THE EFFECTS OF MESCALINE ON THE UPTAKE AND RELEASE OF ^{3}H-DOPAMINE BY THE RAT CAUDATE NUCLEUS

by

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Approved

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To my wife and daughters

for their understanding and

encouragement

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ABBREVIATIONS

EDTA	Ethylenediaminetetraacetate
LD_{50}	Half lethal dose
s.c.	subcutaneously
i.p.	intraperitoneally
i.v.	intravenously
TMPA	3,4,5-trimethoxyphenylacetic acid
TMPE	3,4,5-trimethoxyphenethanol
MAO	Monoamine oxidase
DAO	Diamine oxidase
LSD	Lysergic acid diethylamide
5-HT	5-hydroxytryptamine
DMT	N,N-dimethyltryptamine
NCS	Nuclear Chicago Solubilizing Solution
CSF	Cerebrospinal fluid
MHPE	3-methoxy-4-hydroxyphenethanol
HVA	Homovanillic acid
S.A.	Specific activity
C	Curie
DOPAC	3,4-dihydroxyphenylacetic acid
MT	3-methoxy-4-hydroxyphenethylamine
MHPG	3-methoxy-4-hydroxyphenylglycol
CPM	Counts per minute

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INTRODUCTION

Mescaline 3,4,5-trimethoxyphenethylamine (Fig.1) is one of the chemical constituents of peyote. The latter, a member of the family cactacea, is commonly referred to in the chemical literature as Lophophora williamsii. Mescaline was first isolated from peyote by Heffter in 1894 (1,2). Shortly thereafter, the presence of mescaline in peyote was confirmed by Lewin (3) who discovered that his β-phenethylamine was the active hallucinatory principle of peyote. The first chemical synthesis of mescaline was realized by Spath in 1919 (4).

The effects of mescaline on the cardiovascular and respiratory systems have been studied by many investigators (5-11). In general, low doses of mescaline (4 mg/kg) have no remarkable effects on blood pressure. Large doses, however, produce a drop in blood pressure, bradycardia respiratory depression and vasodilation (5-8, 10,12). Strong solutions of mescaline arrest the perfused frog heart in diastole (5). Mescaline produces sensitization to the cardiodepressant action of sodium EDTA (13). In cats and dogs,

it has been shown that the action of mescaline on the cardiovascular system is potentiated by pretreatment with reserpine (14). A threshold dose of 152 μg/kg, i.v., causes slight potentiation of the effect of epinephrine on the nictitating membrane of the anesthetized cat (15). Mescaline was shown to be only slightly antagonistic to the vasoconstricting effect of 5-hydroxytryptamine (serotonin) (Fig. 2), perfused through the isolated rabbit leg (16).

Mescaline stimulates the escape reflexes in mice in low doses and inhibits them in high doses (17). In small doses, Mescaline enhances the potentiating effect of serotonin but blocks the prolongation effect of reserpine on hexobarbital hypnosis in mice (18). Mescaline, in low doses, increases brain serotonin levels and suppresses the protective effect of reserpine against toxicity of amphetamine in mice (19). Mescaline produces hyperthermia in most animals (20,21), and this effect can be potentiated by pretreatment with iproniazid (22). Delay, Gerard and Thuillier (23), Friedman and Burnstein (24), and Mayer-Gross (25) have studied the toxicology of mescaline in rats, mice, frogs and guinea-pigs. The LD_{50} of mescaline varies with the

route of administration, 157 mg/kg i.v., 330-410 mg/kg i.p., and 534 mg/kg s.c. Mescaline, in small doses, has been shown to cause excitation in rats (26). Increasing the dosage delays the onset of the excitation period. In high doses, however, mescaline causes sedation in these animals (26). Mescaline has been shown to produce experimental catatonia in mice, guinea-pig, cats, monkeys and other animals (27-34). The catatonic manifestation, however, is inhibited by chlorpromazine and reserpine (35). Mescaline causes reduction in the behavior pattern of contentment and sociability, but increases excitement, aggressiveness and hostility (36-38). Mescaline given intracranially produces aggressive tendencies and paroxysms of ear scratching in mice (39) by doses ineffective by other routes. In humans, mescaline in large doses produces visual and auditory hallucinations accompanied by personality disturbances (40,41). The disorganization in psychic integration is reported to be more apparent in schizophrenic patients than in normal subjects receiving mescaline (42). Mescaline has been shown to precipitate symptoms of schizophrenia in persons suffering from latent schizophrenia (43). It also has caused relapse

of symptoms in patients who have improved after psychosurgery (43,44,45).

The earliest studies on the absorption, and tissue distribution of mescaline were done by Tarsitano in 1945 (46). Working on dogs injected subcutaneously, the author found the highest concentrations of mescaline in the liver and kidneys. Very little of the drug was found in brain and none in the blood. Cochin et al (47), found the plasma concentration of mescaline highest immediately following the intravenous injection of the drug. The drug disappeared from the blood stream six to eight hours post injection. The findings of Tarsitano were later confirmed by Block and his co-workers (48-52). Using ^{14}C-mescaline, these authors found the highest concentrations of radioactivity in the liver and kidneys of the mouse, shortly after intraperitoneal injection. Little or no radioactivity was found in the brain. Neff et al (53) found, after administering radioactive mescaline intravenously into cats, that the highest concentration of radioactivity in the brain was localized in the hypophysis, thirty minutes later. Relatively high levels

of radioactivity were also found in the cortical and subcortical gray matter. The maximum concentration of mescaline in the brain is attained between thirty minutes and two hours post injection. This period has been shown to coincide with the time of maximal intoxication (53). The biological half life of mescaline in plasma and cerebrospinal fluid is one and a half to two hours. However, Charalampous et al (54), found significant concentration of radioactivity in the human cerebrospinal fluid throughout a period of 9.5 hours after administration of ^{14}C-mescaline.

The metabolism of mescaline has been investigated in the dog by Cochin, Wood and Seevers (47), Spector (55), and in rats by Block and Patzid (56), Goldstein, Friedhoff, Pomerantz, Simmons and Cointrera (57), Friedhoff and Goldstein (58). The major metabolite of mescaline was found to be 3,4,5-trimethoxyphenylacetic acid (TMPA) (Fig.3), a pharmacologically inactive compound (55-59). Block et al (56) have shown that rats and mice injected intraperitoneally excrete ^{14}C-mescaline in their urine as unchanged mescaline and the

acid metabolite, 3,4,5-trimethoxyphenylacetic acid. Musacchio et al (60) and Musacchio and Goldstein (61) have identified in addition to TMPA, N-acetylated-O-demethylated metabolites of mescaline in the urine of rats. Neffs et al (53), however, found TMPA to be the sole metabolite of mescaline identified in the cat brain, plasma, cerebrospinal fluid and urine. Human metabolic studies of mescaline have been done by Salomon, Gabrio and Thale (62), Harley-Mason, Laird and Smythies (63), Charalampous (54), Friedhoff and Hollister (64), and Patel (65). Mescaline taken orally is recovered substantially unchanged. In addition to TMPA, 3,4,5-trimethoxyphenethanol (TMPE) (Fig.3), and the N-acetylated-O-demethylated metabolites have been identified in both urine and cerebrospinal fluid. The enzyme, monoamine oxidase (MAO), presumably responsible for the oxidative deamination of most monoamines was shown to have little or no effect on mescaline (66,67). A specific mescaline oxidase isolated from the rabbit liver (68) was claimed to be responsible for the conversion of mescaline to TMPA. Some investigators (69) postulate that mescaline oxidase,

while different from monoamine oxidase, is identical to diamine oxidase (DAO). Still other investigators (70,71,72) believe, however, that the oxidative deamination of mescaline can be effected by monoamine oxidase, or both. Nevertheless, highly purified human plasma monoamine oxidase was shown to have little or no effect on mescaline (73). Several in vivo studies have revealed that different organs within the same animal metabolize mescaline differently. Thus, rabbit lung preparations produce N-methylation of mescaline preferentially (74,75), while rabbit liver preparations cause O-demethylation, along with oxidative deamination of mescaline (76).

As can be predicted, alterations in the aromatic ring substituents and the side chain have been shown to cause substantial changes in the psychopharmacological properties of mescaline (77,78,79). Smythies and Levy (80) have demonstrated that the removal of the 5-methoxy group from mescaline causes a 50% loss of activity. The same authors have also shown that the removal of the 4-methoxy group from mescaline results in a complete loss

of activity. Ernst (81) has confirmed the presence of the 4-methoxy group to be essential for the manifestation of the hypokinetic rigid syndrome exhibited in cats. N-methylmescaline (Fig.4) has been shown to be less potent than its parent compound. N,N-dimethylmescaline has been shown to produce no mental disturbances in man (82) and has negligible inhibition on the conditioned avoidance response in cats (83,84). Friedman et al (24), however, have found little or no difference in the activities of the N-methyl-β-hydroxy derivative and mescaline itself. In man, α-methylmescaline has been found to produce visual hallucinations (85) in doses lower than that required by mescaline. Shulgin (86,87,88) has demonstrated, from tests conducted in humans with several compounds analogous to α-methylmescaline, that while the presence of the 3,4-methylenedioxy group (Fig.4) does not change the activity of the parent compound, enlargement of this heterocyclic ring or elongation of the aliphatic side chain results in loss of activity.

In spite of this vast amount of data published on the absorption, tissue distribution, metabolism and the pharmacodynamic properties of mescaline, very little,

however, is known about the site or mode of action of this drug. Various hypotheses have been formulated in order to explain the hallucinatory effect of mescaline. Quastel and Wheatly (89), Fisher (90), Gordon (91), Hoffman (92), Friedhoff and Goldstein (58), have suggested that mescaline *per se* may not be active. These investigators have postulated the *in vivo* formation of an active abnormal metabolite of mescaline. Speck (11) has suggested a competition between mescaline and noradrenaline for the central noradrenergic receptors. Most investigators (39, 93,94) have agreed, however, that the various pharmacological and behavioral effects induced by mescaline and other hallucinogens are predominantly of central origin.

The most potent hallucinogenic drug yet known to man is d-lysergic acid diethylamide (LSD-25). This drug has been shown by Cerletti (95) and others (96-99) to have strong antiserotonin action. These observations have aroused considerable interest since serotonin (5-HT) is a biogenic amine whose distribution in the brain closely parallels that of norepinephrine (100). For some time,

it has been postulated that the hallucinogenic effects of mescaline may also be due to its action on the metabolism of serotonin. It has been shown, however, that mescaline has no antiserotonin action (101). Moreover, brom-lysergide (Fig.5), has been found to have strong antiserotonin action similar to that of lysergide (95) but is not hallucinogenic itself. There is little likelihood of discovering a unitary explanation of the mode of action of all the hallucinogenic drugs. Even the central sympathomimetic effect, common to all hallucinogens, is not specific since drugs with low hallucinogenic potential such as the amphetamines (102-105) are potent sympathomimetics. It may be possible that a certain hallucinogen may act by blocking certain brain centers and pathways while enhancing others. Which centers of the central nervous system are blocked and which centers are facilitated may depend entirely on the specific properties of the drugs and on the administered doses. This view is supported by the fact that only a few, if any, of the drug-evoked behavioral or physiological changes in animals are common to all the hallucinogenic drugs. Certain effects are believed to be

characteristic of one type of hallucinogen, for example, the postural change in rat evoked by mescaline but not LSD-25. Several compounds such as dimethyltryptamine (DMT), 4-hydroxydimethyltryptamine (psilocin) and 4-phosphodiester psilocin (psilocybin) and LSD-25 may indeed owe their hallucinogenic effects to their interactions with brain serotonin metabolism (96,105,106,107). This assumption is based primarily on the similarity in the structure of these drugs and serotonin (5-HT) (Fig.2). In keeping with this view, Harley-Mason is reported by Osmond and Smythies (108) to have suggested that schizophrenia, may be caused by an interaction between adrenaline and an abnormal metabolite structurally ralated to mescaline. In mammals, the peripheral chemical adrenergic neurotransmitter is noradrenaline, a catecholamine (109). The catecholamines, noradrenaline and its precusor dopamine are also widely distributed in the mammalian brain (110-114). The highest concentration of noradrenaline is found in the hypothalamus (110,111). Dopamine has been found in the mammalian brain in amount comparable to or exceeding noradrenaline (111,112,113). Dopamine, however, has a different

regional distribution in the brain from that of noradrenaline. The highest concentration of dopamine is found in the corpus striatum in animals and in man (114). From current evidence (115-124) it has been concluded that these two amines function as chemical transmitters in the central nervous system. Almost all of the criteria for chemical transmission have been met by these amines. For example, dopamine and noradrenaline have been shown to localize within the nerve endings (115) where they are protected from enzymatic destruction by being stored within nerve granules or vesicles (115). Also, the presence in the brain of the enzymes responsible for their synthesis (116-119) and inactivation (120,121) has been demonstrated. Finally, it has been shown that dopamine and noradrenaline can be released from the central nervous system upon appropriate stimulation *in vitro* (122-124) and *in situ* (125-132). A positive correlation has been reported between brain catecholamine levels and mood (133,134). Thus depression has been associated with a relative brain deficiency of catecholamines, whereas elation has been associated with an excess of such amines. Catecholamine depleting agents, of which

reserpine is a prototype, can produce, in some subjects a syndrome indistinguishable from the depression observed in the disease state (135). Correspondingly, mood elevation has been reported in depressed patients treated with monoamine oxidase inhibitors (136,137) or other agents capable of increasing the concentration of the amines at the receptor sites (138).

