on dry ice. The results are expressed as cpm/mg of residual protein. Protein was isolated as described in the methods section for *in vivo* protein synthesis I. Vertical bars represent the standard error of the mean.

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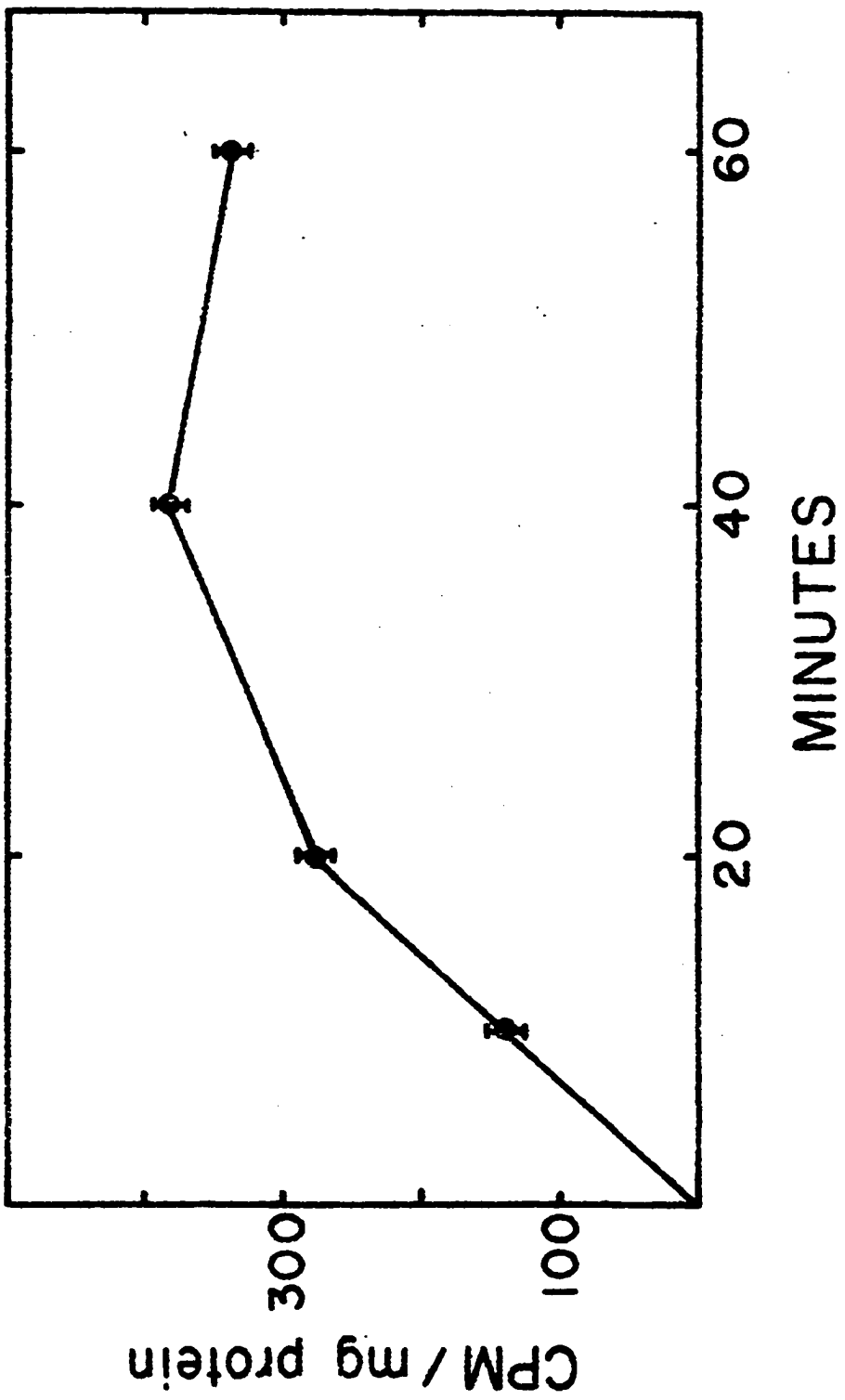

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tritiated uridine, yielded the results shown in Figures 7 and 8 for brain and liver, respectively. The data indicated that reserpine inhibits the incorporation of uridine into RNA greater than 50% from about 4 to 24 hours after this alkaloid. This inhibition reached a maximum of about 60 to 80% inhibition between 6 and 12 hours in both brain and liver.

Decreased rates of fixation of uridine into RNA by reserpine could be due to interference by reserpine with free mixing of the radioactive precursor with the endogenous pool of precursor. In order to obviate this problem, the pool size of uridine nucleotides was determined in the livers of mice treated with reserpine. In Table 1 it is apparent that reserpine does not significantly alter the precursor pool size of uridine nucleotides.

Experiments analogous to those described in Figures 7 and 8 for the incorporation of radioactive uridine into RNA of brain and liver were carried out for the synthesis of protein. The effects of reserpine on the incorporation of 1-^{14}C-valine into protein of brain and liver are shown in Figures 9 and 10. A marked similarity between the time courses of inhibition by reserpine of uridine utilization for the formation of RNA and valine for the synthesis of protein exists. Thus, inhibition of the synthesis of protein was greater than 50% from 4 to 24 hours with maximal inhibition of from 60 to 80% being observed from 6 to 12

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Legend to Figure 7

TIME COURSE OF THE INCORPORATION OF TRITIATED URIDINE INTO RNA OF BRAIN AFTER TREATMENT WITH RESERPINE

Mice received an intravenous injection of 2.5 mg/kg of reserpine phosphate at time zero. At the indicated times, they received ^{3}H-uridine (100μC, 500μC/mg), as described in Figure 3, and were killed 20 minutes later. The tissue was processed as described in Figure 3, except that the deoxyribose content was determined as a measure of the amount of tissue in place of the frozen weight. The results are expressed as cpm fixed in the hot acid soluble fraction/μmole of total deoxyribose of the tissue. Each point represents the mean of from 3 to 15 animals. Control is approximately 2,000cpm/μmole deoxyribose.

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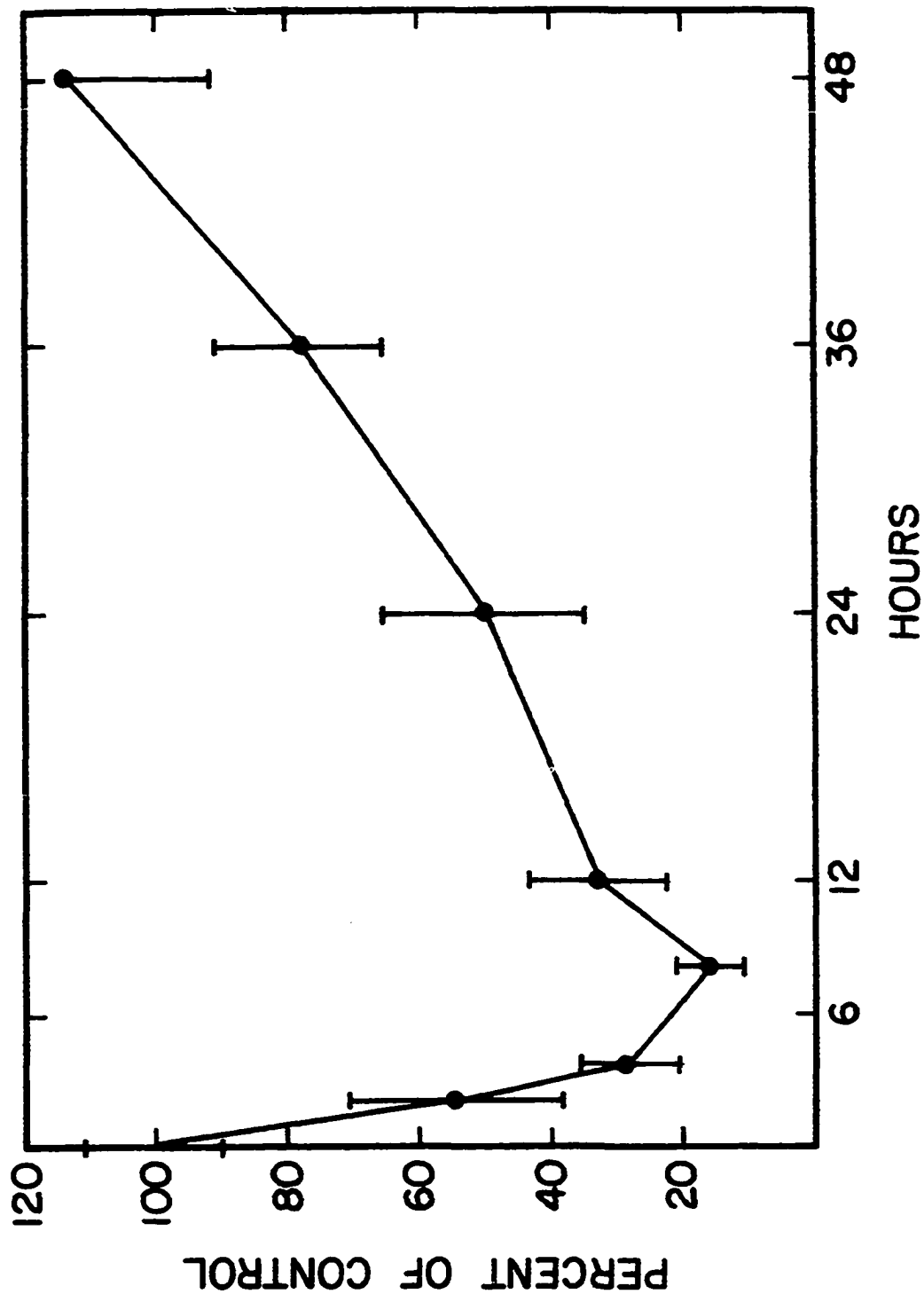

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Legend to Figure 8

TIME COURSE OF THE INCORPORATION OF TRITIATED URIDINE INTO RNA OF LIVER AFTER TREATMENT WITH RESERPINE

Mice received an intravenous injection of 2.5 mg/kg of reserpine phosphate at time zero. At the indicated times, they received ^{3}H-uridine (100μC, 500μC/mg), as described in Figure 3 and were killed 20 minutes later. The tissue was processed as described in Figure 3 except that the deoxyribose content of the tissue was determined as a measure of the amount of tissue in place of the frozen weight. The results are expressed as cpm fixed in the hot acid soluble fraction/μmole of total deoxyribose of the tissue. Each point represents the mean of from 3 to 15 animals. Control is approximately 40,000cpm/μmole deoxyribose. Vertical bars represent the standard error of the mean.

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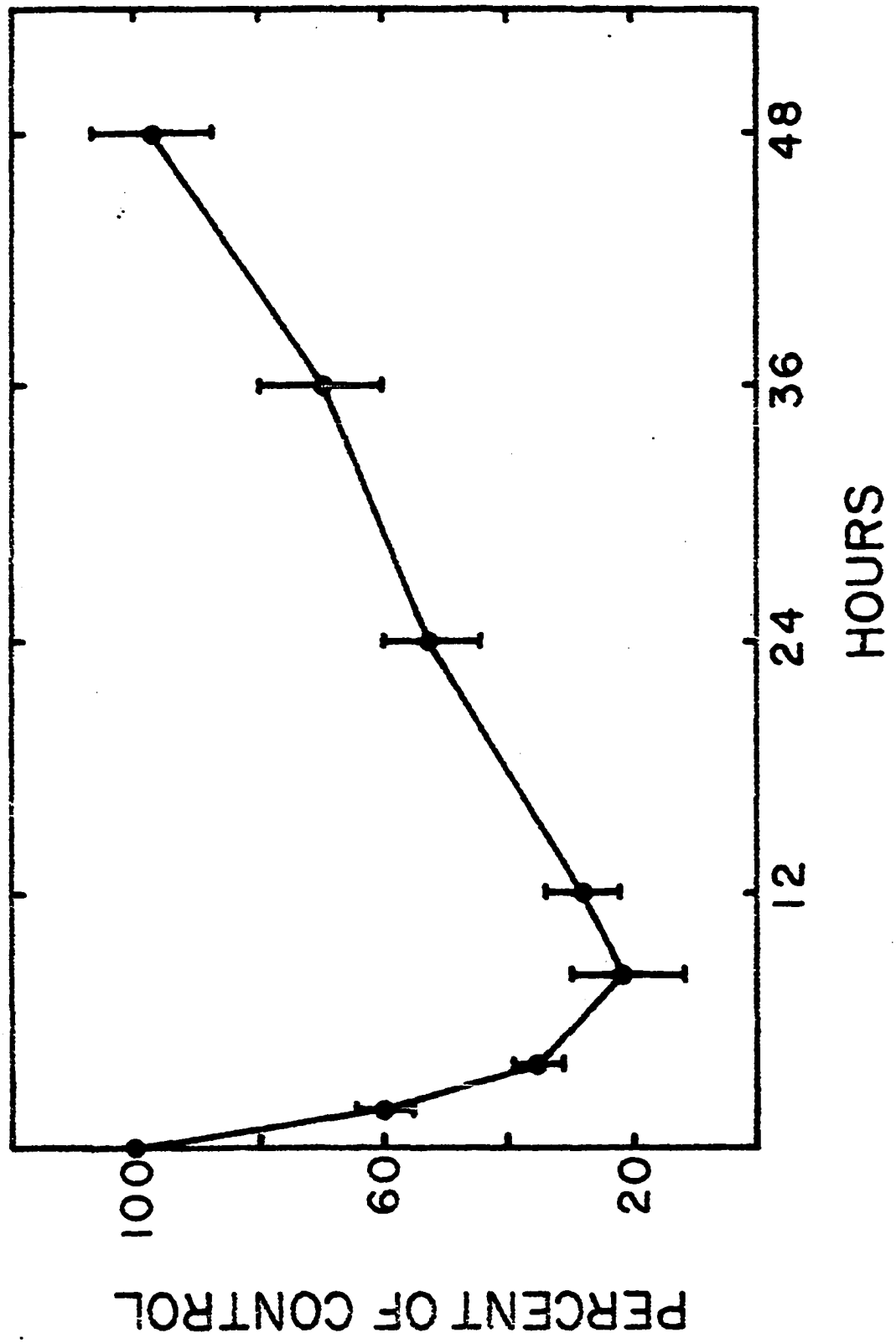

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TABLE 1

SPECIFIC ACTIVITY OF THE URIDINE NUCLEOTIDE POOL IN LIVER OF NORMAL AND RESERPINIZED MICE

TREATMENT	SPECIFIC ACTIVITY (cpm/mmole)
NONE	280 ± 15
RESERPINE	265 ± 75

The specific activity of the phosphorylated uridine nucleotides was determined 6 hours after intravenous administration of 2.5 mg/kg of reserpine phosphate by collecting the acid-soluble fraction from liver, and hydrolyzing the uridine nucleotides to 5'-UMP with 0.1M PCA. The UMP was separated from the other nucleotides by thin layer chromatography and the absorbance at 260mμ of UMP and its radioactivity determined. The results are expressed as cpm/mmole of UMP. The results are the average of duplicates.