The contention that the catecholamines may, in some way, be implicated in the psychotomimetic action of mescaline is supported by the fact that many anti-catecholamine agents, of which chlorpromazine is a prototype strongly antagonize the central effect of mescaline (139,140). More recently, Shah (141) has observed that mescaline-induced behavioral changes in mice can be prevented by pretreatment with reserpine. The mechanism of action of reserpine appears to involve an impairment of catecholamine retention within the storage vesicles in the nerve endings. This results in a rapid intraneuronal destruction of newly formed dopamine by monoamine oxidase and subsequent depletion of brain catecho-

lamines. If binding and release of brain catecholamines are involved in the mechanism of action of mescaline, some direct evidence can be obtained by focussing attention on the subcellular localization of the drug, preferably in the regions of the brain rich in the central neurotransmitters, noradrenaline and dopamine. The brilliant studies of De Robertis et al (142) and Gray and Whittaker (135) have made possible the isolation of the nerve endings particles or synaptosomes where the highest concentration of these amines are found. Recently, Shah (141) showed that ^{14}C-mescaline injected intraperitoneally into mice can be recovered in the particulate fraction of the brain homogenate. A considerably larger amount of radioactivity, however, has been found predominantly associated with the soluble supernatant. Nevertheless, the author failed to analyse the ratio of radioactivity in the subfractions of the particulate. These subfractions consisting of myelin, synaptosomes and mitochondria can be easily separated by modern, accurate methods employing ultracentrifugation (142,143). The aim of this dissertation is to explore the subcellular binding of mescaline in brain tissue as well as to examine the relationship which may exist between this hallucinogen and the brain catecholamine, dopamine.

MATERIALS

Beckman/spinco Model L3-50 Ultracentrifuge with SW-41 Ti rotor and types 40 and 50.1 rotors.

International Clinical Centrifuge Model CL#75290H

Packard Radiochromatogram Scanner Model 7201 with Packard Recording Ratemeter Model 385

Beckman Liquid Scintillation System LS-200B with Automatic Teletype

Buchler Instruments Constant Temperature Water Bath

Rinco Instrument Flash Evaporator

Junior Coleman Spectrophotometer Model 6A

Dubnoff Metabolic Shaking Incubator

Mettler Analytical Balance Model H 20 T

Mettler Balance Model P-1200

Eastman Chromagram Sheet #6064

Eastman Chromagram Developing Jars and Tanks

Disposable Pipettes (pasteur)

Buchler Dekastaltic Pump

Beckman Research pH Meter with Combination Microelectrode

Con-Torque Motor Driven Homogenizer

Thomas Tissue Grinding Vessel with Teflon Pestel with 1 mm. clearance

Wohelm Neutral Aluminum Oxide Grade 1

Intramedic Polyethylene Tubing #PE 60

Yale Hypodermic Needles 21G

Vertis Portable Lyopholyzer

Beckman Polyallomer Tubes, 10 ml. capacity

Beckman Cellulose Nitrate Tubes, 14.5 ml. capacity

Hamilton Microsyringe with Stop Guide Model 710-N

Corning Glass disposable Micro-Sampling Pipettes

Exax Culture Tubes 1 ml. capacity

Wheaton Scintillation Vials, 22 ml. capacity

New England Nuclear Scintillation Solution (AQUASOL)

Nuclear Chicago Protein Solubilizing Solution (NCS)

Mescaline-8-^{14}C Hydrochloride Specific Activity = 3.3 mcuries/mMole, New England Nuclear Corp. Boston, Mass.

3,4-Dihydroxyphenethylamine - H^3 (G) Specific Activity = 15 curies/mMole, New England Nuclear Corp. Boston, Mass.

3,4-Dihydroxyphenethylamine 7 - ^{14}C Hydrochloride, Specific Activity = 55 mcuries/mMole, Amersham/Searle Arlington Hgts, Ill.

Mescaline Hydrochloride, Aldrich Chemical Co. Milwaukee, Wisc.

Diabutal (Sodium Pentobarbital), Diamond Laboratories Inc.

Cerebrospinal Fluid (CSF) made according to Merlis (149)

Serpasil (R), Reserpine USP, 2.5 mg/ml, Ciba

Urea-^{14}C Specific Activity = 50 mcuries/mMole,

New England Nuclear Corp. Boston, Mass.

METHODS

Dissection of the Rat Brain.- Male wistar rats with an average weight of 250g were used. The animals were kept in metabolic cages; food and water were restricted for eight hours preceding the experiments.

The rats were removed from the metabolic cages and killed by decapitation. The animals heads were blotted with paper towels and a mid-saggital incision was made through the skin. The skull bone was cut between the eyes with a bone cutter and the temporal bones scored along their sides, anterior-posteriorly. The entire brain was gently but quickly removed from the cranial cavity by cutting the cranial nerves and the medulla oblongata. The brain was washed in physiological saline to remove the excess blood, blotted and immediately embedded in crushed ice. For experiments in which the whole rat brain was used, the freshly dissected brain was blotted, weighed and immediately transferred into a homogenizing vessel containing ice-cold 0.32 M sucrose solution, 0.5 ml per gram of tissue.

Anatomical Localization.- For experiments in which certain regions of the brain, such as frontal cortex, caudate nucleus, mid-brain, brain stem and cerebellum were needed, an average of three rat brains were pooled.* The anatomical localization, after removal of the brain from the cranial cavity, was done in the following manner:

The dissected brain was placed on its ventral portion in a culture dish mounted on ice. A thin slice of the cortex was cut out along the midline, posterior-anteriorly. This portion was placed into a small plastic container labeled frontal cortex and was frozen on dry-ice. A midline sagittal cut was made, dividing the two hemispheres and exposing the lateral ventricles, the right and the left caudate nuclei. The two semi-spherical bodies forming the caudate nuclei were carefully removed by cutting anterior-posterior and ventrally. They were labeled caudate nuclei. The remainder of the cortical envelop along with the hyppocampi was removed and discarded. A vertical cut was made anteriorly to the pons. The portion comprising

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* See Fig.6 for description of brain areas taken for study.

the ventral section of the brain from the optic chiasm crossing to the pons was labeled, mid-brain. The cerebellum was removed from the remainder of the brain. Finally, a vertical cut was made posteriorly to the medulla to comprise the pons and the medulla, that portion was labeled, brain stem. The rest of the brain, mostly spinal cord, was discarded. In experiments using caudate tissues, only the caudate nuclei were removed from the dissected rat brain.

Preparation of Tissues.- Whole rat brains up to 2 g. were homogenized in a Thomas glass grinding vessel with teflon pestle (see materials) with 10 ml of ice-cold 0.32 M sucrose. Larger masses were homogenized in 12.5 ml. The homogenization lasted one to two minutes with approximately 7 to 10 up and down strokes. All preparations were performed in an ice bath. Small masses of brain tissue up to 400 mg were homogenized with 5.0 ml of ice-cold 0.32 M sucrose. Perchloric acid solution of 0.4 N was used in some experiments.

Preparation of Subcellular Fractions.*- The isolation of the subcellular fraction was performed according to the method of Gray and Whittaker (143). The brain was homogenized in an ice cold solution of 0.32 M sucrose. The homogenate was centrifuged at 900 x g in an International Clinical Centrifuge for 10 minutes, to remove the nuclei, red blood cells, connective tissues and other cell debris. Unless otherwise specified, all centrifugation was done between 0° and 4°C. The supernatant was withdrawn with a pipette and kept in the cold. The precipitate was resuspended in cold 0.32 M sucrose and recentrifuged at 900 x g for an additional 10 minutes and the precipitate discarded. The supernatant was then combined with the original. The combined supernatant was centrifuged in a Spinco Beckman Ultracentrifuge Model L3-50, at 11,500 x g for 20 minutes, using a fixed angle rotor Type 40. The resulting supernatant contained the microsomes, and soluble supernate contaminated by some ruptured myelin and synaptic vesicles. The myelin, synaptosomes and mitochondria precipitated as a softly packed pellet. The supernatant was discarded and the precipitate was resuspended in a volume

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*See Figs. 7 and 8 for outlines.

of 0.32 M. sucrose equivalent to one half that from which it precipitated. The suspension was again recentrifuged at 11,500 x g for 20 minutes. The supernatant was discarded and the washed pellets were then resuspended in cold 0.32 M sucrose. This suspension was layered over a discontinuous gradient composed of 0.8 M and 1.2 M sucrose. The filled tubes (cellulose nitrate, 13 ml total capacity) were centrifuged at 54,000 x g for 2 hours in the Spinco Beckman Model L3-50 Ultracentrifuge, using a horizontal swinging bucket rotor type SW-41 Ti. After completion of centrifugation, three distinct layers of subcellular fractions could be seen. These fractions were myelin in the upper layer, synaptosomes in the middle layer and mitochondria in the bottom layer (Fig.8). Good homogeneity of the fractions was obtained as indicated by their electron microscopic analysis (Plates 1,2,3). Samples from all three fractions were analyzed by electron microscopy, using the positive staining technique (144,145). Samples were taken from each fraction for protein and isotope determinations.

Preparation of synaptic vesicles.- Synaptic vesicles were prepared by either the method of Whittaker (146) or that of De Robertis (147). In either case, the pure synaptosomal or the crude mitochondrial preparations obtained from the 11,500 x g fraction (see above) were osmotically shocked with cold distilled water for 5 minutes. The osmotically shocked subcellular preparations were centrifuged at 17,500 x g for 20 minutes. The pellets containing synaptic ghosts and other heavy components were discarded. The supernatant was withdrawn and recentrifuged at 100,000 x g for 30 minutes. The resulting gel-like sediment represented the synaptic vesicles which were liberated during the osmotic shock treatment. In spite of the osmotic shock that had ruptured the synaptosomal membrane, a high proportion of the vesicles showed no membranal disruption. Moreover, norepinephrine has been found to be highly concentrated in this fraction, when isolated from the rat hypothalamus (142,146,147).

Preparation for Liquid Scintillation Counting.- Brain tissue preparations up to 250 μl were pipetted into 22.0 ml capacity

glass counting vials, to which 1.0 ml of Nuclear Chicago Solubilizing Solution (NCS) was added. The mixtures were digested in capped vials at 55 ^{o}C in a constant temperature water bath. After completion of the digestive period, the vials were allowed to cool to room temperature and 15.0 ml of cold Aquasol (New England Nuclear Liquid Scintillation Cocktail) was added. The vials were shaken vigorously and then placed in a Beckman LS-200 B Liquid Scintillation Spectrometer. Samples were allowed time for sufficient cooling in order to avoid high background. Each sample was counted for a minimum of 100 minutes. The background was automatically substracted, except in cases where a background count was desired for a batch of Aquasol-NCS mixture.

Protein Determination.- The method used for determining tissue protein content was a slight modification of that described by Lowry et al (148). Briefly, 100 μl or so of the tissue sample, depending on the expected protein value, was diluted in 1.0 ml of 0.1 N NaOH. The volume was adjusted to 50 ml in a volumetric flask with distilled water.

A standard albumin solution with a final concentration of 100 μg/ml was treated the same way. Aliquots of 1.0 ml of both standard and samples were reacted with copper-tartrate reagent prepared without NaOH then with a phenol reagent. After color development, the tubes were read in a Junior Coleman Spectrophotometer, Model 6 A at 550 mμ.

Intraventricular Injection of Labeled Dopamine.- Male wistar rats were lighly anesthetized with ether (Anesthesia Grade). The animal head was placed in a horizontal position in line with the long axis of the body. A small beaker (15 ml capacity) in which was placed a piece of cotton wet with ether was held close to the animal's nose in order to maintain prolonged anesthesia throughout the operation. A mid-sagittal incision was made through the skin from the eyes to the ears. The skull bone was scraped clean of connective tissues, the sagittal and coronal crossing suture lines were exposed. With the head of the animal held in a horizontal position against a small animal rack, slight but firm pressure, with rotary motion was carefully applied on the skull with a 21 gauge hypodermic needle. A small hole just

deep enough to penetrate the skull bone was bored by this technique. The hole was made right on the coronal suture line, 1.5 mm lateral to its crossing point with the sagittal suture (see Fig. 9A). A 50 μl capacity Hamilton syringe having a stop at 4.0 mm from its tip (Fig. 9B) was used for injection. The animal was then put into deep anesthesia with ether. With the plunger drawn to 10 μl with ^{3}H-dopamine in 0.01 N acetic acid, the radioactive solution was rapidly injected into the left lateral ventricle. The needle was held in place for 10 seconds and was then withdrawn. The index finger was immediately placed over the hole in the skull to prevent leakage of the injected material. The incision was closed with 2 to 3 wound clips (Clays Adams, 9 mm clips). The animal was placed back into its metabolic cage, where it recovered fully within 3 to 5 minutes post surgery. No abnormal behavior such as ipsilateral or contralateral turning syndrome, characteristic of striatal damages or lesions, was observed in the recovered animal.

Implantation of Cannulae and Cerebral Ventricular Perfusion.- In addition to the small hole bored on the coronal suture of the rat skull, for intraventricular injection (see above) another hole of the same size and at the same distance from the sagittal suture, was made on the right side. Two hours after the intraventricular injection of the radioactive material (see Methods), the animal was put under deep anesthesia with an intraperitoneal injection of sodium pentobarbital, 20 mg/kg body weight. The animal was immobilized on a small animal rack, and a cannula made from a ground hypodermic needle #21 G was inserted into each hole. The ground needle was first inserted into polyethylene tubing #PE-60, leaving exposed only the short ground portion 4.0 mm from its tip (see Fig.10). Artificial cerebrospinal fluid, prepared as described by Merlis (149), was withdrawn through the polyethylene tubings prior to the insertion of the cannulae into the skull. The inlet tubing was always inserted at the injection site. Artificial cerebrospinal fluid (CSF) was then infused into the left ventricle at a rate of 100 μl per minute with a Buchler Multistages Dekastaltic Pump. The effluent was collected every minute

from the right lateral ventricle into 1.0 ml size culture tubes containing 20 μl of 5 N acetic acid. The body temperature was maintained with the aid of a heating lamp. For alumina extraction and chromatographic analysis, pooling of samples collected over a 10 minute period was necessary. In some experiments, it was necessary to start the flow of the effluent by pulling gently on the plunger of a syringe attached to the polythylene tubing. No leakage of perfusing fluid was observed during the entire perfusion period. The exact location of the cannulae was verified by perfusing with an inert blue dye at the end of each experiment. The animal was killed immediately after and its ventricular walls exposed (see under methods). The dye solution was rinsed away with physiological saline solution and both caudate nuclei removed for radioactive assay.

Analysis for H^3-Dopamine and Metabolites.- Determination of H^3-Dopamine and its metabolites in brain tissue homogenate, synaptosomal preparations or superfusate was achieved by the method of Anton and Sayre (150). Briefly, the catecholamine, was extracted from perchloric acid supernatant or from

artificial cerebrospinal perfusion effluent at pH 8.6 with 400 mg of activated aluminum oxide (151), in the presence of 200 mg ethylenediaminetetraacetate disodium salt (EDTA) and 10 mg sodium metabisulfite per sample. The mixture was centrifuged at low speed and the supernatant was set aside and subsequently assayed for O-methylated metabolites. The alumina was then washed several times with 10 ml portions of distilled water. The labeled catechol was eluted with 3 ml of 0.1 N hydrochloric acid. Aliquots of 100 μl were transferred into scintillation counting vials for radioactivity determination. The remainder of the alumina eluate was evaporated to dryness under nitrogen gas and the residue taken into 200 μl of methanol. To this was added 5 μg each of unlabeled dopamine hydrochloride and DL-norepinephrine hydrochloride. The mixture was applied, as a band, on a 1½ inch wide Eastman chromagram cellulose coated strip. The chromatogram was developed in a buthanol: acetic acid: ethanol system (35 : 10 : 10) until the solvent front ascended within 2 cm of the edge of the strip. Dopamine, and norepinephrine were identified by the color reaction with diazotized sulphanilic reagent. The appro-

priate areas were scraped off the chromatogram and transferred into counting vials. 1.0 ml distilled water was added to the vials to form a suspension with the cellulose powder. To this suspension was added 15 ml of Aquasol and the sampled were counted in a Beckman Liquid Scintillation Spectrometer Model LS-200 B. Known amounts of ^{3}H-dopamine and ^{3}H-norepinephrine from standard stock solutions were also carried through the entire procedure in parallel experiments in order to determine their recoveries at each step of the analysis. Alumina extraction recoveries for dopamine and norepinephrine were 82 and 85 per cent, respectively. Thin layer chromatographic recoveries were 35 per cent for both catecholamines. Correction of the final result were made for chromatographic recoveries and counting efficiency.

The effluent from the alumina was adjusted to pH 6.0 with 0.1 N HCl and placed on columns of Dowex 50 W X 8 (H^{+} form 6 X 40 mm). The columns were washed with a small amount of distilled water and the wash collected with the Dowex effluent. Aliquots of 0.5 ml were counted in 15 ml Aquasol, the radioactivity of the combined effluent-wash

represented the labeled deaminated-O-methylated metabolites. Subtraction of the radioactivity found in that fraction from that of the alumina effluent gave the amount of labeled-O-methylated metabolite (methoxytyramine). The remainder of the Dowex effluent-wash was lyophylized in a Virtis portable lyophylizing apparatus. The residue was taken up in methanol and centrifuged. The clear supernatant concentrated to 200 µl was applied on Whatman paper No. 1, 1½ inch wide strip, along with 200 µg each of unlabeled homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenethanol (MHPE). The strips were developed overnight in isopropanol: ammonia: water system, (8:1:1). Identification of the added reference compounds was accomplished by spraying with the diazotized sulphanilic reagent. The appropriate stained bands were cut out, eluted in 1.0 ml of water and counted in scintillation vials containing 15 ml of Aquasol. Unfortunately chromatographic recovery for HVA and MHPE could not be achieved due to the unavailability of the labeled authentic compounds.

Hydrolysis of the Alumina Effluent.- In a few experiments, the alumina effluent was adjusted to pH 5.4 with 1.0 N acetic acid and hydrolyzed for 3 hours at 37°C with 0.1 ml of glusulase (Endo Laboratories) containing 106,100 units of glucuronidase and 73,800 units sulfatase per ml of solution. The hydrolysate was cooled to room temperature, the pH adjusted to 7.0 and extracted with 3 times the volume with ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was taken up in a small volume of ethyl acetate and evaporated to dryness under nitrogen gas. The final residue was dissolved in 300 μl of methanol. Radioactive content was determined in a 10 μl aliquot. Authentic reference compound (3-methoxy-4-hydroxyphenethanol, 25 μg, Regis Chemical Co.) was added to the alcohol solution. The mixture was chromatographed on a cellulose coated thin layer strip. Identification and radioactive assay were performed as described previously.

Experimental Protocols

1- Measurement of ^{14}C-Mescaline Binding to Whole Rat Brain Homogenate, in vitro.

Male wistar rats with an average weight of 250g were sacrified by decapitation. Their brains were immediately removed, rinsed with cold physiological saline, weighed and homogenized as described in methods for tissue preparation. Aliquots of 1.0 ml were withdrawn from the total homogenate and transferred into clean test tubes placed in an ice cold water bath. To each tube was added 1 x 10^{-2} μmole of ^{14}C-mescaline HCl (s.a.3.3 mc/mMole, New England Nuclear Corporation) in 50 μl of 0.32 M sucrose. The samples were incubated for 30 minutes at 37^{o}C under shaking in a Dubnoff metabolic shaking incubator. At the end of the incubation period, the tubes were placed in a 0^{o}C ice cold water bath for 10 minutes. The incubates were then transferred into 10.0 ml capacity Beckman polyallomer centrifuge tubes fitted with metal caps. The tubes were centrifuged at 100,000 x g

for 30 minutes in a Spinco-Beckman Model L3-50 Ultra-centrifuge using type 40, fixed angle rotor. After centrifugation, the supernatant fluid was discarded, and the resulting pellets resuspended in 10.0 ml of 0.32 M sucrose. The tubes were recentrifuged at the same speed for 30 minutes. The liquid phases were decanted and the walls of the centrifuge tubes were wiped dry with the aid of filter paper. The semi-dry pellets were transferred into 22.0 ml capacity counting vials (Wheaton Scintillation vials) containing 1.0 ml of Nuclear Chicago Solubilizing solution (NCS). The radioactivity was determined (see under preparation for scintillation counting).

2 - Measurement of ^{14}C-Mescaline Binding to Tissue Homogenate Isolated from Various Regions of the Rat Brain in vitro.

Male wistar rats were sacrificed by decapitation. Their brains were removed and dissected into various anatomical regions (see under methods for anatomical localization). These tissues were quickly homogenized in 0.32 M sucrose and duplicate aliquots of 1.0 ml were withdrawn from each homogenized section. Radioactive mescaline was added to all the

tubes. The protocol described earlier for measurement of ^{14}C-mescaline binding to whole rat brain homogenate, in vitro was followed thereafter.

3- Determination of Localization of ^{14}C-Mescaline in Subcellular Particles after Incubation with whole Rat Brain Homogenate.

The protocol described for the measurement of ^{14}C-mescaline binding to whole rat brain homogenate, in vitro, was followed up to the end of the incubation period. After 0^{o} C cooling, the tubes were centrifuged at low speed, (900 x g) at 4^{o} C in an International Clinical Centrifuged (model, CL), for 10 minutes. The supernatant was withdrawn. Three samples were pooled in order to have enough tissue for subcellular fractionation. The pooled supernatant was subjected to a discontinuous sucrose density gradient centrifugation (see Methods section). The radioactivity and the protein contents were determined for each subcellular fraction.

4- Measurement of ^{14}C-Mescaline Binding to Synaptosomes Isolated from Various Brain Regions, in vitro.

Synaptosomes were prepared (see under Methods section) from pooled anatomically identical brain sections. These subcellular particles were centrifuged at 30,000 x g for 30 minutes to remove the hypertonic 0.8 M sucrose solution from which they were isolated. The particles were resuspended into cold 0.32 M sucrose. Duplicate aliquots of 1.0 ml were withdrawn from each preparation and incubated with ^{14}C-mescaline. The radioactivity was determined as before (see under Methods section).

5- ^{14}C-Mescaline Binding to Caudate Synaptosomes, in vitro Following in vivo Treatment with Reserpine.

Male wistar rats received injection of reserpine (SERPASIL (R) Ciba) 6.25 mg/kg intraperitoneally, and sacrificed eight hours later. The brain was removed and the caudate nuclei removed. Synaptosomes subsequently isolated from these brain tissues (see Methods section) were incubated with ^{14}C-mescaline and the radioactivity determined. (see under Preparation Liquid Scintillation).

6- <u>The Effect of Prior Incubation with Unlabeled Mescaline on the Incorporation of H^3-Dopamine into synaptosomes Isolated from Caudate Nucleus.</u>

Caudate nuclei pooled from three rat brains were homogenized in 0.32 M sucrose. Synaptosomes isolated from these tissues (see under Methods section) were incubated for 15 minutes with unlabeled mescaline hydrochloride in Beckman polyallomer centrifuge tubes. After the incubation period, the tubes were rapidly cooled to $0^{o}C$. then centrifuged at 100,000 x g for 5 minutes. The supernatant was discarded and cold 0.32 M sucrose to a final volume of 1.0 ml was added to resuspend the pellets. To each tube was then added 39,000 CPM of ^{3}H-Dopamine and the samples were reincubated for 30 minutes. After the second incubation, the tubes were recentrifuged at 100,000 x g for 5 minutes to remove the excess of radioactive material. The resulting pellets were resuspended in 10 ml of cold 0.32 M sucrose and the tubes centrifuged at 100,000 x g for thirty minutes. The radioactivity was determined according to the method described earlier (see under Preparation for Liquid Scintillation)

7- The Effects of Prior in vivo Administration of Unlabeled Mescaline on the in vivo uptake of Intraventricularly Injected ^{3}H-Dopamine by Rat Caudate.

Pairs of rats which were already prepared surgically for intraventricular injections (see Methods section) were used. Unlabeled mescaline hydrochloride (Aldrich Chemical Co.) was administered intraperitoneally fifteen minutes before the intraventricular injection of ^{3}H-dopamine, and the animal was killed by decapitation one-half hour later. The brain was removed, the caudate nuclei dissected and synaptosomes were isolated for radioactive assay (see appropriate sections under Methods).

8- Injection of ^{14}C-Mescaline in vivo to Determine its Subcellular Localization in Various Brain Regions.

^{14}C-mescaline was injected intravenously (via the tail vein) in pairs of rats. These animals were killed forty-five minutes later. The brains were removed, dissected into anatomically differentiated regions (see under Methods section) and subcellular fractions were prepared from each brain section. Radioactive assays were performed according

to the method described earlier (see Methods section).

9 - <u>The Effects of Mescaline on the Content of ^{3}H-Dopamine and its Metabolites in the Effluent of the Perfused Cerebroventricular System of the Rat.</u>

Two hours after intraventricular injection of ^{3}H-dopamine (G), specific activity = 15 C/mmole (New England Nuclear Corp.) into the rat, the cerebroventricular system was perfused with artificial cerebrospinal fluid (CSF) (see under Methods for Implantation of Cannulae and Cerebral Ventricular Perfusion). The effluent was collected at one minute intervals and aliquots of 10 μl from each collection were assayed for radioactivity content in aquasol. The perfusion continued with artificial CSF up to sixty minutes, at this time, a fairly steady baseline of activity in the collected perfusion effluent has been established. At this time, the ventricular system was perfused for 10 minutes with CSF containing various concentrations of mescaline hydrochloride or amphetamine sulfate. Perfusion with drug alternated with drug-free CSF. The radioactivity content was determined for each perfusion effluent collection made.

Effluent samples corresponding to CSF or CSF plus drug over a ten minutes perfusion period were pooled and analyzed for ^{3}H-dopamine and its metabolites (see Methods section).

RESULTS

1- Measurement of ^{14}C-Mescaline Binding to Whole Rat Brain Tissue Homogenate.- Tissue homogenate of whole rat brain binds ^{14}C-mescaline HCl added to the incubation medium. A continuous rise in the amount of ^{14}C-mescaline HCl bound per mg of tissue protein was observed, as illustrated in Table 1. This can be interpreted as indicating two things: 1) That the binding sites were not saturated within the concentration range used. 2) That mescaline, in contrast to dopamine (113,114) and norepinephrine (103,152) was not taken up into a saturable number of vesicles or granules. Of these two possibilities, the latter seems more likely. Snyder et al (152) and Hendley et al (153) have detected saturation of ^{3}H-norepinephrine and ^{3}H-dopamine accumulation in the various areas of the rat brain using much lower concentrations of the amines.