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Legend to Figure 9

TIME COURSE OF INHIBITION BY RESERPINE
OF THE INCORPORATION OF VALINE-1-^{14}C
INTO PROTEIN OF MOUSE BRAIN

The rate of synthesis of protein in mouse brain was determined as in Figure 5. Each value represents the mean of from 3 to 12 animals. Protein isolated from untreated animals had a specific activity of about 65cpm/mg residual protein. The vertical bars represent the standard deviation of the mean.

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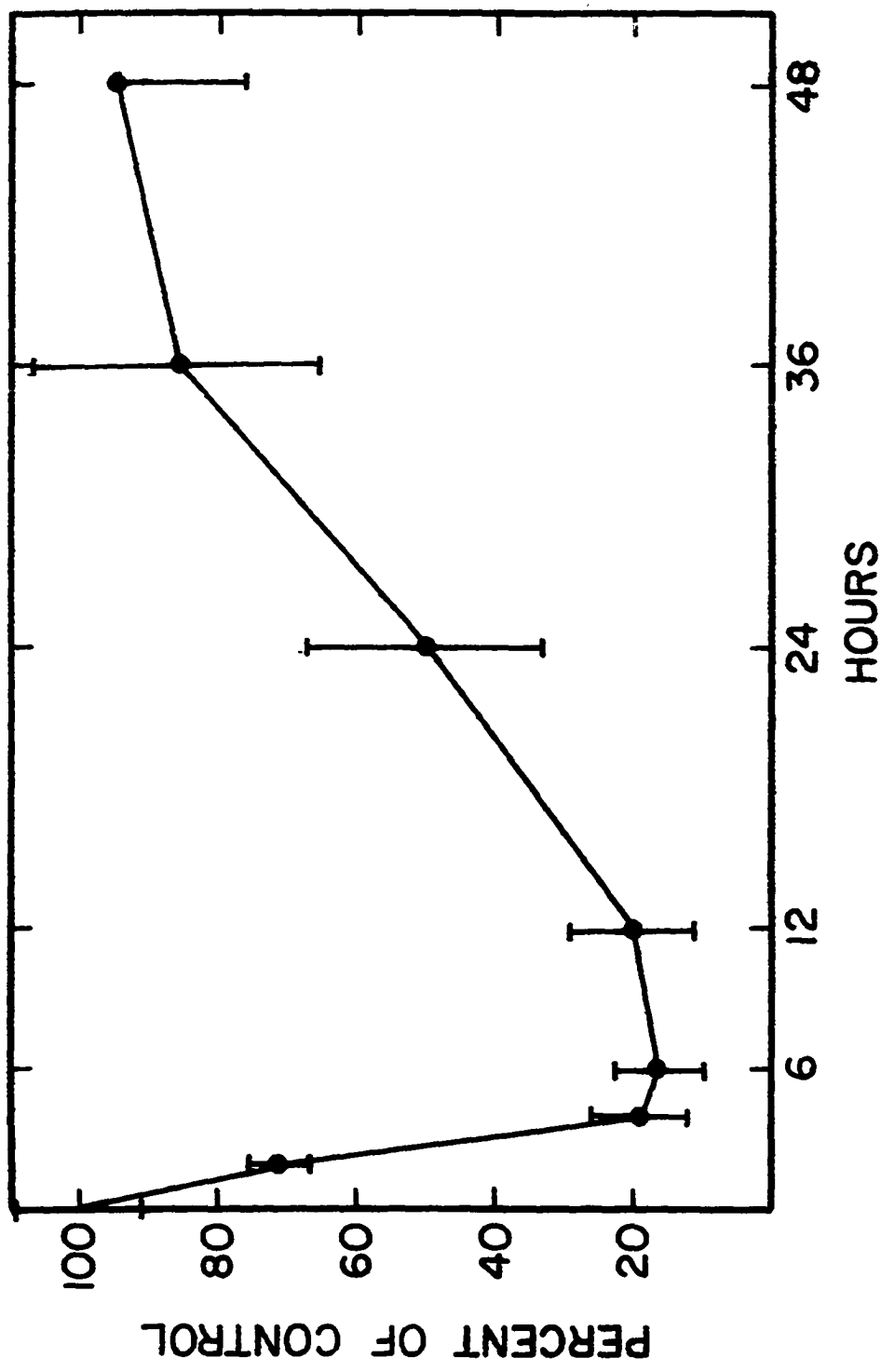

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Legend to Figure 10

TIME COURSE OF INHIBITION BY RESERPINE
OF THE INCORPORATION OF VALINE-1-^{14}C
INTO PROTEIN OF MOUSE LIVER

The rate of synthesis of protein in mouse liver was determined as in Figure 6. Each value represents the mean of from 3 to 12 animals. Protein isolated from untreated animals had a specific activity of 380cpm/mg of residual protein. The vertical bars represent the standard error of the mean.

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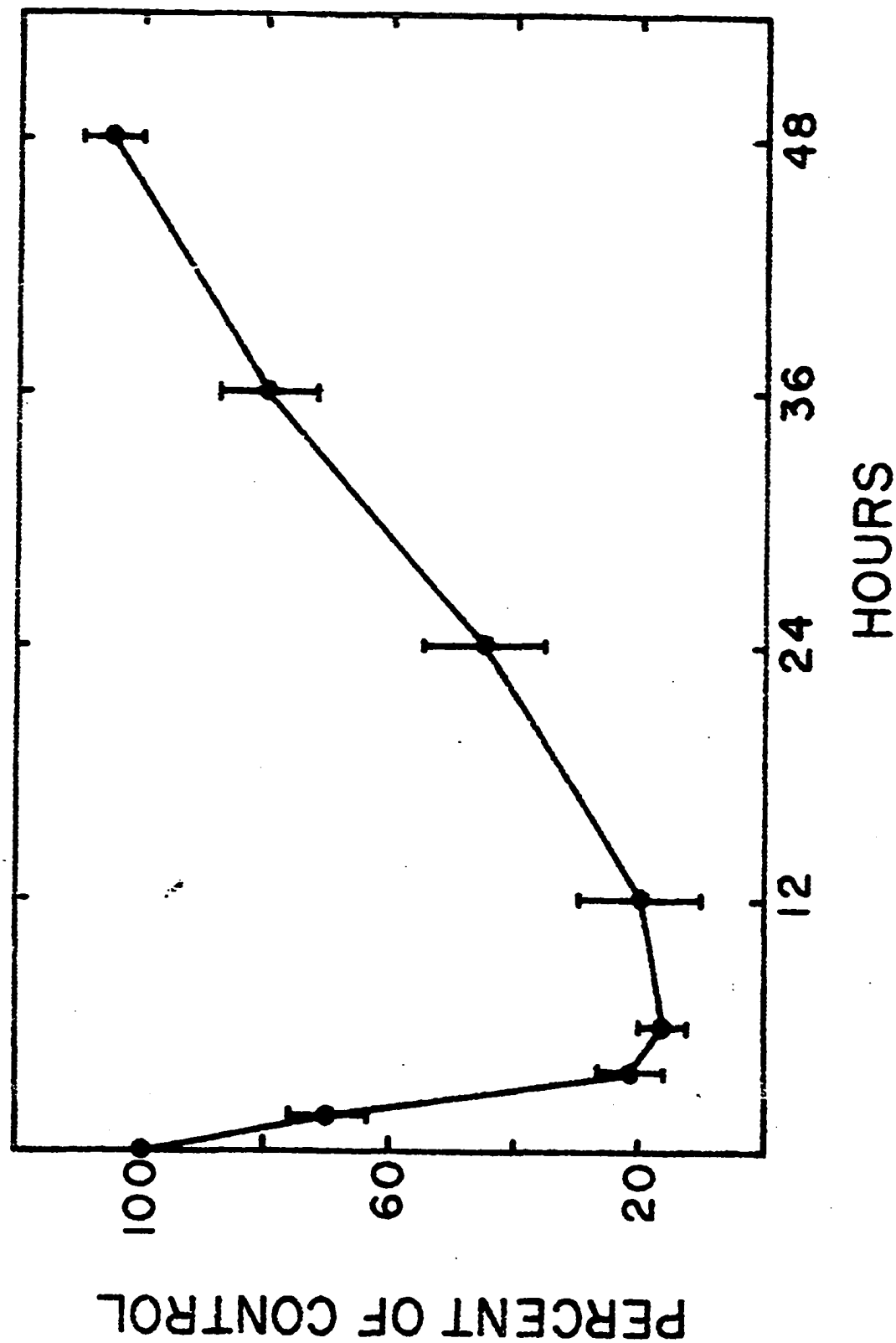

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TABLE 2

INHIBITION BY RESERPINE OF INCORPORATION OF ^{14}C-PHENYLALANINE INTO PROTEIN OF LIVER

TREATMENT	SPECIFIC ACTIVITY (cpm/mg)
CONTROL	470 ± 31
RESERPINE	90 ± 17

Six hours after the intravenous administration of 2.5 mg/kg of reserpine phosphate, mice were given 5μC of uniformly labeled ^{14}C-phenylalanine (7.5μC/μmole). After permitting the radioactive precursor to incorporate for 20 minutes, the liver was excised and the specific activity of the protein was determined as counts per minute per milligram of protein ± the standard deviation (51).

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hours after treatment with this alkaloid.

To demonstrate that these results were not unique to valine, the action of reserpine on the incorporation of ^{14}C-phenylalanine into protein was also determined. The results shown in Table 2 indicate that incorporation of this amino acid into acid-insoluble material was decreased to about the same extent as that observed with valine.

The dose of reserpine (2.5 mg/kg of reserpine phosphate i.v.) used in these experiments described (Figures 7-10) is considered relatively large by pharmacologists who employ this drug to decrease biogenic amine levels, although doses from 10 to 15 mg/kg have been used (57, 58) when a more extreme effect of the drug is desired. Nevertheless, no fatalities occurred in several hundreds of intravenous administrations in these experiments. It was of interest to determine if smaller doses of this drug would also interfere with the synthesis of macromolecules. It is apparent that one-half of a 2.5 mg/kg dose of reserpine phosphate did not interfere with macromolecular synthesis at any of the time points examined.

Reserpine induces a rapid release of biogenic amines, essentially saturating the affected tissues with these molecules and their metabolites; therefore, it was important to determine if the process of amine release after treatment

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TABLE 3

INHIBITION OF RNA SYNTHESIS BY RELATIVELY SMALL DOSES OF RESERPINE PHOSPHATE

DOSE OF RESERPINE	TIME AFTER RESERPINE (hr)	PER CENT OF CONTROL	
		BRAIN	LIVER
1.25	4	97 ± 15	91 ± 11
1.25	6	94 ± 21	101 ± 9
1.25	12	103 ± 14	87 ± 6
1.25	24	84 ± 17	107 ± 14
0.625	6	97 ± 3	110 ± 17

At various times after the intravenous administration of either 1.25 or 0.625 mg/kg of reserpine phosphate, the rate of synthesis of RNA was determined as in Figures 5 and 6. Each number represents the mean of 3 determinations. The results are expressed as cpm in RNA/μmole of total deoxyribose of the tissue ± the standard error. Control for liver is 40,000cpm/μmole deoxyribose and brain was 2,000cpm/μmole deoxyribose.

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TABLE 4

INHIBITION OF PROTEIN SYNTHESIS BY RELATIVELY SMALL DOSES OF RESERPINE PHOSPHATE

TREATMENT	SPECIFIC ACTIVITY (cpm/mg)	
	BRAIN	LIVER
CONTROL	67 ± 7	370 ± 31
RESERPINE	63 ± 4	400 ± 14

Six hours after the intravenous injection of 1.25 mg/kg of reserpine phosphate, the rate of synthesis of protein was determined as in Figures 9 and 10. Each number represents the mean of 3 separate determinations ± the standard error.