2- Subcellular Distribution of Bound ^{14}C-Mescaline.- In order to test the hypothesis that mescaline binding to brain tissue homogenate is not specific, the

brain tissue homogenates were incubated with ^{14}C-mescaline and the incubates were subjected to discontinous sucrose gradient fractionation, after removal of the heavy nuclei. The largest accumulation of ^{14}C-mescaline per mg of tissue protein was found in the synaptosomal fractions (Table 2). This is of interest since endogenous and exogenous catecholamines, dopamine and norepinephrine are also found to accumulate in those sub-fractions (142,154,155). However, when the synaptosomes were lysed with cold distilled water to liberate their vesicles (142), no radioactivity was found in the granule fraction. This implied that ^{14}C-mescaline either is not specifically bound within the synaptic vesicles or that osmotic shock treatment may have released the content of ^{14}C-mescaline from the vesicles. The latter seems unlikely, since catecholamines have been found in a substantial proportion of vesicles isolated in this way. Futhermore, rats that were treated with reserpine, to empty the synaptic vesicles of their content of catecholamines, did not show any variations over the control animals in the accumulation of ^{14}C-mescaline (Table 3). This indicates that mescaline,

in contrast to the catecholamines, is not taken up into the synaptic storage vesicles.

3- Measurement of ^{14}C-Mescaline Binding to Synaptosomes from various Regions of the Rat Brain.- The accumulation of ^{14}C-mescaline in synaptosomes was investigated as to regional specificity in the rat brain, in vitro. When crude synaptosomal preparations* from various areas of the rat brain were incubated with ^{14}C-mescaline HCl, no regional differences in the accumulation of the labeled compound were observed (Table 4). This tends to suggest that accumulation of ^{14}C-mescaline is probably due to a simple diffusion through the synaptosomal membrane. In similar experiments where ^{14}C-dopamine was incubated with synaptosomal preparations from the striatum, the cerebral cortex and the cerebellum, differences were noted in the accumulation of the catecholamine into each of these regions (Table 5). These findings are in agreement with those of Snyder et al (152), Glowinski and Iversen (155), Hendley et al (153), and Glowinski, Snyder and Axelrod (156). Working with ^{3}H-norepinephrine,

*The crude synaptosomal preparations used were the 11,500 x g fractions containing synaptosomes contaminated by myelin and mitochondria.

these investigators were able to show that the striatum accumulated the most and the cerebellum the least of the added labeled catecholamines. Furthermore, these authors have also demonstrated that between 80 and 90 per cent of the accumulated radioactivity was unchanged catechols. This ability of the striatum to accumulate exogenous catecholamines against a concentration gradient is attributed to an active process or mechanism facilated by ATP (157). In an effort to study the kinetics of ^{14}C-mescaline accumulation into nerve ending particles, pure striatal synaptosomes were incubated with ^{14}C-mescaline HCl at various concentrations. The striatum was chosen because of its richness in nerve terminals (158,159). Saturation of the binding sites was not achieved by mescaline in the range of concentrations used (Table 6). The affinity of mescaline for the synaptosome is low since less than 0.5% of the added mescaline was bound.

4- Inhibition of ^{3}H-Dopamine Uptake into Synaptosomes by Prior Incubation with Mescaline HCl.- Dopamine is found highly concentrated in the striatal synaptic particles,

When pure synaptosomes isolated from the rat caudate were pre-incubated with mescaline HCl at concentrations varying from 1.0 x 10^{-4}M to 1.0 x 10^{-2}M and then incubated with a known concentration of ^{3}H-dopamine, strong inhibition in the uptake of the labeled amine was detected (Table 7). At relatively high concentration, 1.0 x 10^{-2}M, mescaline inhibited the ^{3}H-dopamine uptake to less than half the control values. From the results in Table 7, it is seen that mescaline in a concentration of 10^{-4}M has only a negligible inhibition on the uptake of exogenous dopamine in isolated nerve ending particles. Only when the mescaline concentration was increased to 10^{-3}M, was the dopamine uptake moderately inhibited. It is unlikely that an effect requiring a concentration of that order of magnitude could be of physiological significance. In analysing these data, one must keep in mind that the effect observed in isolated synaptosomes may not be a true reflection of events in the intact brain.

5 - Inhibition of ^{3}H-Dopamine Uptake by Rat Caudate After Treatment with Mescaline. In order to study the effect of mescaline *in vivo*, the uptake of exogenous

^{3}H-dopamine was investigated in rats pretreated with mescaline. Glowinski and Axelrod (160) have demonstrated that labeled catecholamines injected intraventricularly were selectively distributed in areas which contained high concentrations of the endogenous catecholamines, notably, the caudate nucleus (dopamine) and the hypothalamus (norepinephrine). Groups of rats were given intraperitoneal injections of mescaline HCl at doses ranging from 25 mg/kg to 200 mg/kg body weight. The uptake of ^{3}H-dopamine was compared between the mescaline treated rats and control animals receiving saline injections in lieu of mescaline. The results in Table 8 show a positive relationship between the degree of inhibition in ^{3}H-dopamine taken up and the dose of mescaline given. This inhibition could be the result of either of two things: 1) The low level of radioactivity was caused by a rapid release of ^{3}H-dopamine due to an inability of the vesicles to store it or protect it from enzymatic destruction. Such observations were made when animals were treated with either chloropromazine or reserpine (135,161,162) or 2) the inhibition in ^{3}H-dopamine uptake resulted from inhibition of the uptake mechanism.

von Euler and Lishajko (163) explained the blocking action of high concentrations of cocaine on the uptake and release of norepinephrine in this way. Only a direct measurement, in vivo, of the net release of catecholamines by mescaline can help settle this question.

6- Subcellular Localization of Intravenously Injected ^{14}C-Mescaline HCl.- Before considering the complex problem of differentiating between release and uptake, it was decided to study the subcellular localization in the brain of ^{14}C-mescaline injected intravenously, via the tail vein. The subcellular distribution of intravenously injected ^{14}C-mescaline HCl was studied in various areas of the rat brain. As can be seen in Fig.11 the regions of the brain which accumulated the largest amount of radioactivity were the cortex and the caudate nucleus. These results find support in the reports by Neff et al (53). In their studies on the distribution of ^{14}C-mescaline HCl in the cat brain, these authors found the largest amounts of radioactivity in the cortex and in the caudate nucleus. Based on the studies done by Barlow et al (164) on the

vascularity of the central nervous system of the cat, it appears that the distribution of radioactivity in the brain was not related to the relative vascularity of the specific brain areas. The most vascular structures of the brain accumulated the least radioactivity. The results in Fig.11 also show that the synaptosomal fractions accumulated the largest amount of radioactivity per mg of tissue protein in all the brain areas studied. After intravenous injection of ^{14}C-mescaline, the highest concentration of radioactivity is found in the caudate nucleus and particularly at the nerve endings. Taking that into account, it was decided to study the effect of mescaline on the catecholamine, dopamine, stored in that region of the brain. These studies were directed toward elucidation of the direct involvement of catecholamines in the central action of mescaline.

7- Perfusion of the Cerebral Ventricles with Artificial Cerebrospinal Fluid after Intraventricular Injection of ^{3}H-Dopamine.- Rats were injected intraventricularly with ^{3}H-dopamine to label dopaminergic neurons in the caudate

(see section under Methods). In these experiments, the left lateral ventricle of the rat was perfused with artificial cerebrospinal fluid and the effluent was collected from the right lateral ventricle. Perfusion was begun 2 hours after ^{3}H-dopamine had been injected. Collections of effluent samples were made every minute and the radioactivity determined. The washout pattern of ^{3}H-dopamine and its metabolites from a typical experiment is shown in Fig.12. Within fifty minutes, the concentration of ^{3}H-dopamine and its metabolites in the effluent had reached a relatively steady level and declined only slightly over the entire length of the experimental period (120 minutes).

8- Effect of Low Dose of Mescaline in the Perfusing Fluid on the Concentration of ^{3}H-Dopamine and its Metabolites in the Perfusion Effluent.* - The results of two representative experiments, from a set of seven, on the effects of low dose of mescaline on the concentration of ^{3}H-dopamine and its metabolites in the perfusion effluent are shown in Fig.13 and Fig.14. (see also Table 9A). Low doses of mescaline caused an immediate increase in the radioactivity monitored in the

*The data for each individual experiment is presented in Appendix I, Table 9A .

perfusion effluent. This spontaneous increase in radioactivity reached a peak in four minutes. Within eight minutes, the radioactivity had already returned to the baseline level even before the end of perfusion with mescaline. No further burst in increased radioactivity was noted when perfusion was continued with mescaline free artificial cerebrospinal fluid. Additional responses in the same animal were noted, however, when the ventricles were again perfused with another low dose of mescaline (Fig.13). The increase in radioactivity was three to four times the base level activity found during perfusion in the absence of mescaline. The effects of 1.0 µg/ml of mescaline were not significantly different from those obtained with 0.2 µg/ml .

9- Effect of Large Dose of Mescaline on the Concentration of ^{3}H-Dopamine and its Metabolites in the Perfusion Effluent.- When large doses of mescaline 10 µg/ml or higher were used to perfuse the lateral ventricles, a drastic reduction in radioactivity below base level was

monitored in the perfusion effluent, (Fig.14 and Fig.15) (see also Appendix I, Table 9A). This effect was not reversible with subsequent perfusion with low doses of mescaline (Fig.15).

10- Effect of d-Amphetamine on the Concentration of ^{3}H-Dopamine and its Metabolites in the Perfusion Effluent*.- Perfusion of the lateral ventricles with artificial cerebrospinal fluid containing d-amphetamine sulfate in concentrations ranging from 0.1 to 1.0 μg/ml did not cause any increases in the radioactivity of the perfusion effluent. Larger doses 50 μg/ml or higher, however, caused a significant increase in radioactivity in the perfusion effluent similar to that observed with low doses of mescaline.

11- Effect of Mescaline on the Washout Pattern of ^{14}C-Urea from the Rat Brain.- Two hours after 10 μcuries of ^{14}C-Urea was injected intraventricularly into the rat brain, the ventricular system was perfused with an artificial cerebrospinal fluid (CSF). The

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*The data are presented in Appendix I, Table 9B .

perfusion effluent was collected from the right lateral ventricle at ten minutes interval for a period of sixty minutes. Thereafter, one minute collections were made. The ventricular system was perfused with artificial CSF containing various concentrations of mescaline. As can be seen in Fig. 16, mescaline has no apparent effect on the washout pattern of ^{14}C-Urea from the structures bordering the ventricular wall.* This indicates that the action of mescaline on the net release of ^{3}H-dopamine from the caudate nucleus (Figs. 13-15) may be specific.

12- Determination of ^{3}H-Dopamine and its Metabolites in the Perfusion Effluent.- When pooled effluent samples were analyzed for ^{3}H-dopamine and its metabolites, significant increases in ^{3}H-dopamine and in the neutral alcoholic metabolite, 3-methoxy-4-hydroxy-phenethanol (MHPE) were noted during mescaline perfusion at low doses (Table 10). The level of the O-methylated metabolite, 3-methoxytyramine (MT) increased only slightly. No measurable amounts of free homovanillic acid (HVA) or 3,4-dihydroxyphenylacetic acid (DOPAC) were detected by the method used. These

* Data for additional experiments are presented in Appendix II, Table 11 .

findings are supported by the works of Taylor and Laverty (165); Glowinski, Kopin, Axelrod (166) Stone (167) and others (168-170). These authors have demonstrated that stored ^{3}H-dopamine and ^{3}H-norepinephrine are metabolized predominantly to their O-methylated neutral alcholic or glycolic metabolites, MHPE and MHPG. In the studies conducted here, 3-methoxytyramine accounted for less than 5% of the total tritium found in the perfusion effluent during both the control and the mescaline perfusion period. These results agree with the present view (165) that the major pathway of the metabolism of actively released dopamine from physiological storage sites is the neutral alcoholic metabolite, 3-methoxy-4-hydroxyphenethanol (MHPE). Most of this metabolite was found conjugated with sulphate. It was freed by hydrolysis with glusulase (Endo Laboratories). No efforts were made to determine the ratio in which the free and the conjugated forms of the neutral metabolite was present in the perfusion effluent.

DISCUSSION

Numerous reports have appeared in the literature concerning the absorption, regional localization, metabolism, and pharmacodynamic and behavioral effects of mescaline in man and other animals (5-88). The mode of action of mescaline in producing psychotomimetic effects is still unknown. As mentioned earlier, various hypotheses have been formulated in order to explain the hallucinatory effect of mescaline (58, 89-92). One consistent observation, however, which emerges from these often contradictory hypotheses is the agreement that the pharmacological and behavioral effects induced by mescaline are predominantly of central origin. At this point, it would be appropriate to ask if the mescaline action is related to any particular areas of the brain.

In the studies reported here, it was demonstrated that the maximum concentration of radioactivity in the brain

was localized in the cerebral cortex and the caudate nucleus, forty-five minutes following i.v. administration of ^{14}C-mescaline into rats. These findings are consistent with those reported by Neff and his co-workers (53) on the regional distribution of ^{14}C-mescaline in the cat brain, thirty minutes following i.v. administration of the labeled compound. This distribution of mescaline appears to be unrelated to the vascularity of the specific brain areas (164).

It was also demonstrated here that i.v. administered ^{14}C-mescaline is preferentially bound to the synaptosomal fraction as compared to the myelin and mitochondrial of cells derived from either the cortex or the caudate nucleus but not other brain areas. Recently, Denber and Teller, studying cell from the cortex (171), demonstrated that a fraction corresponding to the nerve endings (synaptosomes) accumulated the highest concentration of radioactivity, forty-five minutes following i.v. administration of ^{14}C-mescaline, when this fraction was compared with other subcellular components. From the results of these

studies, it appears that mescaline may have a specific affinity for the synaptosomal components of the cortex and caudate nucleus, at least when administered i.v.

It might be expected that cortical function would in some way be involved in the mediation of disturbances of higher functions such as occur with mescaline. A role for the caudate in the modulation of perceptual and mentational function is not well established. These findings with mescaline are, however, consistent with the conclusions of Snyder (172) from his ingenious studies of the mechanism of amphetamine psychosis. From these studies, Snyder concluded that psychotogenic effects of amphetamine are probably mediated by dopamine in the caudate, while the arousal effects seem to be mediated by norepinephrine in the brain activating system. Interestingly, mescaline has also been shown to affect neural transmission in the caudate. Schwartz has shown that mescaline behaves like dopamine in producing inhibition of electrical activity at the dopaminergic neuronal receptors in the caudate nucleus of the anesthetized cat (173). Gonzalez-Vegas (174)

has shown that in the brain stem neurons, however, mescaline antagonizes the actions of both dopamine and norepinephrine on electrical activity. Unfortunately, the latter author did not investigate the effect of mescaline on caudate neurons. If the results of the studies done by Schwartz and Gonzalez-Vegas are valid it would appear that mescaline has opposing effect on brain stem and caudate neurons. Such opposition may be involved in mediating some aspects of the central action of mescaline.

If mescaline is acting by disrupting neural transmission at caudate and cortical synapses, what is the mechanism by which this effect is produced? The studies carried out here have not been concerned with cortical mechanism. There is growing evidence, however, from these studies as well as others (11, 139-141, 173-176) that mescaline may affect the mechanism in the caudate nucleus involved in the release and re-uptake of dopamine in neural transmission. Denber and Merlis (139), Deegan and Cook (140) and Sturtevant and Drill (35) have demonstrated that chlorpromazine, which among its many effects,

blocks dopaminergic receptors to some extent and also enhances the deamination of dopamine (177), strongly antagonizes the central effect of mescaline. Shah (141) has demonstrated that reserpine, which depletes dopamine and other biogenic amines, blocks the gross behavioral changes associated with mescaline intoxication. Katz and Kopin (175) have shown that highly concentrated solutions of mescaline (10^{-2}M) block the stimulation-evoked release of catecholamines from rat brain slices. Also, Leonard and Tongue (176) have demonstrated and increase in endogenous brain dopamine levels in rats treated with mescaline in doses ranging from 25 to 50 mg/kg i.p.

When all these findings are considered in concert, it would appear that mescaline may either be acting as a false transmitter (173) or interfering with the normal transmitter function of dopamine. From the studies reported in this dissertation, it would appear that the latter action may also be involved. In studies reported here, it has been demonstrated that rats pretreated with unlabeled

mescaline in pharmacological doses (25 to 200 mg/kg weight) showed a dose dependent decrease in the retention of intraventricularly injected ^{3}H-dopamine by caudate synaptosomes presumably as a result of increased release or reduced uptake. It was also demonstrated in these studies that synaptosomal preparations isolated from the rat caudate nucleus accumulated less exogenous ^{3}H-dopamine when pre-incubated with unlabeled mescaline. However, high concentrations of mescaline (10^{-3} M or higher), were necessary to obtain this effect, _in vitro_. These results together with those reported by Katz and Kopin (175), Leonard and Tongue (176) suggest that high doses of mescaline may block the efflux of dopamine from its synaptosomal storage sites or block its uptake or both.

Further studies were necessary in order to determine whether mescaline could affect the efflux of stored dopamine _in vivo_. In the study described above, in which rats were pretreated with pharmacological doses of unlabeled mescaline, it was not possible to determine whether mescaline actually affected dopamine _in situ_

in the synapse or simply blocked its access to storage sites. Although further evidence on this point could have been obtained by administering ^{3}H-dopamine intraventricularly and then subsequently administering mescaline, it was felt that temporal and *in situ* dose relationship could be better explicated through use of a brain perfusion technique devised for this purpose. This technique, as described in the method section, involves the perfusion of the lateral ventricle and collection of effluent through a second exit point in the opposite ventricle. Brain perfusion has been sucessfully used in larger animals such as the cat (178). The caudate nucleus and hypothalamus lie in close proximity to the lateral ventricle so that perfusion of this cavity with an artificial cerebrospinal fluid makes it possible to monitor the release of catecholamines from these paraventricular structures. Thus, Portig (129) and Vogt (130) were able to confirm the presence of dopamine and homovanillic acid (HVA) in the cerebrospinal fluid of the cat *in vivo*, by perfusing the ventricular system. Carr and Moore (126) have demons-

trated the efflux of exogenous ^{3}H-norepinephrine and its metabolites during cerebroventricular perfusion of the cat brain with d-amphetamine sulfate. More recently, von Voigtlander and Moore (132) have shown an increase in ^{3}H-dopamine appearing in the perfusate during perfusion of the cat lateral ventricle with a highly concentrated solution of amantadine (300 μg/ml).

In the studies reported in this dissertation, it was demonstrated that perfusion of the lateral ventricle of the rat with an artificial cerebrospinal fluid containing high concentrations of unlabeled mescaline (10 μg/ml or higher) decreased the concentration in the efflux of radioactive dopamine administered previously intraventricularly. It was found, however, that perfusion with lower concentrations of mescaline (0.1-1.0 μg/ml) in the perfusing fluid rapidly and significantly increased the concentration of ^{3}H-dopamine and its metabolites in the efflux. This effect appears to specific, since the hallucinogen was shown to have no effects on the washout pattern of ^{14}C-Urea,

which had been previously injected into the brain intraventricularly. The results of these studies suggest that mescaline has a biphasic dose effect on dopamine. This may be related to its biphasic temporal effect on behavior reported by Smythies (26) and Bridger (179) in which it has been shown that early effects of mescaline are excitatory and later effects inhibitory.

Mescaline at lower doses could induce the increase in ^{3}H-dopamine in the perfusion effluent by any one of the following mechanisms: 1) inhibition of the enzymatic degradation of dopamine; 2) interference with the reuptake process of dopamine and finally, 3) enhancement of the spontaneous release of dopamine from the caudate nucleus. The first possibility cannot be ruled out entirely since the ratio of unmetabolized dopamine to metabolites is higher during mescaline induced efflux. However, mescaline has previously been shown to have no inhibitory effect on monoamine oxidase activity, both in vivo and in vitro (180). The studies carried

out here do not make it possible to differentiate effects on release from those on re-uptake.

Mescaline in high doses could produce a decrease in the total radioactivity content of the perfusion effluent by either one of the following mechanisms: 1) facilitation of the re-uptake of released dopamine through an increase in the permeability of the cell membrane; or 2) inhibition of the release of dopamine. The first possibility seems unlikely since: a) *in vivo* pretreatment with large doses of mescaline causes a decrease in the retention of intraventricularly injected ^{3}H-dopamine; b) large concentrations of mescaline in the incubation medium inhibit the uptake of exogenous labeled dopamine by rat caudate synaptosomal preparations and finally, c) concentrated solutions of mescaline ($> 10^{-4}$ M) inhibit the stimulation-evoked release of catecholamines from brain slices (175). That large concentrations of mescaline in the superfusing fluid decrease the efflux of tritium by blocking the release of dopamine seems most likely.

It has been suggested by some investigators that the psychotomimetic actions of mescaline are mediated by one of its metabolites (90-92). However, the very rapid efflux of ^{3}H-dopamine upon ventricular perfusion with mescaline, at low concentrations, weighs against this insofar as the release of dopamine is concerned. Studies carried out here indicate that mescaline does not have a profound effect on the metabolism of released dopamine. In the perfusion experiments in which the concentration of ^{3}H-dopamine was increased in the perfusate, there was also an increase in metabolites but to a lesser extent. Although all metabolites were not isolated separately, there was no differential increased of MHPE or other metabolites studied.

The results of the studies reported here, indicate that mescaline, in low doses, enhances the release or blocks the re-uptake of dopamine at the synapse of caudate neurons. In large doses, mescaline presumably blocks the release mechanism at the synapse of these neurons.

Although the mechanism of the hallucinogenic action of mescaline is not clear, an interaction with dopamine has been shown. This interaction of mescaline with dopaminergic neurons in the central nervous system merits further investigation, both to determine the mechanism of action of this drug and to probe the normal behavioral regulatory functions of the caudate.

SUMMARY

The binding of mescaline in the central nervous system and the effects of such binding on the uptake and release of exogenous dopamine have been investigated in the caudate nucleus and other brain regions of the rat. *In vitro*, the amount of ^{14}C-mescaline bound per mg of tissue protein was small. The synaptosome-rich fractions had the highest concentrations of radioactivity of the cell fractions studied. However, synaptic vesicles isolated from these synaptosomes were found void of radioactivity. Pre-incubation of synaptosomal preparations

from the rat caudate nucleus with unlabeled mescaline in concentrations varying from 10^{-4}M to 10^{-2}M shows a dose dependent inhibition in the uptake of ^{3}H-dopamine by these particles. In vivo, prior administration of unlabeled mescaline, i.p., inhibits the uptake of ^{3}H-dopamine, injected intraventricularly. This inhibition has a positive relationship to the dose of mescaline administered. Forty-five minutes after the intravenous injection of ^{14}C-mescaline, only 0.02% of the injected dose was found in the whole rat brain. Most of the bound drug was localized in the synaptosomal fractions of the caudate nucleus and the cerebral cortex. Rapid release of ^{3}H-dopamine in vivo was demonstrated using the cerebroventricular perfusion technique. Perfusion with an artificial cerebrospinal fluid (CSF) containing small amounts of mescaline (0.2 - 1.0 μg/ml) caused an immediate increase in the efflux of ^{3}H-dopamine and its metabolites. However, perfusion with artificial CSF containing high concentrations of mescaline (10 μg/ml or higher), caused a reduction in the tritium appearing in the perfusion

effluent. Mescaline, was shown to have no effects on the washout pattern of ^{14}C-Urea, which had been previously injected into the rat brain through the intraventricular route. The results of these studies suggest that mescaline, in low concentrations, may induce the increase in the efflux of ^{3}H-dopamine by enhancing the release of dopamine from the structures bordering the lateral ventricles or blocking its re-uptake or both. At high concentrations, however, mescaline appears to block the release of endogenous dopamine.

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TABLES

Table 1

^{14}C-Mescaline Binding to Tissues of Whole Rat Brain Homogenate

N	Concentration of Mescaline·HCl in incubation medium	^{14}C-Mescaline Bound Per mg Tissue Protein in mμmole
5	3.0×10^{-6}M	$1.18 \times 10^{-2} \pm 0.10$
5	9.0×10^{-6}M	$3.48 \times 10^{-2} \pm 0.20$
12	1.2×10^{-5}M	$5.78 \times 10^{-2} \pm 0.10$

Whole rat brain homogenate was incubated for 15 minutes with ^{14}C-Mescaline·HCl at various concentrations. Accumulation is expressed in mμmole/mg/15 minutes incubation ± S.E.M.

N = number of experiments

Table 2

Subcellular Distribution of Bound ^{14}C-Mescaline in Tissue From Whole Rat Brain

N	Subcellular Fraction	^{14}C-Mescaline Bound Per mg Synaptosomal Protein in mμmole
9	Myelin	$3.04 \times 10^{-2} \pm 0.07$
	Synaptosomes	$5.21 \times 10^{-2} \pm 0.10$
	Mitochondria	$3.01 \times 10^{-2} \pm 0.06$

Whole rat brain tissue homogenate was incubated for 15 minutes with ^{14}C-Mescaline·HCl, 1.2×10^{-5}M, and then subjected to subcellular fractionation. (See Methods section). Accumulation is expressed in mμmole/mg/15 minutes incubation ± S.E.M.

Table 3

Effect of Reserpine Pretreatment *in vivo* on the Binding of ^{14}C-Mescaline by Rat Caudate Synaptosomes *in vitro*

N	Treatment	^{14}C-Mescaline Concentration in incubation medium	^{14}C-Mescaline Bound Per mg Synaptosomal Protein in mμmole
8	Saline (Control)	3.0×10^{-5}M	$4.5 \times 10^{-2} \pm 0.21$
	Reserpine	3.0×10^{-5}M	$4.8 \times 10^{-2} \pm 0.46$

The animals received two intraperitoneal injections of reserpine 6.25 mg/kg body weight at 12 hour intervals and were sacrificed four hours following the last injection. Synaptosomes isolated from the caudate nuclei were incubated in 0.32 M sucrose with ^{14}C-Mescaline·HCl. Accumulation is expressed in mμmole/mg/15 minutes incubation ± S.E.M.