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TABLE 5

THE EFFECT OF TWO DOSES OF RESERPINE ON THE INCORPORATION OF RADIOACTIVE PRECURSORS INTO THE RNA AND PROTEIN OF BRAIN AND LIVER

	PER CENT OF CONTROL			
	RNA		PROTEIN	
TREATMENT	BRAIN	LIVER	BRAIN	LIVER
RESERPINE (zero time)	124 ± 16	89 ± 11	131 ± 14	92 ± 2
RESERPINE (zero time and 42 hours)	29 ± 7	38 ± 4	17 ± 3	27 ± 7

Reserpine phosphate, 2.5 mg/kg intravenous, was given either at zero time or zero time and again at 42 hours, and the rate of ^{3}H-uridine incorporation into RNA was determined at 48 hours. The rate of RNA synthesis was measured as described in Figures 7 and 8. Each value represents the mean of 3 determinations ± the standard deviation.

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with this alkaloid could be responsible for the effects of the drug on macromolecular synthesis. A comparison of Figures 7 and 18 shows that the rates of incorporation of ^{3}H-uridine with RNA had returned essentially to normal at 48 hours after reserpine treatment, but that the tissue levels of biogenic amines at this time were depleted to between 0 and 10% of control levels. A second dose of reserpine at 42 hours after the first dose can thus only release a small amount of amine (at most 10% of normal); measurement of the rates of synthesis of RNA and protein with such a protocol makes it possible to determine if the release of amines produced by the first dose of reserpine was involved in the inhibition of macromolecular synthesis. It is apparent from the data shown in Table 5 that release of the relatively large amount of amine is not involved in the inhibition of such synthesis by this alkaloid, as the incorporation of ^{3}H-uridine into RNA was depressed by the alkaloid in a situation in which little amine was available to be released.

In the same way that it is possible to reduce biogenic amine levels before a dose of reserpine (as in Table 5), administration of inhibitors of monoamine oxidase will increase the amounts of biogenic amines present, even after reserpine when given in proper sequence (59). The data in Tables 6 and 7 show that pretreatment of mice with either pargyline or iproniazid results in substantial reversal of or protection

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TABLE 6

THE EFFECT OF PARGYLINE ON THE INHIBITION OF RNA SYNTHESIS BY RESERPINE

TREATMENT	PER CENT OF CONTROL	
	BRAIN	LIVER
RESERPINE	19 ± 7	25 ± 3
PARGYLINE + RESERPINE	75 ± 9	71 ± 11
PARGYLINE	105 ± 14	97 ± 5

Pargyline (100 mg/kg ip) was given 24 hours before sacrifice, reserpine (2.5 mg/kg iv) 6 hours before sacrifice. The drugs were administered together on the same schedule, and tritiated uridine was given 20 min before sacrifice. The results were obtained as in Figures 7 and 8 and represent the mean of 3 determinations ± the standard deviation.

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TABLE 7

THE EFFECT OF IPRONIAZID ON THE INHIBITION OF RNA SYNTHESIS BY RESERPINE

TREATMENT	PER CENT OF CONTROL	
	BRAIN	LIVER
RESERPINE	27 ± 9	14 ± 2
IPRONIAZID + RESERPINE	81 ± 14	69 ± 15
IPRONIAZID	91 ± 7	111 ± 17

Iproniazid (100 mg/kg ip) was given 24 hours before sacrifice. Reserpine (2.5 mg/kg iv) was given 6 hours before sacrifice. The drugs were administered together on the same schedule, and tritiated uridine was given 20 min before sacrifice. The results were obtained as in Figures 7 and 8 and represent the mean of 3 determinations ± the standard deviation.

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from the inhibition of RNA synthesis brought about by reserpine.

Reserpine inhibited the incorporation of amino acids into protein by greater than 50% from 4 to 24 hours after the alkaloid (Figures 9 and 10). To determine if reserpine was selective in its inhibition of the synthesis of protein in various cellular fractions, the nucleus, mitochondria, microsomes, and cytoplasm were isolated and the specific activities of the protein determined in each. Inhibition of protein synthesis by this alkaloid appeared to be non-specific, as all of the fractions were inhibited to the same extent.

Winnick et al first described a system of components isolated from rat liver which, in the absence of intact cells, would incorporate amino acids into peptide bonded polymers of diverse constitution (60). Since this original report, such systems have been described for many different species and tissues (61). One of the more universal traits of such systems is that they are generally sensitive to drugs which inhibit the *in vivo* synthesis of protein in concentrations analogous to those employed to inhibit such synthesis *in vitro*. Cycloheximide (62), puromycin (63), and emetine (64) generally fit into this class, although there are some additional processes that these drugs have been shown to

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TABLE 8

THE EFFECT OF RESERPINE ON THE INCORPORATION OF 1-^{14}C-VALINE INTO PROTEIN FRACTIONS FROM MOUSE LIVER

FRACTION	RESERPINE	SPECIFIC ACTIVITY (cpm/mg)	RATIO
MICROSOMAL	+	3120	46
	-	6700	
SUPERNATANT	+	960	40
	-	2370	
NUCLEAR	+	20	50
	-	40	
WHOLE HOMOGENATE	+	1250	41
	-	3080	

Various fractions were prepared from mouse as described in the methods section and their specific activities determined. The mice had received 2.5 mg/kg of reserpine phosphate 6 hours prior to killing or were controls. Each value represents the average of 2 determinations, and the results are expressed as cpm/mg of Lowry protein (51) $\pm$ the standard deviation.

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TABLE 9

THE EFFECT OF RESERPINE ON THE INCORPORATION OF AMINO ACIDS IN A CELL-FREE PROTEIN SYNTHETIC SYSTEM DERIVED FROM LIVER

METHOD OF DRUG EXPOSURE	CPM/mg
None	450 ± 25
In Vitro (5×10^{-4}M)	390 ± 27
In Vivo (2.5 mg/kg)	410 ± 39

The microsomal and supernatant fractions were isolated from mouse liver as described in the methods and were incubated in the presence of final concentrations of 10mM $MgCl_2$, 20mM KCl, 20mM Tris pH 7.4, 1mM ATP, 20mM creatine phosphate, and a mixture of tritiated amino acids. The system was linear for around 30 minutes and was allowed to incorporate for 25.

In vitro drug exposure constituted adding reserpine to a final concentration of 5×10^{-4}M. In vivo exposure was performed by treating mice with 2.5 mg/kg of reserpine phosphate 6 hours prior to sacrifice and isolating the microsomes and supernatant from livers of treated mice. Each value represents the mean of 4 separate determinations ± the standard deviation and is expressed as cpm/mg of Lowry protein of the microsome fraction (51).

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Legend to Figure 11

THE EFFECT OF RESERPINE ON THE POLYSOME PROFILE OF MOUSE LIVER

Nuclei and mitochondria were removed by centrifugation in medium containing 0.25M sucrose, 15mM $MgCl_2$, 20mM KCl, and 0.1M Tris-HCl (pH 7.4). The supernatant was layered over a 20 to 50% sucrose gradient ionically identical to the above medium. The tubes were centrifuged at 280,000 x g for 70 minutes; fractions were collected using an Isco Density Gradient Fractionator, and the absorbence at 258mμ was determined.

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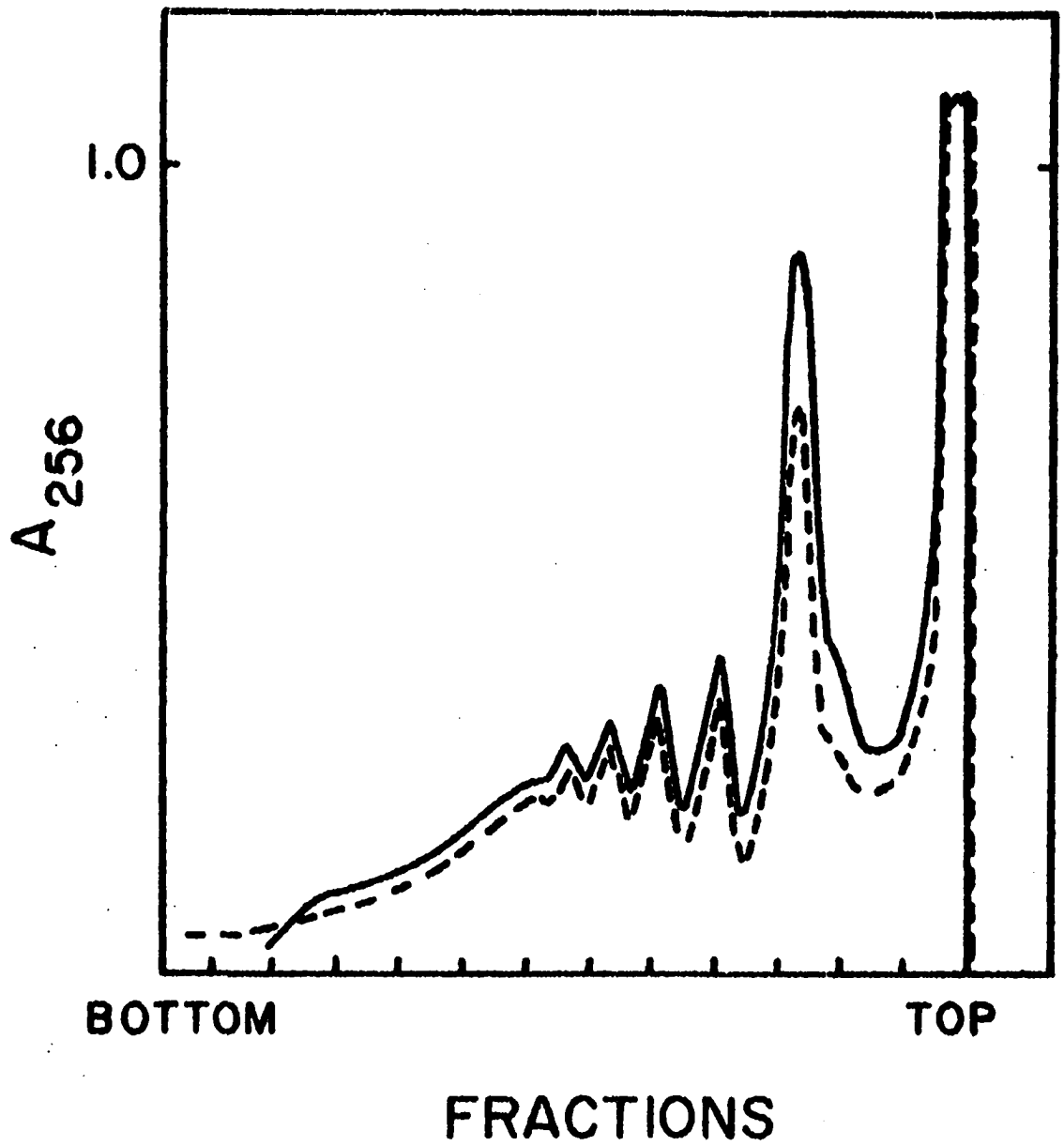

for these results. Recently it has become apparent that there are several RNA synthetic enzymes in higher organisms (70), and these enzymes have highly selective liability to drugs. It is possible that reserpine inhibits one of these enzymes, but that this enzyme has no effect on the polysome profile. The other explanation might lie in the fact that it has been reported that reserpine (71) also inhibits the breakdown of RNA, and, although it is only implied, the shift from poly- to monosomes with drugs is thought to be a consequence of the ongoing metabolism of the RNA (72).

Since reserpine substantially inhibited the synthesis of both RNA and protein *in vivo* (Figures 7 through 10), it seemed desirable to further characterize this inhibition by studying it *in vitro*. To accomplish this, slices of liver were incubated in Krebs II buffer with reserpine (1.4×10^{-4} M) and either ^{14}C-adenine or ^{14}C-valine. The presence of reserpine in the incubation mixture did not appear to alter the rate of incorporation of ^{14}C-adenine and ^{14}C-valine into RNA and protein, respectively. Since reserpine is quite insoluble under these conditions, it was possible that the drug was not able to penetrate the tissue. Tissues were therefore prepared from animals that had been pretreated with reserpine (2.5 mg/kg, *i.v.*) 6 hours prior to excision of the liver and the slices were once again incubated with either ^{14}C-adenine or ^{14}C-valine. No inhibition of either RNA or protein synthesis was observed under these conditions.

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Legend to Figure 12

THE EFFECT OF RESERPINE ON THE INCORPORATION OF ^{14}C-ADENINE INTO SLICES OF MOUSE LIVER

Slices were prepared from normal animals or from mice that had received 2.5 mg/kg of reserpine phosphate prior to sacrifice. The slices were then incubated for the indicated periods of time in Krebs II (53) containing 0.2 mM 8-^{14}C-adenine (1 μC/ml, 5 μC/μmole) in a total volume of 5 ml; flasks were purged with 95% O_2 : 5% CO_2 prior to incubation. A fraction of post-incubation homogenate was taken for determination of the amount of protein present by the technique of Lowry (51), and the results are presented as cpm/mg of protein. Untreated, ○—○; treatment *in vitro*, □—□; treated *in vivo*, ■—■.