Table 4

Binding of ^{14}C-Mescaline to Synaptosomes Isolated from Various Areas of the Rat Brain

N	Brain Area	^{14}C-Mescaline Bound Per mg Synaptosomal Protein* in mμmole
9	Caudate	$5.7 \times 10^{-2} \pm 0.20$
3	Cortex	$5.1 \times 10^{-2} \pm 0.14$
3	Cerebellum	$5.0 \times 10^{-2} \pm 0.15$

*The synaptosomal preparations used were the 11,500 x g particles containing synaptosomes contaminated by myelin and mitochondria. These particles were incubated for 15 minutes with ^{14}C-Mescaline·HCl, 3.4×10^{-5}M. Accumulation is expressed in mμmole/mg/15 minutes incubation ± S.E.M.

Table 5

^{14}C-Dopamine Uptake by Synaptosomes Isolated from Various Areas of the Rat Brain

N	Brain Area	^{14}C-Dopamine Uptake Per mg Synaptosomal Protein* in mμmoles
15	Caudate	$5.06 \times 10^{-2} \pm 0.10$
9	Cortex	$1.78 \times 10^{-2} \pm 0.10$
9	Cerebellum	$1.01 \times 10^{-2} \pm 0.17$

*The synaptosomal preparations used were the 11,500 x g particles containing synaptosomes contaminated by myelin and mitochondria. These particles were incubated for 15 minutes with ^{14}C-Dopamine·HBr 3.3×10^{-6}M. Accumulation is expressed in mμmole/mg/15 minutes incubation.

Table 6

Binding of ^{14}C-Mescaline to Rat Caudate Synaptosomal Preparations*

N	^{14}C-Mescaline Concentration	^{14}C-Mescaline Bound Per mg Synaptosomal Protein in mμmoles
3	1.0×10^{-5}M	$4.07 \times 10^{-2} \pm 0.10$
12	1.2×10^{-5}M	$5.98 \times 10^{-2} \pm 0.10$
5	3.4×10^{-5}M	$15.20 \times 10^{-2} \pm 0.80$

*Pure synaptosomes were isolated from pooled rat caudate (3 rats per experiment) and incubated for 15 minutes with ^{14}C-Mescaline.HCl. Accumulation is expressed in mμmole/mg/15 minutes incubation ± S.E.M.

Table 7

Inhibition of ^{3}H-Dopamine Uptake into Synaptosomes by Mescaline·HCl*

N	Concentration of Mescaline·HCl in the Incubation Medium	Percent Inhibition
5	1.0×10^{-4}M	1.2 ± 0.37
8	5.0×10^{-4}M	17.4 ± 0.70
10	1.0×10^{-3}M	29.2 ± 0.30
7	5.0×10^{-3}M	44.1 ± 0.90
5	1.0×10^{-2}M	45.2 ± 1.80

*Pure synaptosomes isolated from the rat caudate were pre-incubated with Mescaline·HCl for 15 minutes at various concentrations. ^{3}H-Dopamine (0.7 μcurie) was then added and the incubation continued for an additional 15 minutes. Results are expressed in percent inhibition of control uptake ± S.E.M.

Table 8

Inhibition of ^{3}H-Dopamine Uptake into Rat Caudate by Pretreatment with Mescaline·HCl*

N	Dose of Mescaline·HCl Given I.P. in mg/kg Body Weight	Percent Inhibition
4	25	4.0 ± 1.47
2	50	9.9 ± 0.90
4	100	16.5 ± 0.95
4	150	40.3 ± 1.10
2	200	80.5 ± 1.50

*Mescaline·HCl was injected i.p. 15 minutes before the intraventricular injection of ^{3}H-Dopamine and the animals were sacrificed 15 minutes later. Results are expressed as percent inhibition of control uptake ± S.E.M.

Table 10

^{3}H-Dopamine and Metabolites in 10 Minute Effluent Collection

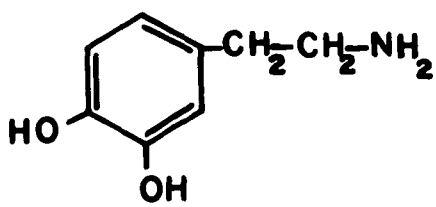
Compounds	(A) Control	(B) Mescaline Perfusion	Ratio B/A
Free ^{3}H-Dopamine	208 ± 10	513 ± 18	2.47 ± 0.16
Free ^{3}H-Methoxy-tyramine	89 ± 6	153 ± 8	1.73 ± 0.13
Free + Conjugated MHPE	442 ± 24	856 ± 23	1.93 ± 0.15
Free HVA	0	0	—
Unidentified	417 ± 13	821 ± 40	1.96 ± 0.20

*Values are expressed in mean CPM ± S.E.M.

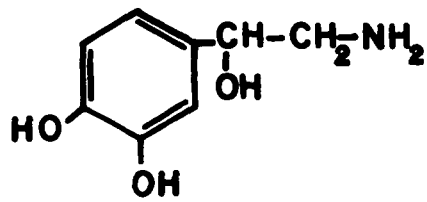
ILLUSTRATIONS

Fig.1 - Illustration of structural similarities between dopamine, norepinephrine and some centrally active β-phenethylamines.

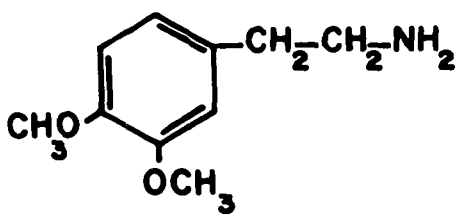
Structures of Dopamine, Norepinephrine and Related β-Phenethylamines



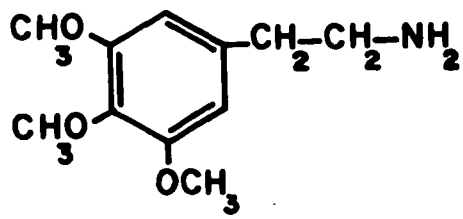
DOPAMINE



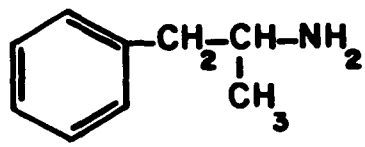
NOREPINEPHRINE



3,4—DIMETHOXYPHEN—
ETHYLAMINE



MESCALINE

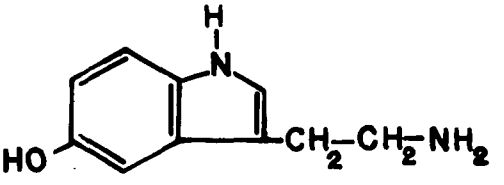


AMPHETAMINE

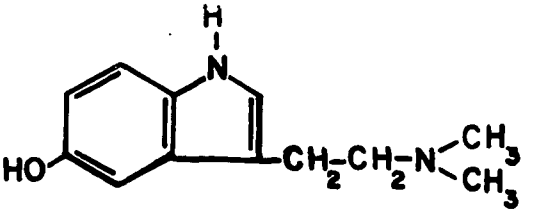
Fig.1

Fig.2 - Illustration of structural similarities between serotonin (5-HT), tryptamine and some centrally active indole-alkylamines.

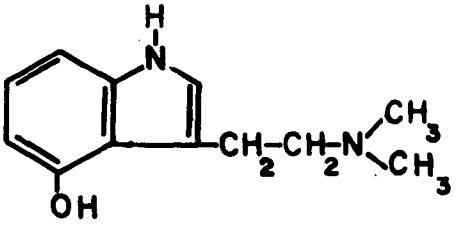
Structures of Serotonin and Related Indole-alkylamines



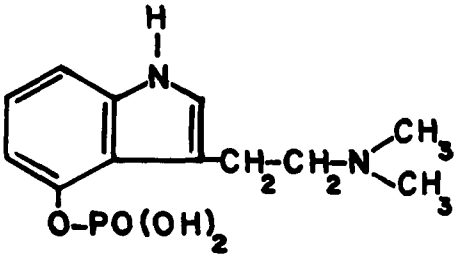
SEROTONIN



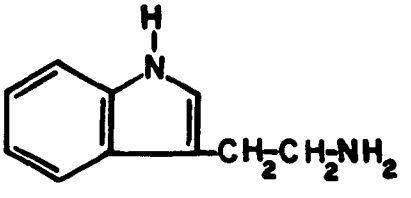
BUFOTENINE



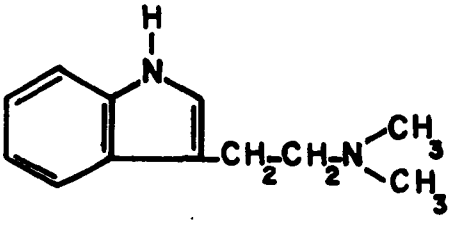
PSILOCIN



PSILOCYBIN



TRYPTAMINE



DIMETHYLTRYPTAMINE

Fig.2

METABOLISM OF MESCALINE

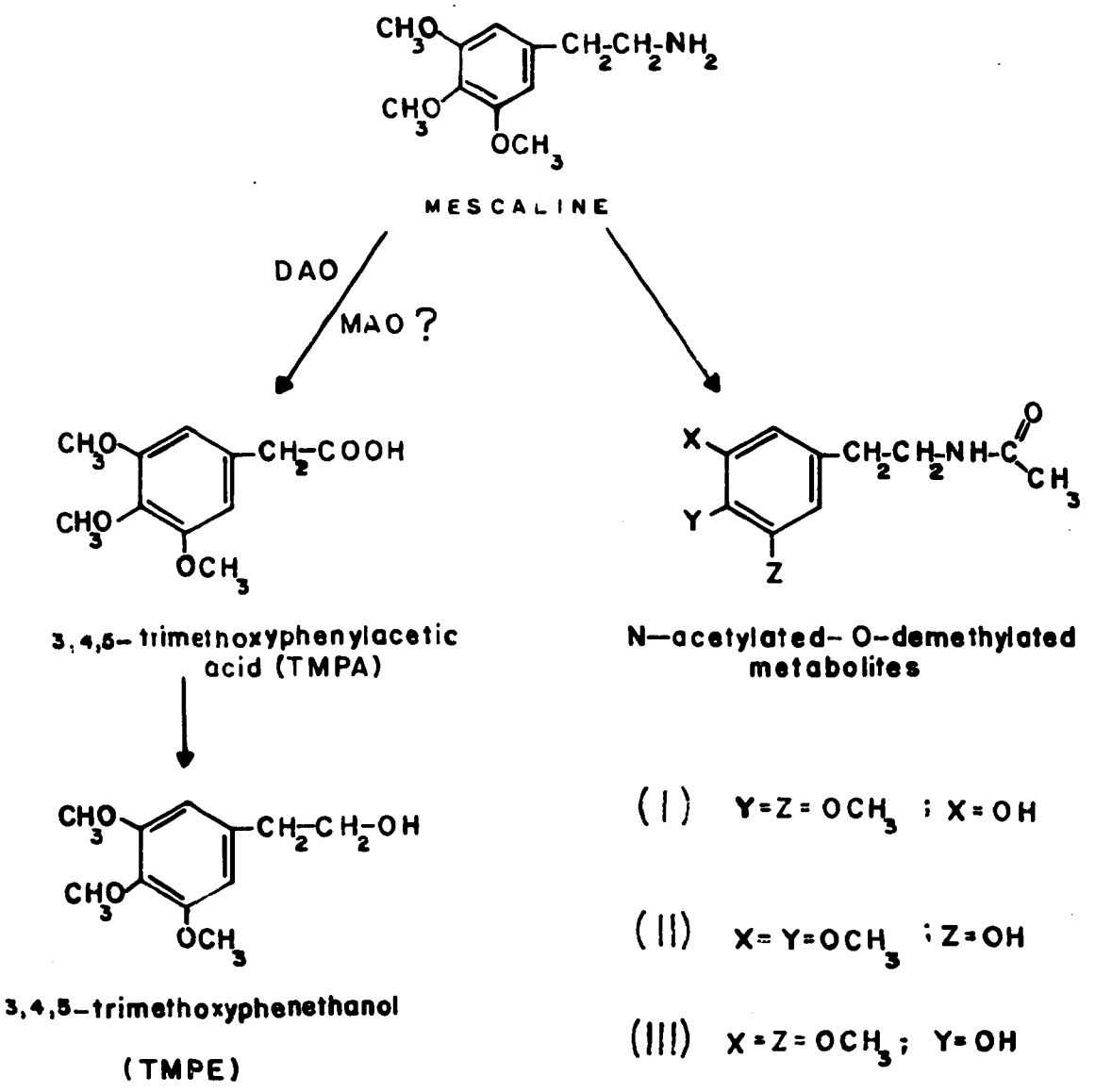
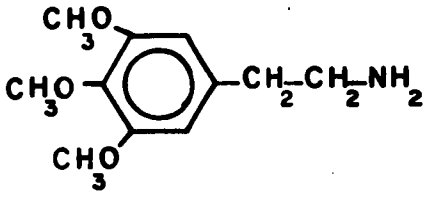
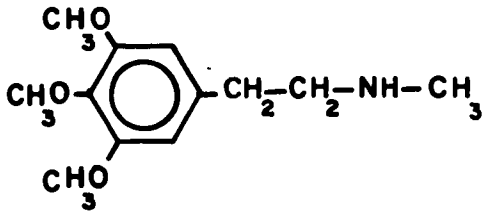


Fig.3 - Pathway for the metabolism of mescaline .

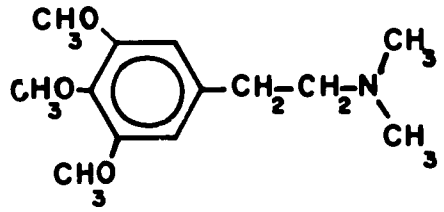
Mescaline and Some Analogous Compounds



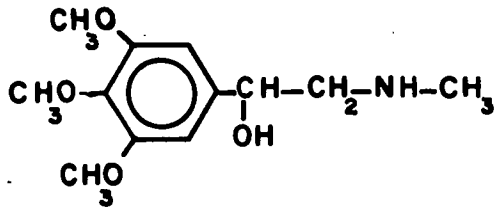
MESCALINE



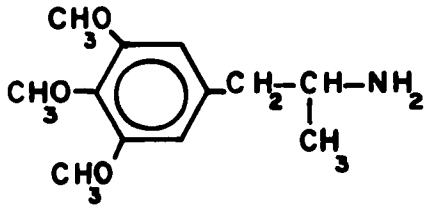
N-METHYLMESCALINE



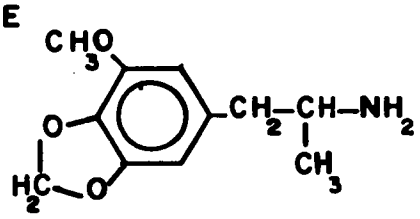
N,N-DIMETHYLMESCALINE



N-METHYL-β-HYDROXY-MESCALINE



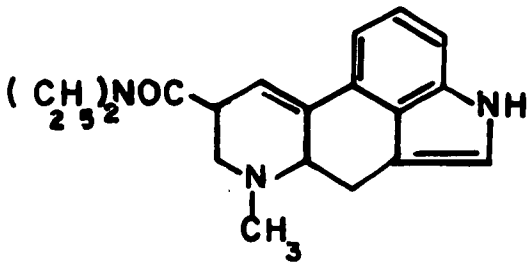
α-METHYLMESCALINE



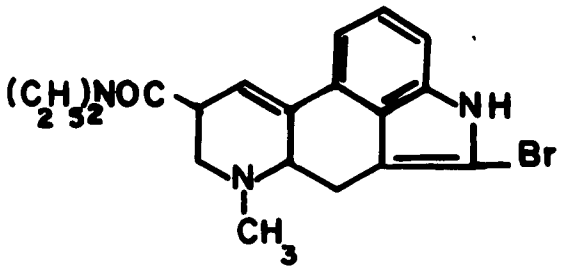
α-METHYL-3,4-METHYLENEDIOXY-5-METHOXYPHENETHYLAMINE

Fig.4 - Mescaline and some centrally active dericatives.

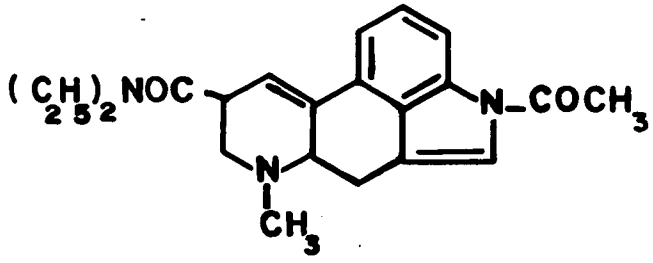
D-Lysergic acid diethylamide and Related Compounds



D-LYSERGIC ACID DIETHYLAMIDE (LSD-25)



BROM-LYSERGIDE (BOL-148)



1-ACETYL LSD

Fig.5 - Structures of LSD-25 and some derivatives.

Fig.6 - The areas taken for study are indicated on this sagittal section of the rat brain. I - Frontal Cortex. II - Caudate Nucleus. III - Mid-brain. IV - Cerebellum. V - Brainstem.

SAGITTAL SECTION OF THE RAT BRAIN

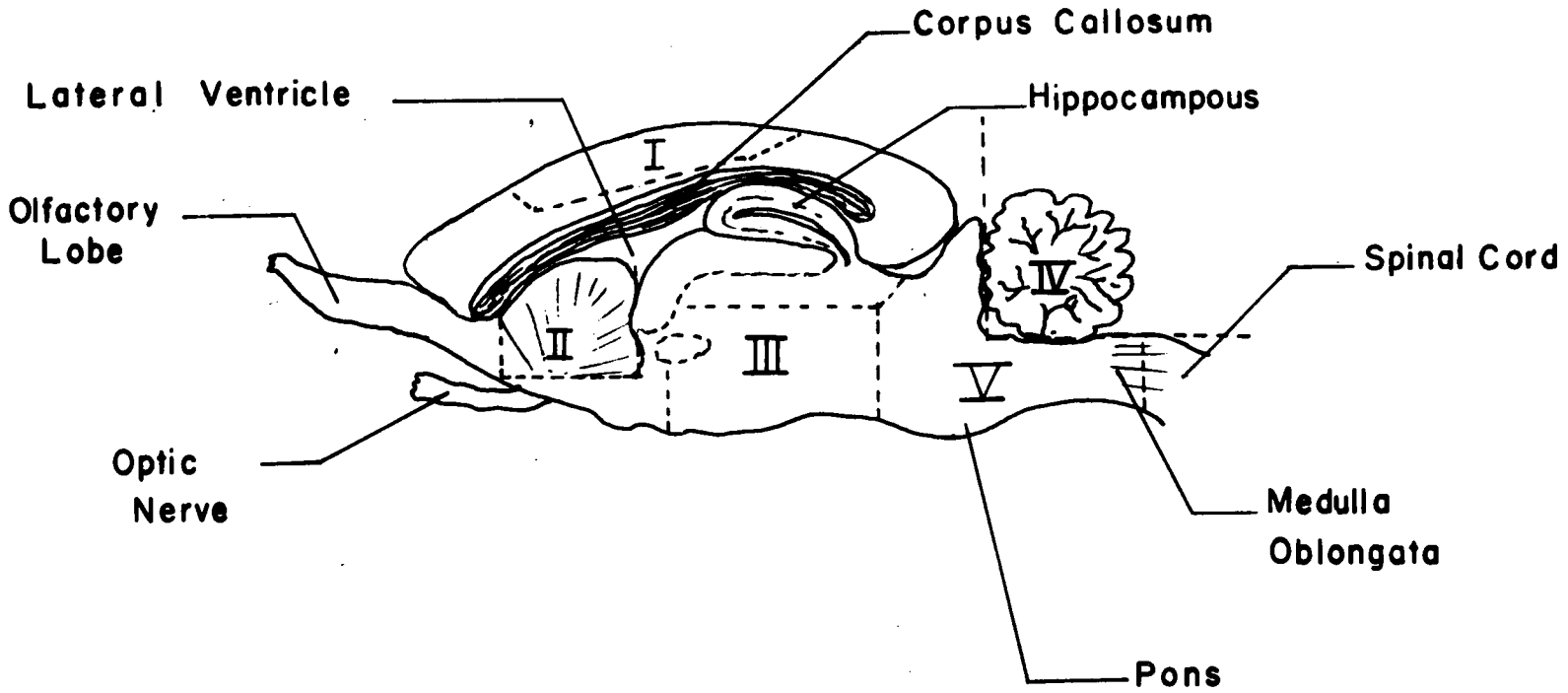


Fig. 6

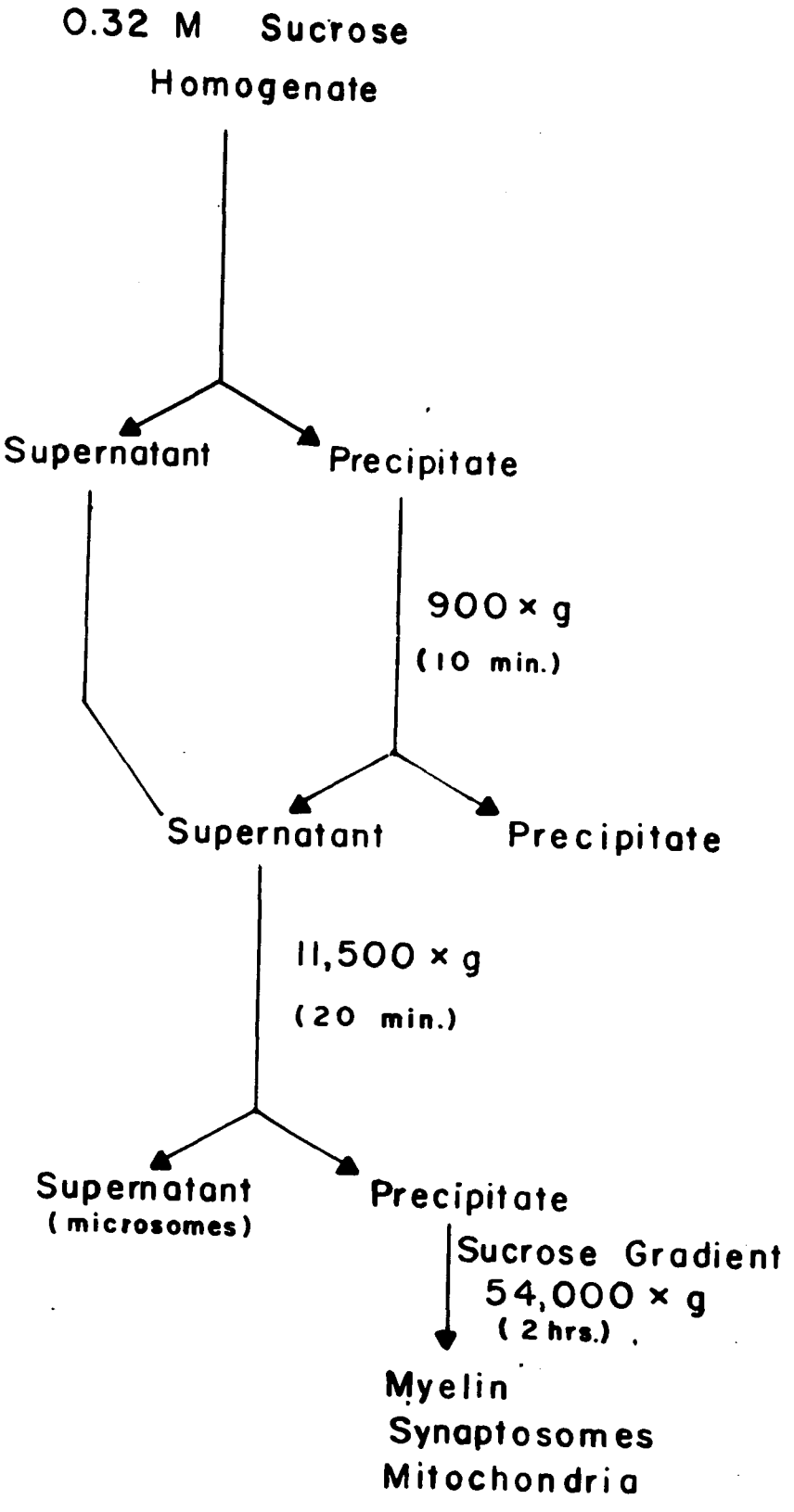


Fig.7 - Schematic representation of the methods used for the preparation of subcellular fractions.

Fig. 8 - Diagram of the subfractionation technique used to separate nerve endings and other components of the mitochondrial fraction obtained at 11,500 x g. Left: beginning of the subfractionation. Right: result of the subfractionation after centrifugation for 2 hours at 54,000 x g.

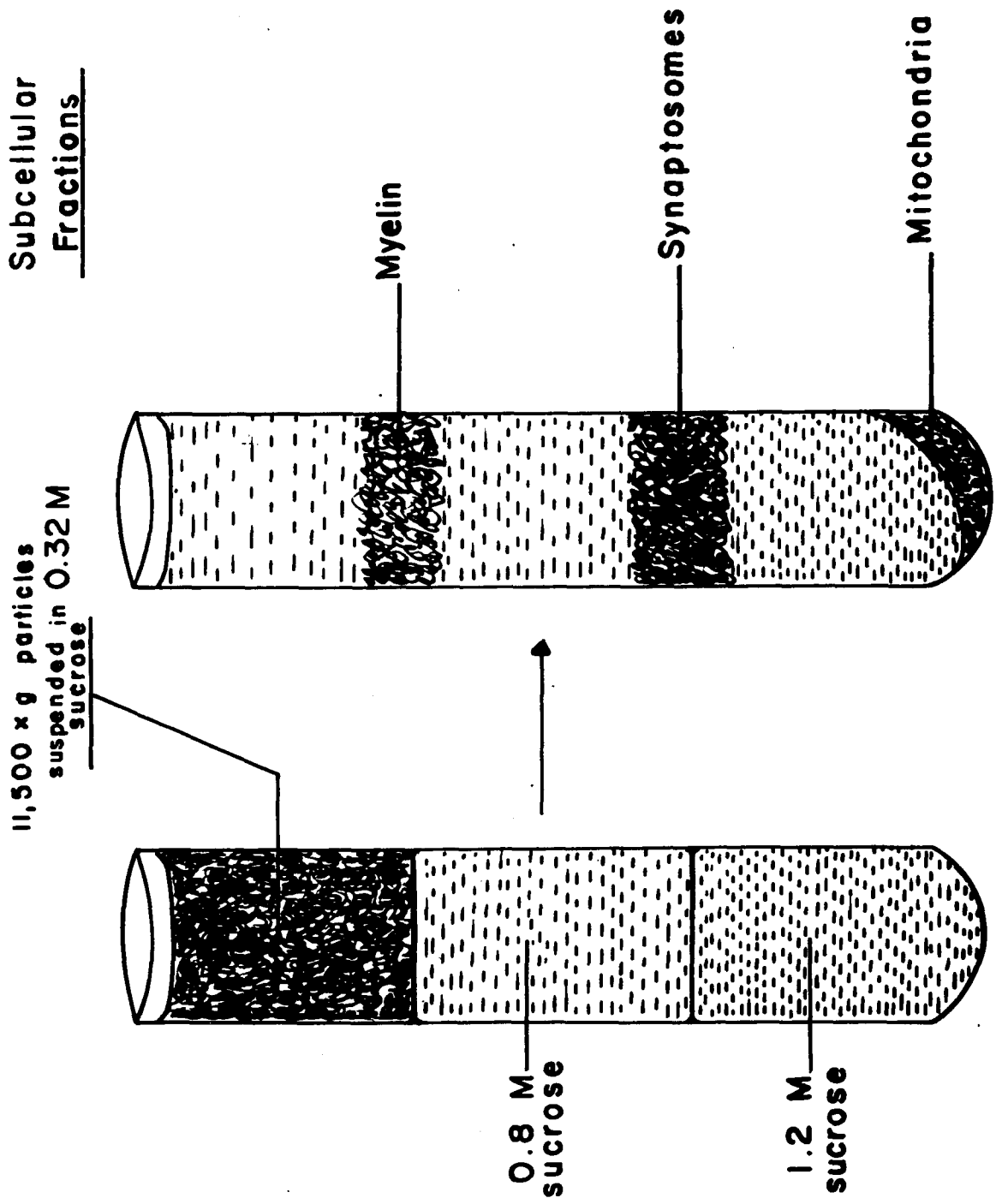

Subcellular Fractions
Myelin
Synaptosomes
Mitochondria
11,500 x g particles suspended in 0.32 M sucrose
0.8 M sucrose
1.2 M sucrose