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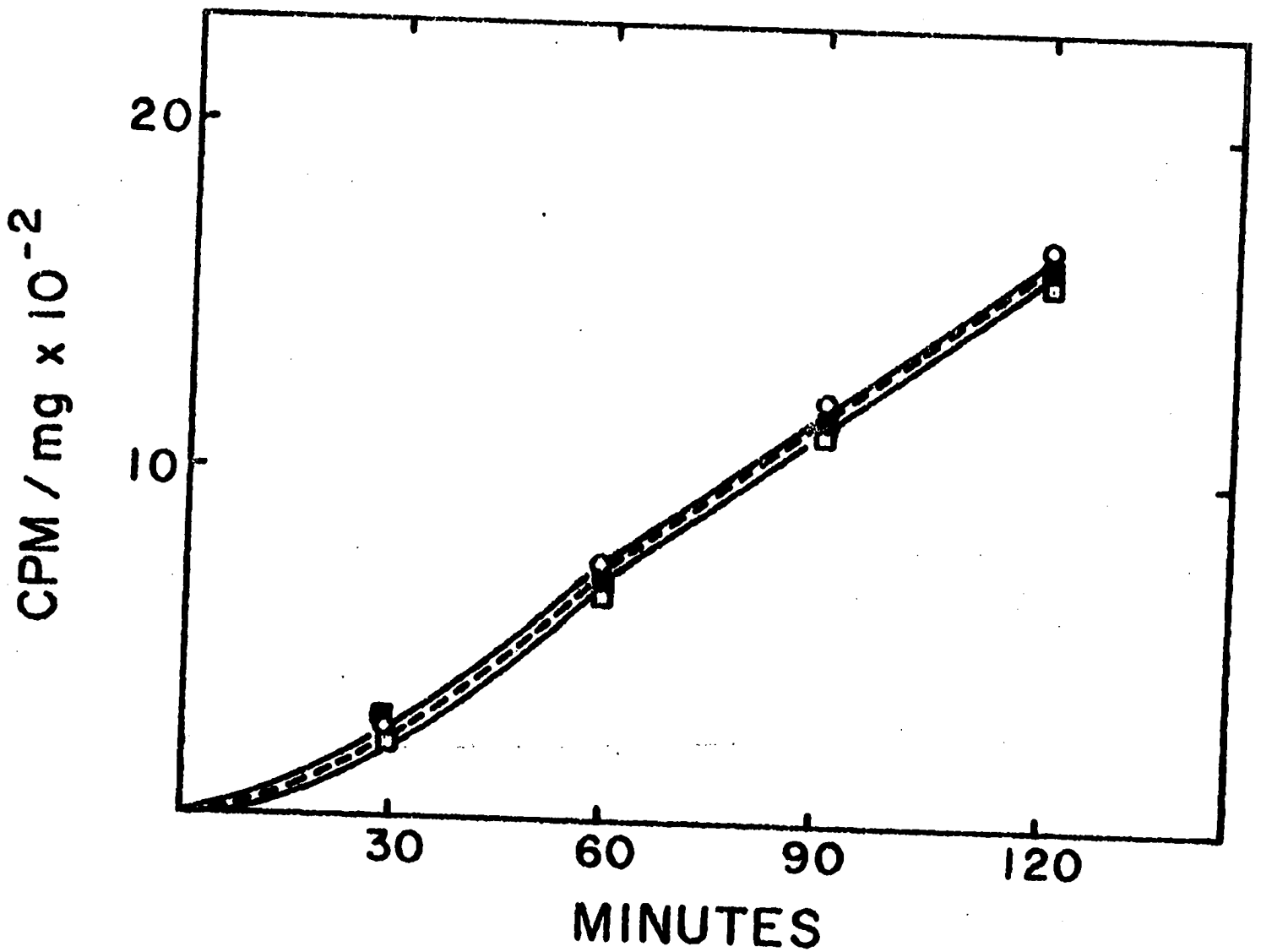

Legend to Figure 13

THE EFFECT OF RESERPINE ON THE INCORPORATION OF ^{14}C-VALINE INTO SLICES OF MOUSE LIVER

Slices were prepared from normal animals or from mice that had received 2.5 mg/kg of reserpine phosphate prior to sacrifice. The slices were then incubated for the indicated periods of time in Krebs II (53) containing 0.1 mM ^{14}C-valine (1 μC/ml, 10 μC/μmole) in a total volume of 5 ml; flasks were purged with 95% O_2 : 5% CO_2 prior to incubation. A fraction of post-incubation homogenate was taken for determination of the amount of protein present by the technique of Lowry (51), and the results are presented as cpm/mg of protein. Untreated, o—o; treatment *in vitro*, □—□; treated *in vivo*, ■—■.

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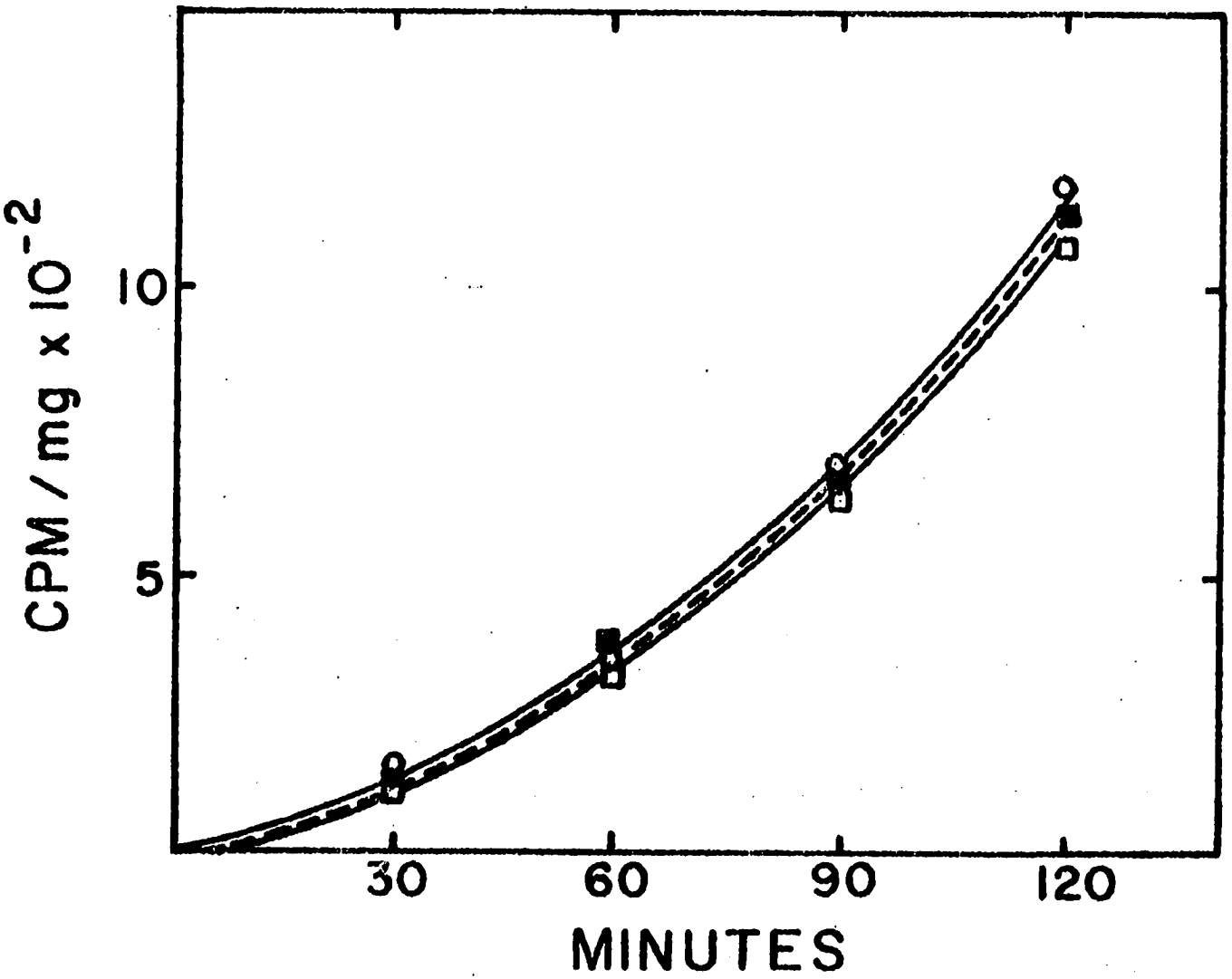

Drugs which inhibit the synthesis of ribonucleic acid *in vivo* have usually been found to also decrease the synthesis of RNA *in vitro*. The synthesis of RNA with bacterial RNA polymerase enzymes is fairly complex; there are three separate steps involved: (a) binding or formation of a complex between the enzyme and the DNA template; (b) initiation of the synthesis of the ribonucleic acid polymer; and (c) elongation of the polymer. Various drugs have been shown to inhibit each of the steps (73). Since reserpine inhibited the synthesis of RNA *in vivo*, it was of importance to determine if the alkaloid would also inhibit the synthesis of RNA by an RNA polymerase. The *E. coli* RNA polymerase was chosen as a model system, since it was the best defined of the enzymes available. In Figure 12 are presented the results obtained when the amount of reserpine phosphate added to the reaction mixture was varied from zero to x 10^{-3} M. A decrease of about 50% was observed with a reserpine concentration of about 4 x 10^{-4} M.

In Figures 13 through 15 are shown similar graphs in which the concentration of reserpine was held constant and the amounts of the reaction components were varied as a means of investigating the mechanism by which reserpine inhibited the synthesis of RNA. It appeared that a complex formed between reserpine and DNA, as an observable flocculance occurred in the reaction mixture. The fact that adding more DNA to the mixture did not reverse the inhibition of the polymerase

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Legend to Figure 14

THE EFFECT OF RESERPINE ON THE SYNTHESIS
OF RIBONUCLEIC ACID BY
ESCHERICHIA COLI RIBONUCLEIC ACID POLYMERASE

RNA polymerase (fraction III) was prepared from Escherichia coli by the method of Chamberlin and Berg (55) and stored at -20° in storage medium (see methods). The reaction mixture contained 10 µmole of Tris-HCl (pH 8.0, 1.0 µmole of $MgCl_2$, and a mixture of 0.1 µmole each of ATP, GTP, UTP, and CTP in a final volume of 0.25 ml and ^{3}H-UTP was present at 1 µC/umole. Each tube also contained 1.2 mg of calf thymus DNA and 0.5 mg of protein from the enzyme preparation. The reaction was incubated at 37° for 10 minutes and terminated by immersion in ice and the subsequent rapid addition of carrier protein and trichloroacetic acid to a final concentration of 5%. The tubes were allowed to remain at 4° for 15 minutes and were then washed 6 times with 5% TCA. Each point represents the mean of from 2 to 4 determinations; the variation between duplicates was less than 5%.

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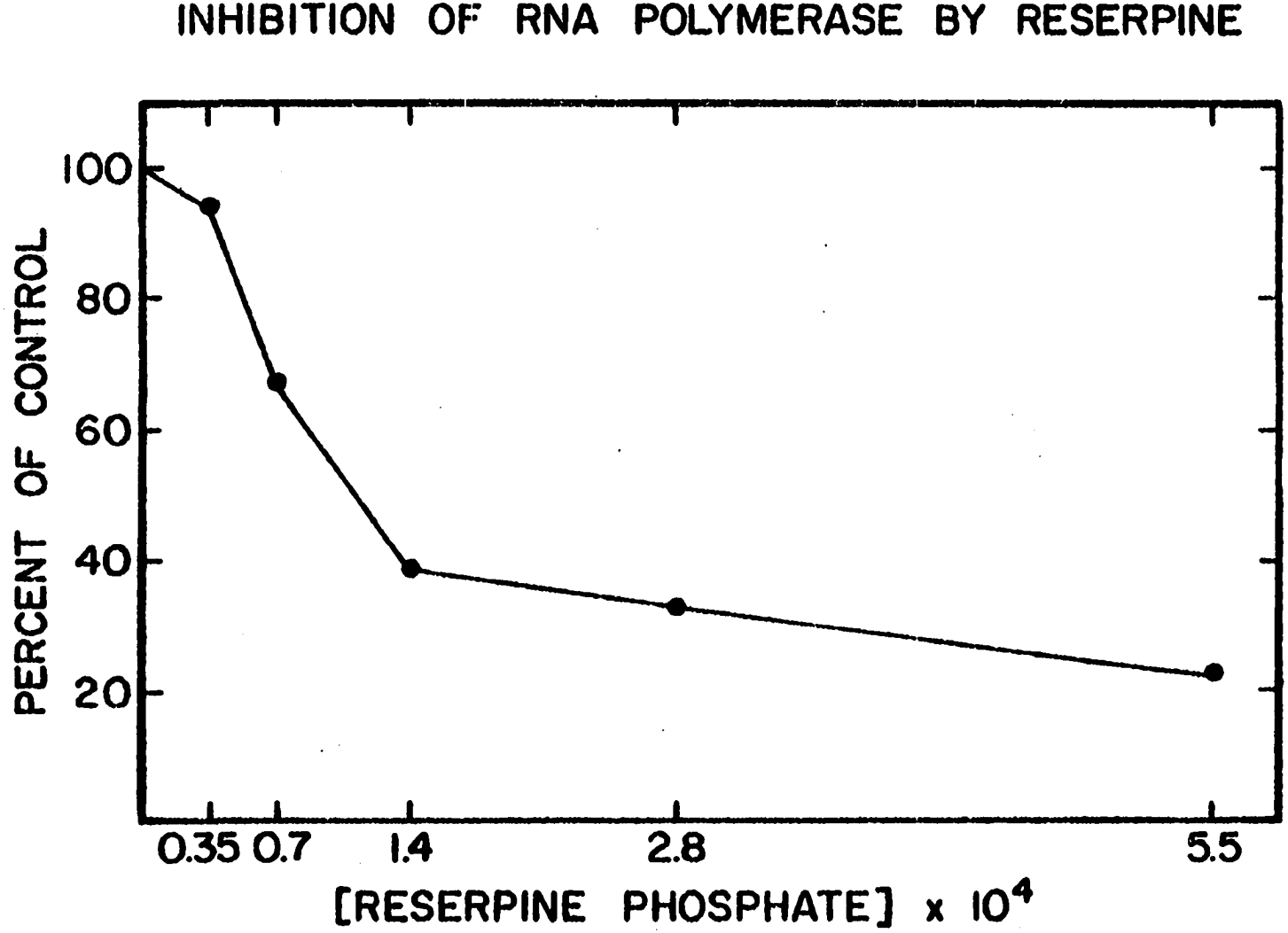

Legend to Figure 15

THE EFFECT OF MAGNESIUM CONCENTRATION ON THE INHIBITION OF RNA POLYMERASE BY RESERPINE

RNA polymerase was assayed as described in Figure 14, except that the amount of $MgCl_2$ was varied from 4 mM to 24 mM.

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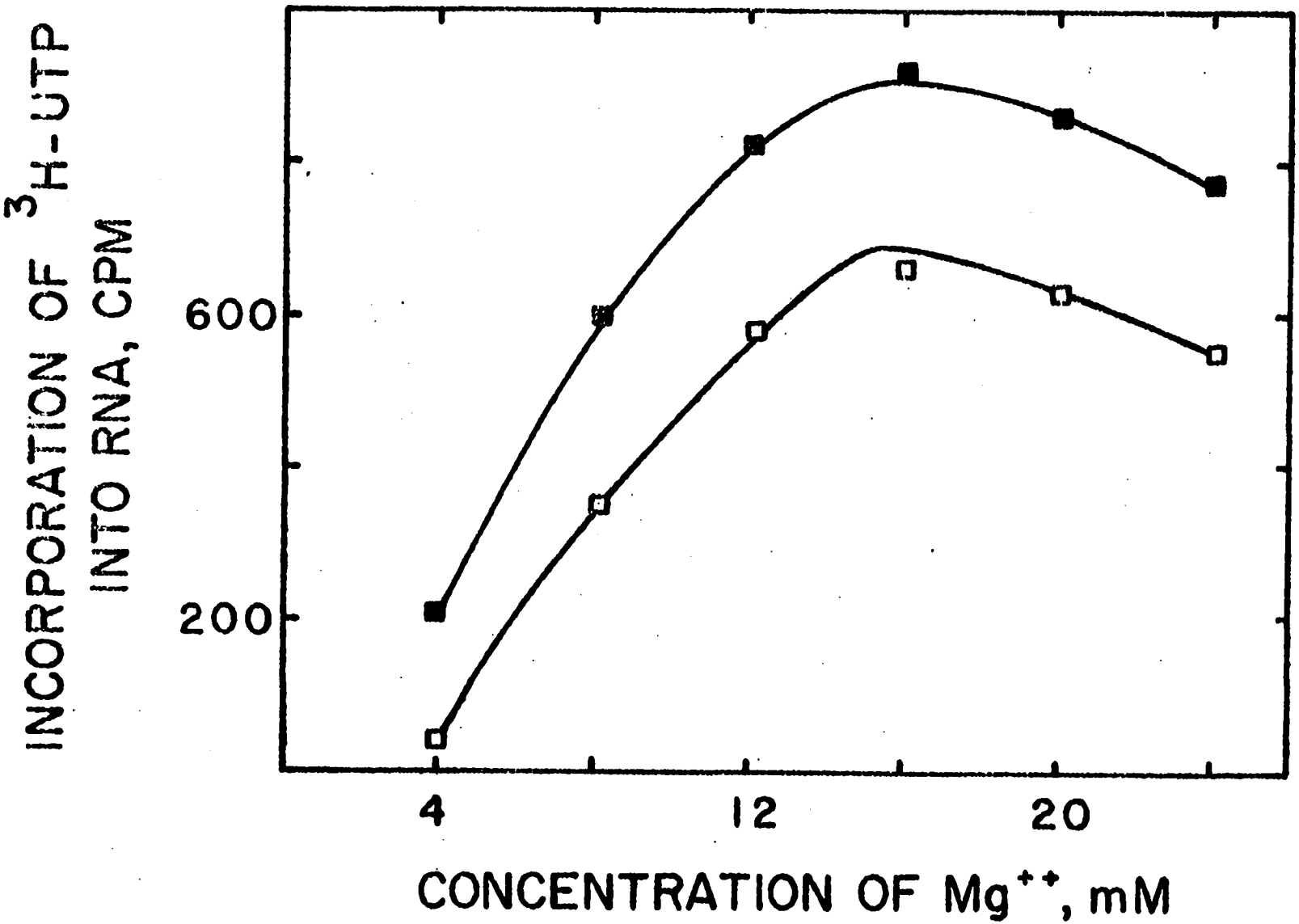

Legend to Figure 16

THE EFFECT OF TEMPLATE CONCENTRATION ON THE INHIBITION OF RNA POLYMERASE BY RESERPINE

RNA polymerase was assayed as described in Figure 14, except that the amount of DNA in each tube was varied from 0.12 mg to 0.72 mg.

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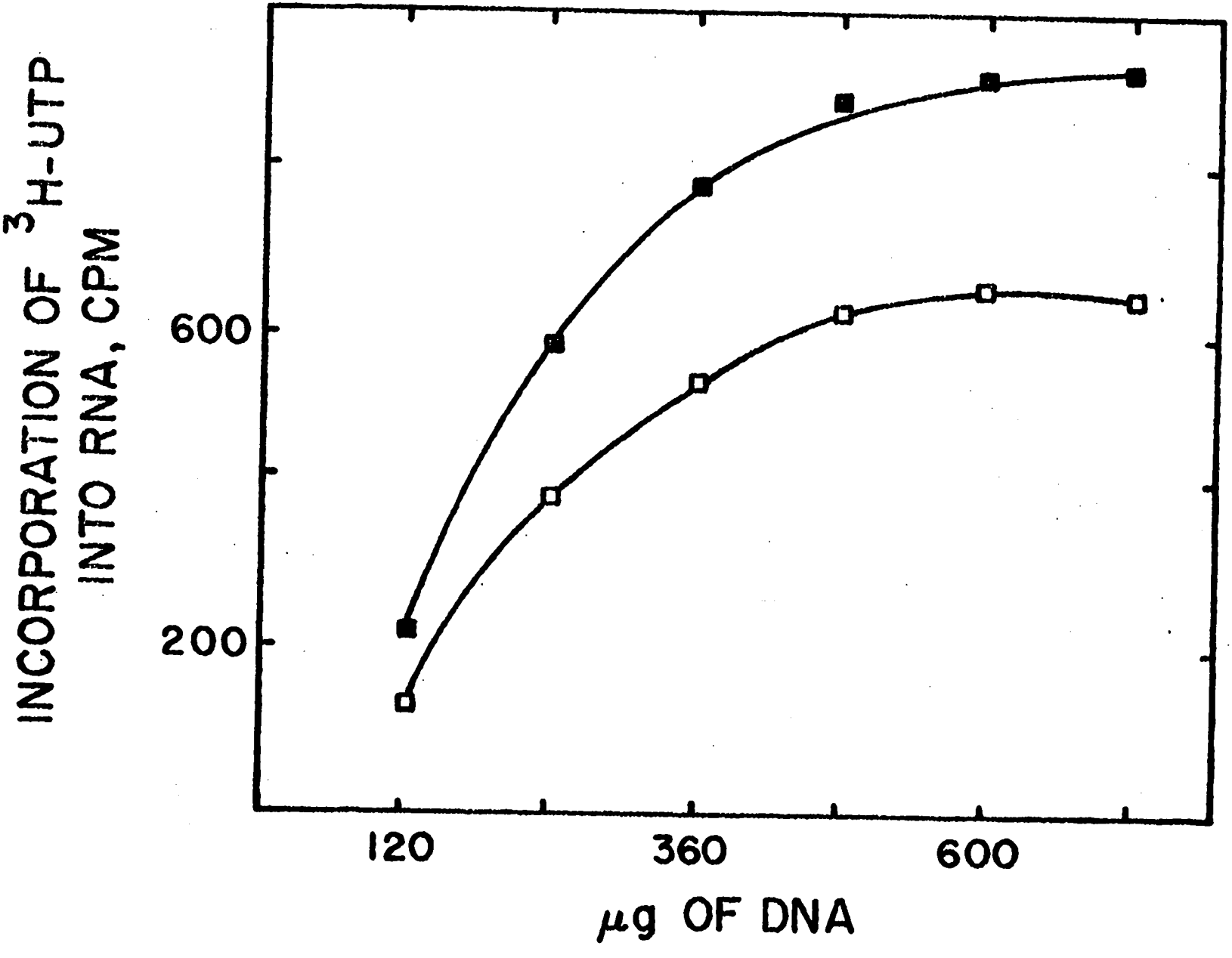

Legend to Figure 17

THE EFFECT OF ENZYME CONCENTRATION ON THE INHIBITION OF RNA POLYMERASE BY RESERPINE

RNA polymerase was assayed as described in Figure 14, except that the amount of enzyme added to each tube was varied from 0.1 mg to 0.5 mg.

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suggests that the reserpine may have precipitated the enzyme DNA complex. Spectrally it was possible to demonstrate shifts in the absorbence of reserpine brought about by the addition of DNA, but it was found difficult to characterize this complex as it appeared to be insoluble in aqueous solvents. The order of addition was varied in each of the experiments shown (i.e., either all reaction components were present before addition of the drug or the drug was added to the tube and the other reagents were added sequentially, thereby permitting the DNA to be exposed to the drug before it could form a complex with the enzyme). The order of addition of the various components had no effect on the extent of inhibition of the synthesis of RNA.

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The data presented in Figures 18 and 19 represent the rates of depletion and repletion of norepinephrine in mouse brain and heart respectively, after a single intravenous dose of 2.5 mg/kg of reserpine phosphate. Several points are noteworthy: (a) depletion is essentially complete in both tissues by about 4 hours; this will be referred to as the depletion phase; (b) following maximal depletion, there is no apparent change in the level of amine for 36 to 48 hours, indicating the existence of a lag phase; and (c) the final phase of amine recovery which is indicative of repletion. The duration of the lag phase is apparently related to the dose of reserpine.

The difference between the rates of repletion in heart and brain is distinct, heart having an appreciably quicker recovery. A major experimental limitation in describing the lag phase is that the amounts of norepinephrine being measured after essentially complete depletion in mouse brain are in the range 10 to 30 ng. The pooling of tissues obtained from several animals provides enough norepinephrine to measure with the present method, but the values obtained are at the lower end of the scale of sensitivity.

It should be stressed that the rate of tissue repletion of norepinephrine at the end of the lag phase appears to be approximately linear. In heart it can be calculated, based upon the rate of amine repletion after the lag phase that had there been no lag, the amount of amine present at 24 hours after reserpine would have been sufficient to be de-

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Legend to Figure 18

THE EFFECT OF RESERPINE ON THE
LEVELS OF NOREPINEPHRINE IN MOUSE BRAIN

Mice were given an intravenous injection of 2.5 mg/kg of reserpine phosphate. At the indicated times, the brain was removed and quickly frozen on dry ice. Subsequently, the norepinephrine of the tissue was extracted as described in the methods, and the fluorescence of the amine developed. The values are the result of five separate determinations on either 2 (first and last points) or 3 (intermediate points) pooled brains. Control levels were 520 $\pm$ 40 ng/g. Vertical bars represent the standard error of the mean.

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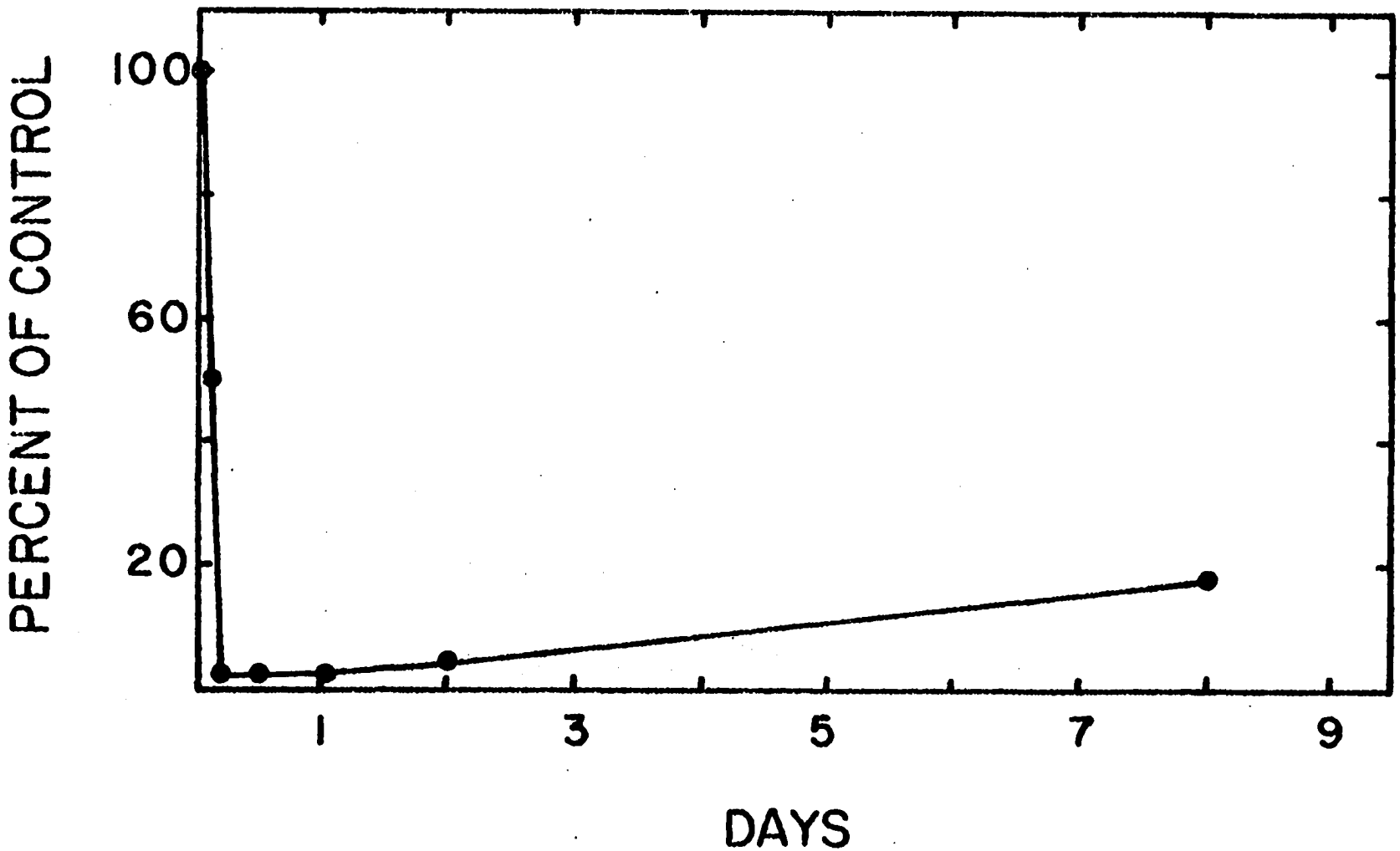

Legend to Figure 19

THE EFFECT OF RESERPINE ON THE
LEVELS OF NOREPINEPHRINE IN MOUSE HEART

Mice were given an intravenous injection of 2.5 mg/kg of reserpine phosphate. At the indicated times, the heart was quickly removed and frozen rapidly on dry ice. Subsequently, the norepinephrine of the tissue was extracted as described in the methods, and the fluorescence of the amine developed. The values are the mean of five separate determinations on either 2 (first and last points) or 3 (intermediate points) pooled brains. Control levels were 1.2 ± 0.1 µg/g. Vertical bars represent standard error of the mean.

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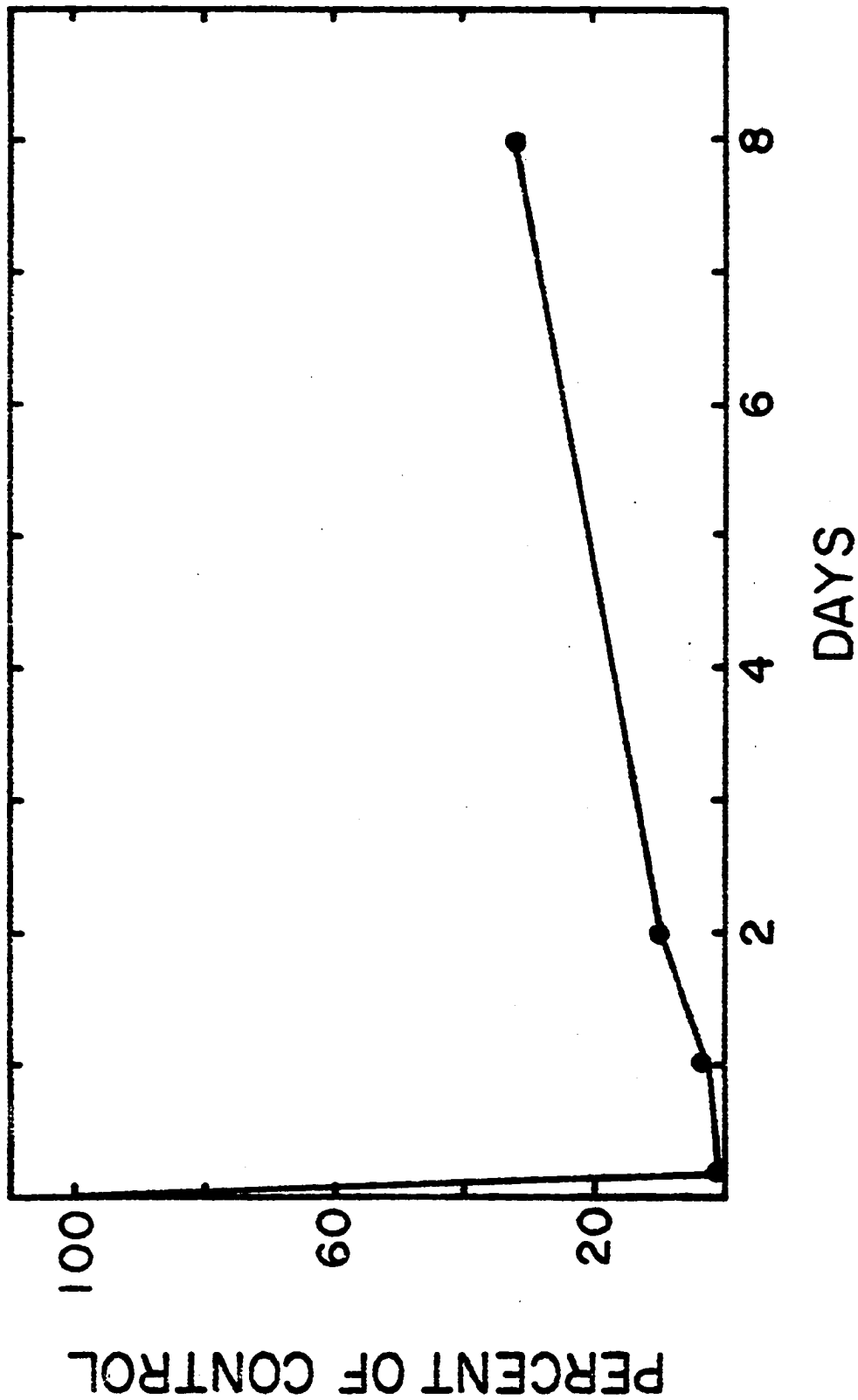

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tectable. This clearly was not the case. In addition, extrapolation of the linear part of the repletion curve does not result in a line that passes through zero time point, even if zero time is taken as the time of complete depletion. In other words, there is a period following complete depletion, which takes about 4 hours, when it is not possible to demonstrate any increase in the minimal amounts of norepinephrine present.

A dose of 2.5 mg/kg of reserpine phosphate was required to inhibit the synthesis of RNA and protein (see Tables 3 and 4); however, no detectable inhibition of macromolecular synthesis was observed at a dose of the alkaloid of 1.25 mg/kg. If the inhibition of RNA or protein synthesis is responsible for the lag phase in the repletion profile, a dose of the drug which did not inhibit such synthesis should not produce the lag and, consequently, the recovery of tissue amine levels would take place more quickly. In Figure 18, it is shown that the rate of repletion of norepinephrine in brain is extremely slow. Eight days after a single dose of reserpine of either 2.5 or 1.25 mg/kg, there is less than 30% recovery of the amount of amine normally present. Because of the extreme slowness of the recovery process and the small amounts of norepinephrine present, it was not possible to distinguish in brain whether a dose of reserpine which does not inhibit macromolecular synthesis permits the more rapid repletion of norepinephrine, but in Figure 19 the data show quite clearly that a dose of reserpine (1.25 mg/kg) which

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Legend to Figure 20

THE EFFECT OF TWO DIFFERENT DOSES OF RESERPINE ON THE REPLETION OF NOREPINEPHRINE IN MOUSE BRAIN

Norepinephrine levels were determined as in Figure 18. Because of the small amounts of norepinephrine which were to be determined, each point represents the mean of 5 assays carried out on 3 pooled brains--except for control and 8 day which were comprised of 1 and 2 organs respectively. Normal animals had norepinephrine levels of 600 $\pm$ 60 ng/g. Vertical bars are standard error of the mean.

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Legend to Figure 21

THE EFFECT OF TWO DIFFERENT DOSES OF RESERPINE ON THE REPLETION OF NOREPINEPHRINE IN MOUSE HEART

Norepinephrine levels were determined as in Figure 19. Because of the small amounts of norepinephrine present, each point represents the mean of 5 assays carried out on 3 hearts pooled together, except for the control and final results which were comprised of 1 and 2 organs respectively. Normal values for norepinephrine in heart were about 1.2 ± 0.1 µg/g. Vertical bars are standard error of the mean.

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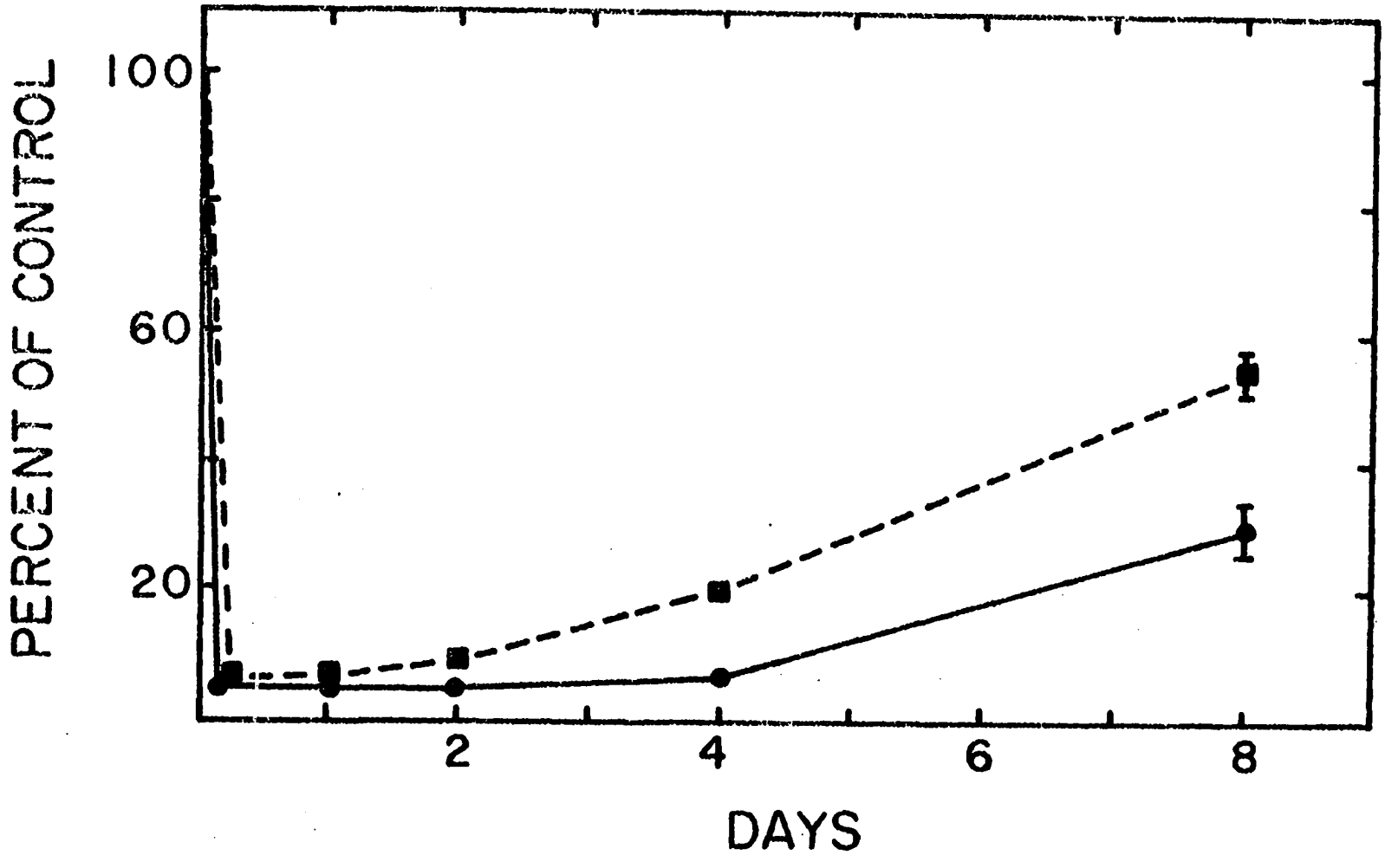

does not affect the formation of RNA and protein allows the amine levels to increase at a more rapid rate. Thus, a significant difference exists between amine levels with different doses of this alkaloid at 8 days after the drug.

Another approach to the proof that new granule synthesis is required for repletion of biogenic amines following treatment with reserpine would be the demonstration that the rate of repletion was slowed by inhibitors of macromolecular synthesis. Actinomycin D has been shown to inhibit the synthesis of RNA in a number of mammalian tissues such as liver, muscle and heart (74, 75, 76). Table 10 shows that norepinephrine levels of heart tissue from mice treated with two doses of actinomycin D (Lyovac Cosmegen, 0.5 mg/kg at 48 hour intervals) were not significantly different from those obtained from untreated animals. In contrast, mice which had been treated with reserpine (1.25 mg/kg, i.v.) plus actinomycin D on days 2 and 4 after the alkaloid showed significantly lower levels of norepinephrine than those that had received reserpine alone.

In Table 11 it is shown that three doses of actinomycin D (0.5 mg/kg) did not produce a decrease in the levels of norepinephrine in heart. This table is included to lend credence to the data of Table 10, as it indicates that an additional dose of actinomycin D does not appear to lower amine levels.

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TABLE 10

THE EFFECT OF ACTINOMYCIN D ON THE RATE OF REPLETION OF NOREPINEPHRINE IN MOUSE HEART AFTER RESERPINE

TREATMENT	NOREPINEPHRINE (ng/gm)
NONE	1362 ± 72
ACTINOMYCIN D	1437 ± 58
RESERPINE	918 ± 87
RESERPINE and ACTINOMYCIN D	686 ± 31

Mice received either no treatment, actinomycin on days 2 and 4 at 0.5 mg/kg ip, 1.25 mg/kg of reserpine phosphate iv on day zero, or a combination of reserpine on day zero and actinomycin on days 2 and 4. The norepinephrine content of the mouse hearts was determined as in Figure 16 on the seventh day after reserpine or three days after the last injection of actinomycin. Each value represents the mean of 4 separate determinations ± the standard deviation.

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TABLE 11

THE EFFECT OF ACTINOMYCIN D ON THE LEVELS OF NOREPINEPHRINE IN MOUSE BRAIN AND HEART

TREATMENT	TISSUE	NOREPINEPHRINE (ng/kg)
NONE	HEART	1400 ± 120
	BRAIN	700 ± 56
ACTINOMYCIN D	HEART	1930 ± 89
	BRAIN	600 ± 73

Animals were either untreated or had received 0.5 mg/kg intraperitoneally of actinomycin D on days zero, two, and four, and they were killed on day six. Norepinephrine was determined as in Figure 16. The results represent the mean and standard deviation obtained from 3 animals.

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In Tables 12, 13, and 14 are shown the results obtained when the amount of norepinephrine present in tissues was assayed after various courses of treatment with either puromycin, emetine, or cycloheximide, respectively. The plan was to employ these inhibitors of protein synthesis in the same way that actinomycin D was used (Table 10) to involve the synthesis of RNA in the repletion of amine stores. According to the hypothesis that posits the resynthesis of granules after reserpine, these drugs should delay the reappearance of amines after reserpine. The one generalization generated from these data is that the stress associated with inhibition of protein synthesis was sufficient to introduce variability into the amine levels, and this made the use of these drugs in conjunction with reserpine impractical.

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TABLE 12

THE EFFECT OF DIFFERENT SCHEDULES OF INJECTION AND DOSES OF PUROMYCIN ON THE LEVELS OF NOREPINEPHRINE IN BRAIN AND HEART

TIME(S)	DOSES mg/kg	NOREPINEPHRINE % OF CONTROL	
		BRAIN	HEART
1 x daily 6 days	25	87 ± 17	58 ± 13
24, 4, 1	25	101 ± 14	137 ± 14
4, 0.5	25	138 ± 11	151 ± 27
1	25	73 ± 19	67 ± 13

The doses of puromycin in water were injected intraperitoneally at the time(s) (in hours) indicated before sacrifice. The tissues were removed, and the amount of norepinephrine/gram wet weight determined as in Figure 18. Each value represents the mean ± standard deviation of from 3 to 10 determinations.

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TABLE 13

THE EFFECT OF DIFFERENT SCHEDULES OF INJECTION AND DOSES OF EMETINE ON THE LEVELS OF NOREPINEPHRINE IN BRAIN AND HEART

TIME(S)	DOSES mg/kg	NOREPINEPHRINE % OF CONTROL	
		BRAIN	HEART
24, 48, 96	20	85 ± 9	111 ± 4
96	20	135 ± 11	115 ± 17
48, 96	20	77 ± 17	85 ± 29

The doses of emetine in water were injected intraperitoneally at the time(s) in hours indicated before sacrifice. The tissues were removed, and the amount of norepinephrine/gram wet weight determined as in Figure 18. Each value represents the mean ± standard deviation of from 3 to 10 determinations.

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TABLE 14

THE EFFECT OF DIFFERENT SCHEDULES OF INJECTION AND DOSES OF CYCLOHEXIMIDE ON THE LEVELS OF NOREPINEPHRINE IN BRAIN AND HEART

TIME(S)	DOSES mg/kg	NOREPINEPHRINE % OF CONTROL	
		BRAIN	HEART
96, 48	10	101 ± 17	97 ± 3
96, 48, 24	10	109 ± 14	96 ± 17
48, 24	10	84 ± 16	121 ± 23
4	10	117 ± 11	63 ± 19

The doses of cycloheximide in water were injected at the time(s) (in hours) before sacrifice. The tissues were removed and the amount of norepinephrine/gram wet weight determined as in Figure 18. Each value represents the mean ± standard deviation of from 3 to 6 determinations.

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DISCUSSION

The reappearance of tissue levels of biogenic amines following depletion by the alkaloid reserpine is a relatively slow process and can require from two weeks to over a month for complete recovery, depending upon the dose of this alkaloid and the tissue in which amine levels were determined (31). This thesis has addressed itself to an understanding of the biochemical events involved in the reappearance of norepinephrine after a single dose of reserpine. A report describing inhibition of RNA and protein synthesis in neoplastic cells by this alkaloid (38) raised the question of the involvement of the syntheses of these macromolecules in the process of amine repletion, and the subsequent demonstration of inhibition of RNA and protein synthesis in amine containing tissues led to the hypothesis that reserpine acted in two ways in its effects on biogenic amines (78). The first is an initial interaction of the drug with the amine-binding granule, which acts to prevent reuptake of norepinephrine; this phenomenon has been long known (79, 80). The second action of reserpine on amine containing tissues as has been shown in this thesis is that reserpine inhibits the synthesis of RNA and protein, and this results in a delay in the amine repletion process (78). Thus, this alkaloid apparently depletes biogenic amine stores by irreversibly inter-

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acting with the amine-binding granule and then delays repletion presumably by inhibiting the synthesis of new granules.

The results obtained in Figures 7 and 8 indicate that reserpine decreases the rate of incorporation of tritiated uridine into RNA of both brain and liver. The rate of incorporation of this precursor is linear for normal animals (Figures 3 and 4) for this period. In Table 1 is shown that there appears to be no appreciable shift in the conversion of uridine into the nucleotide pool. This assay measures total UMP after degradation of UTP and UDP to the mononucleotide level, thus, inhibition of phosphorylation beyond the mononucleotide level is not revealed by this procedure. Decreased fixation of precursor into RNA with no apparent change in specific activity of the precursor pool of nucleotides presumably demonstrates real inhibition of RNA synthesis. Furthermore, actinomycin D inhibits uridine incorporation in this system.

The results shown in Figures 9 and 10 demonstrate that reserpine decreased the rate of incorporation of ^{14}C-valine into protein of brain and liver. The rate of precursor incorporation was demonstrated to be linear for normal animals (Figures 5 and 6) during this period. That incorporation of valine represents the synthesis of protein is suggested by finding that emetine and cycloheximide inhibit valine fixation into acid-insoluble material and that the incorporation of phenylalanine into this fraction is also inhibited (Table 2).

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Actinomycin D has been shown to inhibit the synthesis of RNA in rat liver (74), the semi-pure E. coli polymerase (81), and in rat liver nuclei (82). Cycloheximide has been shown to inhibit the synthesis of proteins in cell-free and intact (in vivo) (62, 83) systems from rat liver. With reserpine, which also apparently inhibits the in vivo synthesis of RNA (Figures 7 and 8), it was anticipated that it would be possible to demonstrate inhibition of RNA synthesis in vitro. Incubation of liver slices in the presence of 8-^{14}C-adenine showed no difference in the ability to incorporate this precursor into RNA in slices incubated in the absence or presence of reserpine (5×10^{-4} M) (Figure 12). Furthermore, slices of liver removed from mice treated with 2.5 mg/kg of reserpine incorporated adenine into RNA at the same rate as did slices prepared from livers of normal animals (Figure 12). It was also anticipated that it would be possible to demonstrate the inhibition by reserpine of the incorporation of labeled amino acids into protein, liver slices; however, it was not possible to discern any difference in the rate of incorporation of radioactive valine into protein in tissue isolated from reserpine pretreated animals or in liver slices exposed to the alkaloid in vitro. Furthermore, in a cell-free protein synthetic system isolated from mouse liver, it was also not possible to demonstrate any inhibition by reserpine of incorporation of valine into protein. However, in a partially purified preparation of E. coli RNA polymerase, it was possible to show marked reduction of the incorporation of

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^{3}H-UTP into cold acid-insoluble material at a reserpine concentration of 4 x 10^{-4} M. Variation of the amount of Mg^{2+}, DNA, or enzyme had no appreciable effect on the extent of inhibition brought about by this drug (Figures 14, 15, 16, 17). Thus, the kinetic mechanism of inhibition of RNA polymerase by reserpine is obscure. Inhibition could possibly be produced at the steps involved either in initiation or propagation without reversal being obligated by variation in the components described. On the other hand, reserpine has been shown to interact with DNA in solution by the production of shifts in the ultraviolet absorption spectrum of this alkaloid (38), and precipitation of a DNA-reserpine complex can occur *in vitro* (84). It is possible, therefore, that under the conditions employed for the assay of RNA polymerase, that reserpine coprecipitates with either DNA, the enzyme, or an enzyme-DNA complex. In support of such a mechanism, reserpine (5 x 10^{-4} M) inhibited the activity of DNA polymerase isolated from *E. coli*, even though the alkaloid did not interfere with the incorporation of H^{3}-thymidine into the DNA of neoplastic cells (38).

The doses of reserpine employed in the early studies of the action of this drug were relatively large (2.5 mg/kg) for amine depletion, but the use of 10 to 15 mg/kg of alkaloid has been reported (85, 86). In Tables 3 and 4 it is shown that a dose of 1.25 mg/kg of reserpine has no effect on the synthesis of RNA and protein, respectively. However, the data in Figures 20 and 21 demonstrate that this smaller dose

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of reserpine has a marked depleting effect on the levels of biogenic amines in both brain and heart. Therefore, it is possible to separate the action of this drug on the process of amine depletion per se from its effect on macromolecular synthesis. The independence of these two effects can be observed by depletion of norepinephrine with reserpine (Table 5) followed by a second dose of the alkaloid, given when the effects of this agent on macromolecular synthesis had worn off (Figures 7 and 8). The second dose of reserpine also inhibits the synthesis of RNA and protein, yet at best under these conditions only about 10% of the total norepinephrine normally present is depleted and therefore released (Figures 18 and 19). Thus, release of amines, as brought about by reserpine, does not appear to play a direct role in the inhibition of macromolecular synthesis by this agent.

Inhibitors of monoamine oxidase have been shown to maintain tissue biogenic amine levels after treatment with reserpine (59), although there are some indications that these amines may no longer be present in their normal storage form (87). It was thus also possible to investigate the effect of apparent non-depletion of norepinephrine on the inhibition of macromolecular synthesis by reserpine. In Tables 6 and 7 are shown the effect of doses of either pargyline or iproniazid on the reserpine-induced inhibition of macromolecular synthesis. In both cases, the inhibitors of monoamine oxidase activity prevented the inhibition of RNA and protein synthesis by reserpine. Thus it would appear that either the

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high levels of unbound amines can compete directly with reserpine for the same site or indirectly compensate at a different site for the effect of this alkaloid on macromolecular synthesis.

The synthesis of the diverse types of RNA in higher cells is under complex control (88), and various drugs, such as actinomycin D and toyocamycin, have been shown to demonstrate some selectivity in the degree of inhibition of various types of RNA (89, 90). Drugs have frequently been shown to alter the polysome profile, and this appeared to be an especially sensitive monitor of the effect of drugs on RNA synthesis (68, 91). In Figure 11 are shown polysome profiles obtained from the livers of normal mice as compared to polysomes isolated from mice treated with a dose of reserpine capable of inhibiting the synthesis of RNA. No apparent difference between these groups was evident. This finding would suggest that reserpine perhaps decreases the rate of turnover of RNA; this hypothesis is supported by the observation that the release of ^{32}P from RNA in rat brain is inhibited by reserpine (71). It was also possible to determine if there was any selectivity in the inhibition of the synthesis of protein in terms of the subcellular distribution of radioactivity after incorporation of ^{14}C-valine into protein following treatment with reserpine. In Table 8, it is shown that there is no apparent selectivity of inhibition in terms of the subcellular distribution of new protein synthesized in the cell.

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In Figures 20 and 21 are shown comparisons of the rate of repletion of norepinephrine after different doses of reserpine in brain and heart, respectively. The doses employed, 2.5 and 1.25 mg/kg iv, both deplete almost completely these tissues of norepinephrine, but only the higher dose influences macromolecular synthesis. In brain the rate of norepinephrine repletion is so slow as to make any distinction between the situation in which reserpine also inhibits RNA and protein synthesis to that in which it does not insignificant. In heart, on the other hand, in the mice in which this alkaloid inhibited the syntheses of macromolecules, the repletion of norepinephrine stores was significantly slower than in tissues in which depletion of norepinephrine was achieved at a level of reserpine which did not inhibit RNA and protein synthesis. This clearly suggests that the synthesis of RNA or protein, or both are involved in the process of amine repletion, probably by influencing resynthesis of a part or whole of the amine binding granule.

It was of interest, therefore, to determine the effect of other more classical inhibitors of macromolecular synthesis on the rates of repletion of amines after treatment with reserpine at a time at which the effects of this alkaloid on RNA and protein synthesis had terminated. In Table 10 is shown that norepinephrine levels in heart in mice treated with reserpine plus actinomycin D returned more slowly than in mice treated only with reserpine. The results shown in Tables 12 through 14 demonstrate that it is difficult to

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employ inhibitors of protein synthesis after reserpine (i.e., cycloheximide, emetine) treatment as probes to determine if the synthesis of protein is involved in amine repletion, as these agents appear to affect the levels of biogenic amines in the absence of other treatment. However, the findings that actinomycin D, an inhibitor of RNA synthesis, or a dose of reserpine which inhibits the synthesis of RNA and protein apparently delays the process of repletion support the thesis that the synthesis of RNA is involved in repletion of amine stores. Magus and Sartorelli (38) and Mueller and Shidemann (39) also employed various metabolic inhibitors after reserpine to attempt to delay repletion of amines; although there is some question as to the use of metabolic inhibitors in the second report, both papers appear to support the hypothesis that both the formation of RNA and protein are required for repletion of biogenic amines after reserpine.

Since the original finding of the depletion of norepinephrine by Carlsson et al. in 1956 (13, 14), a number of laboratories have reported results similar to those shown in Figures 18 and 19, which are included to indicate that in mouse the rates of repletion of norepinephrine in the organs tested generally correspond to previous findings. The rates of repletion of norepinephrine in brain and heart after treatment with reserpine are quite different; this result leads to the question of the mechanism involved in the control of the rate of amine repletion in various tissues of a reserpine-depleted animal.

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In the introduction section an experiment was described (35) in which rats which had two ligations on the sciatic nerve were treated with tetrabenazine; recovery of fluorescence due to the presence of norepinephrine occurred above, between and below the two ligations. A similar experiment in rats treated with reserpine, however, resulted in the reappearance of fluorescence only above the top ligation, and this occurred only after a period of time which corresponded to the time required for migration of granules to the ligation from the nerve cell body. The conclusion drawn from this and other experiments described in the introduction was that the amine binding sites (i.e., the granules) were synthesized in the nerve cell body and subsequently transported proximodistally; thus, repletion of amines after treatment with reserpine requires the processes of both resynthesis of the amine storage granule and transport of the granule to its active site. With a reversible drug, such as tetrabenazine, however, the granules are not irreversibly damaged and are again able to take up and bind norepinephrine following disappearance of the drug. Hillarp and his colleagues (92, 93, 94) have demonstrated the rapid transport of such granules from their site of synthesis in the nerve cell body somatofugally, but the question remains unclear as to what process is the rate-limiting step. Figure 20 represents a schematized model of the potentially rate-limiting steps in the biosynthesis and logistic disposition of the amine storage granules.

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Legend to Figure 22

A SCHEMATIC REPRESENTATION OF THE POTENTIAL RATE-LIMITING STEPS IN THE REPLETION OF AMINES AFTER RESERPINE

The numbers in this diagram represent: (1) the nuclear synthesis of RNA, (2) the cytoplasmic synthesis of protein, (3) the transition between synthesis and the process of rapid active transport down the axon, (4) the transport of granules down the axon, and (5) the functional area of the granules in the varicosities.

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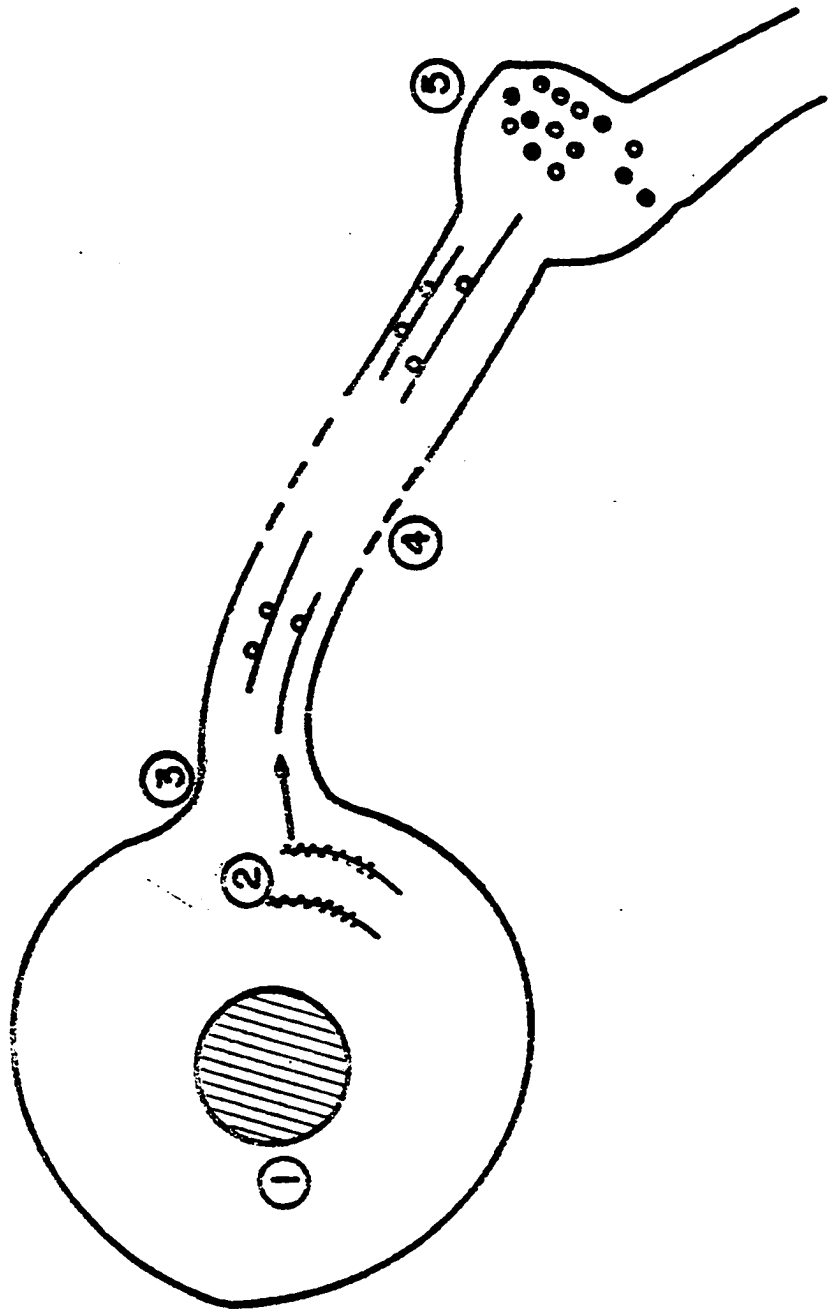

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This hypothetical model presents some of the major events involved in the synthesis and disposition of the granules, under both normal conditions and those of reserpine depletion. In the nerve cell body, the proteins of the granule are synthesized, and this process presumably requires the synthesis of RNA as well (95). As the new granule nears completion, it will either be released into the cytoplasm as a free particle or it will become associated with some apparatus for rapid transport down the axon. Once in the axon, the granules are apparently associated with microtubules (96, 97) which affect the rapid dislocation of granules towards the varicosities. Upon exposure of the granules to a synaptic region, they appear to undergo a change in character, in that they seem to divide in two (98).

With this model in mind, some predictions of the steps which most likely are rate-limiting can be made. The synthesis of RNA or protein has frequently been implicated as limiting in the synthesis of induced enzymes (99, 100), but there are other examples in which the enzyme level is increased as degradation is slowed by substrate protection (101). In the unusual case of the nerve cell with enormous distances between the site of synthesis of a protein and the site at which it subsequently is to be used, modulation of the rate of synthesis of RNA and protein appears to be the only positive control that the cell would possess. The possibilities for communication between nucleus and axoplasm appears increasingly remote as the material proceeds down the

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axon. One of the apparent complexities of adrenergic neurons is the way in which they release their stores of amine transmitters. It seems likely that the granules which migrate down the axon release biogenic amines, at least for the first time, by means of exocytosis, and in this process also release some protein (102). There also remains the possibility that this protein influences the synthetic activity of the nerve cell body.

Proteins are generally thought to be synthesized on the rough endoplasmic reticulum (103). Upon completion of the protein, it is released into the cytoplasm. In the case of granular proteins which are intended for export into the axoplasm, unless they are synthesized under circumstances which then control their eventual disposition, they would move randomly in the perinuclear region until they associated with the microtubules which appear to affect transport in the axon. It appears that the site of synthesis may be important for proteins which are to be secreted (104), and it also seems as though the nucleus contains ramifications of the microtubular apparatus (105).

The potential for Nos. 4 or 5 of Figure 20 to be the rate-limiting steps is unlikely, as the transport system has been shown to be relatively rapid, and it is difficult to imagine how intra-axonal events at the varicosities could influence events at the nerve cell body.

Of the three remaining possibilities for rate-limiting process, the synthesis of RNA or protein seems most likely;

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otherwise there would be an accumulation of fluorescence in the nerve cell body as the granules accumulated amines. The nerve cell body is only weakly fluorescent at any time, and no period of intense fluorescence occurs after treatment with reserpine (35). These facts would appear to rule against initiation of transport as the rate-limiting step, which also may suggest that this process is an organized event. It also probably indicates that Nos. 1 or 2 of Figure 20 are the rate-limiting steps in the repletion of biogenic amines after a single dose of reserpine.

The fact that it was possible to demonstrate inhibition of the synthesis of RNA and protein and that there were different rates of repletion after doses of this alkaloid which apparently depleted biogenic amines equally well but only one of which inhibited macromolecular synthesis led to the hypothesis presented in Figure 21. An initial phase of interaction of reserpine with the amine binding granules leads to their depletion and apparent destruction. A second phase, which occurs only with doses of this alkaloid which inhibit the synthesis of RNA and protein, introduces a lag in the new appearance of amines. The third phase then represents the biosynthesis of new granules after the inhibition of macromolecular synthesis has terminated.

It seems clear that there is a delay in repletion of norepinephrine after higher doses of reserpine (31, 106). This delay can apparently be prolonged with the use of inhibitors of macromolecular synthesis (38, 39). There are currently available antibodies to dopamine-β-oxidase (107) and

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Legend to Figure 23

A SCHEMATIC REPRESENTATION OF THE EFFECT OF INHIBITION OF MACROMOLECULAR SYNTHESIS ON AMINE REPLETION

This scheme represents a model of the postulate advanced to relate the inhibition of macromolecular synthesis by reserpine and the depletion of amines by this alkaloid.

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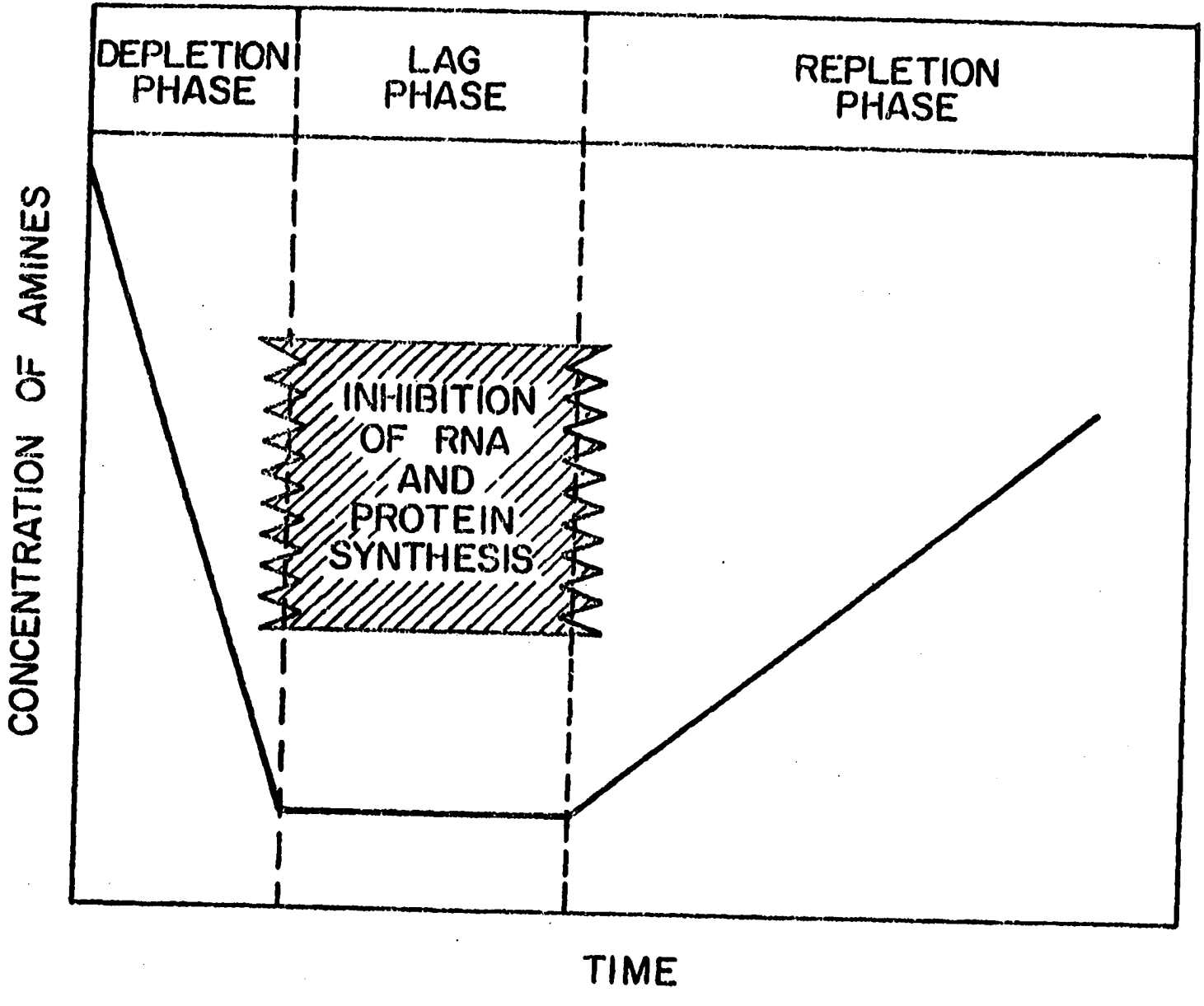

structural components (43, 47) of the granule. The techniques for measuring subcellular distribution of amine (108) and very small amounts of them localized in areas of axons (109) have also become available. If these approaches were combined, it would be possible to have a clear understanding of the events involved in the reconstitution of the amine binding capacity of adrenergic neurons.

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