Fig.8

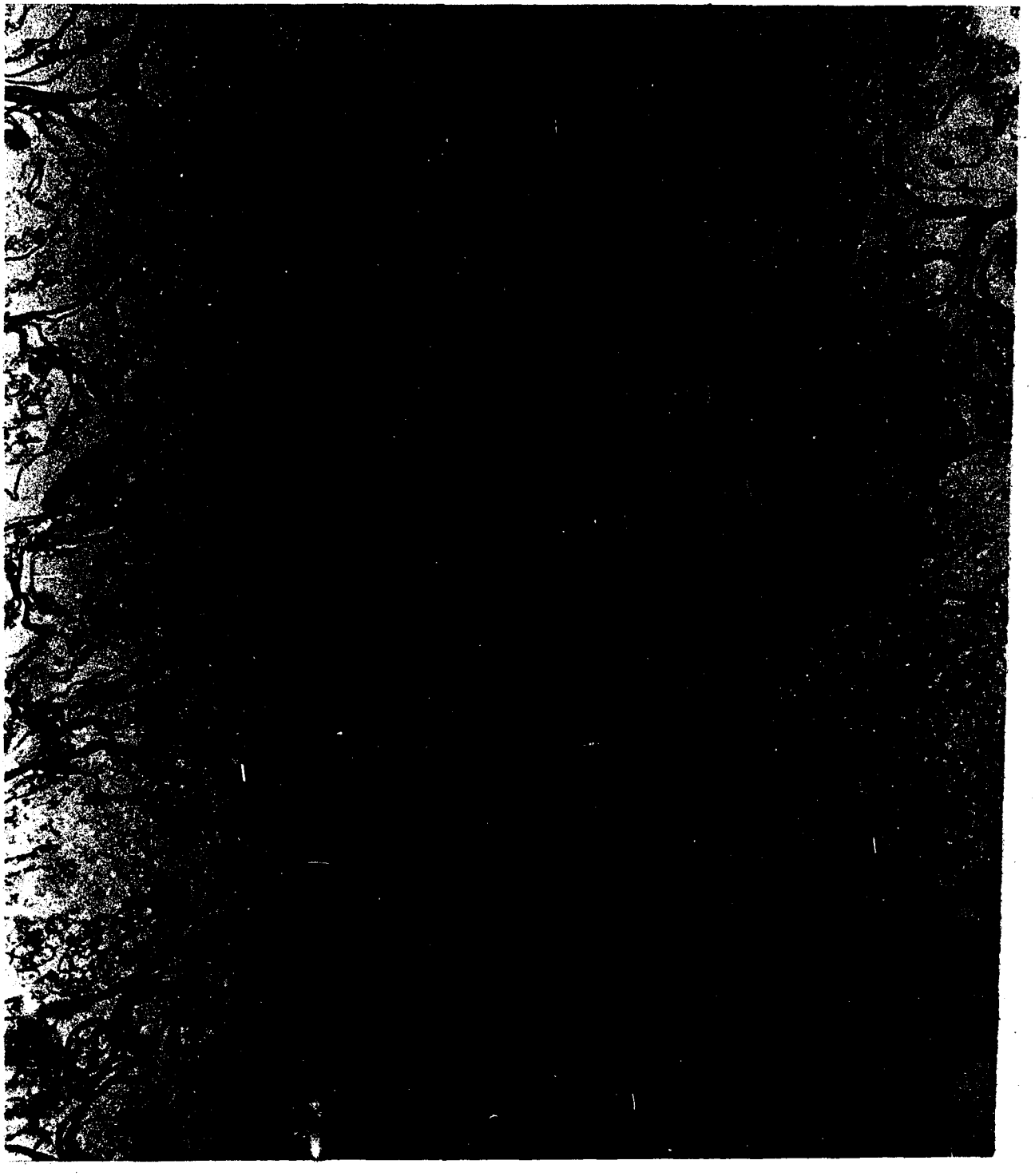


Plate 1 - Electron micrograph of the upper layer, showing exclusively myelin fragments (x 60,000).

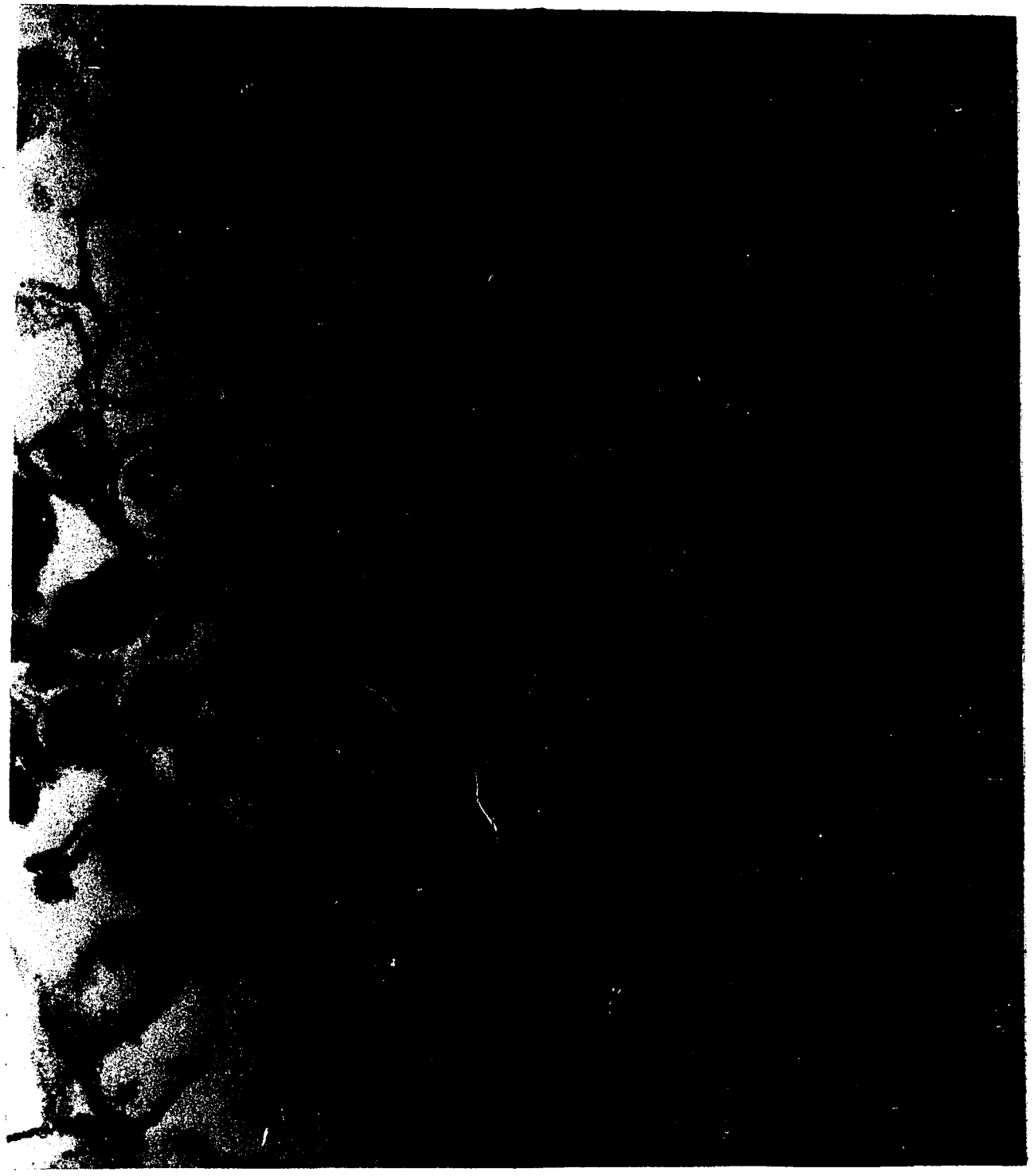


Plate 2 - Electron micrograph of the middle layer containing almost exclusively synaptosomes and their "ghosts" (x 60,000).

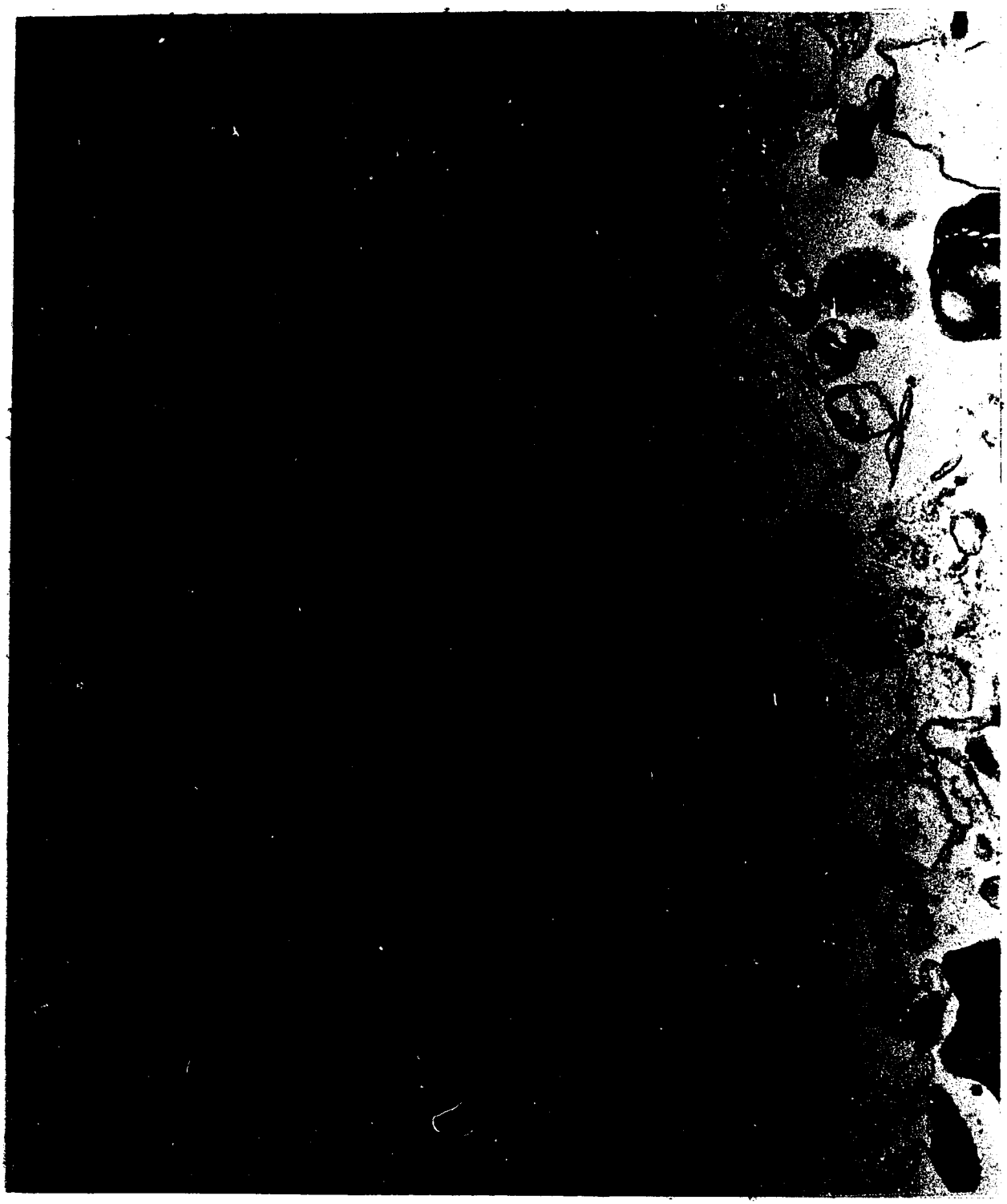


Plate 3 - Electron micrograph of the bottom layer containing free mitochondria with some contamination by synaptosomes (x 60,000).

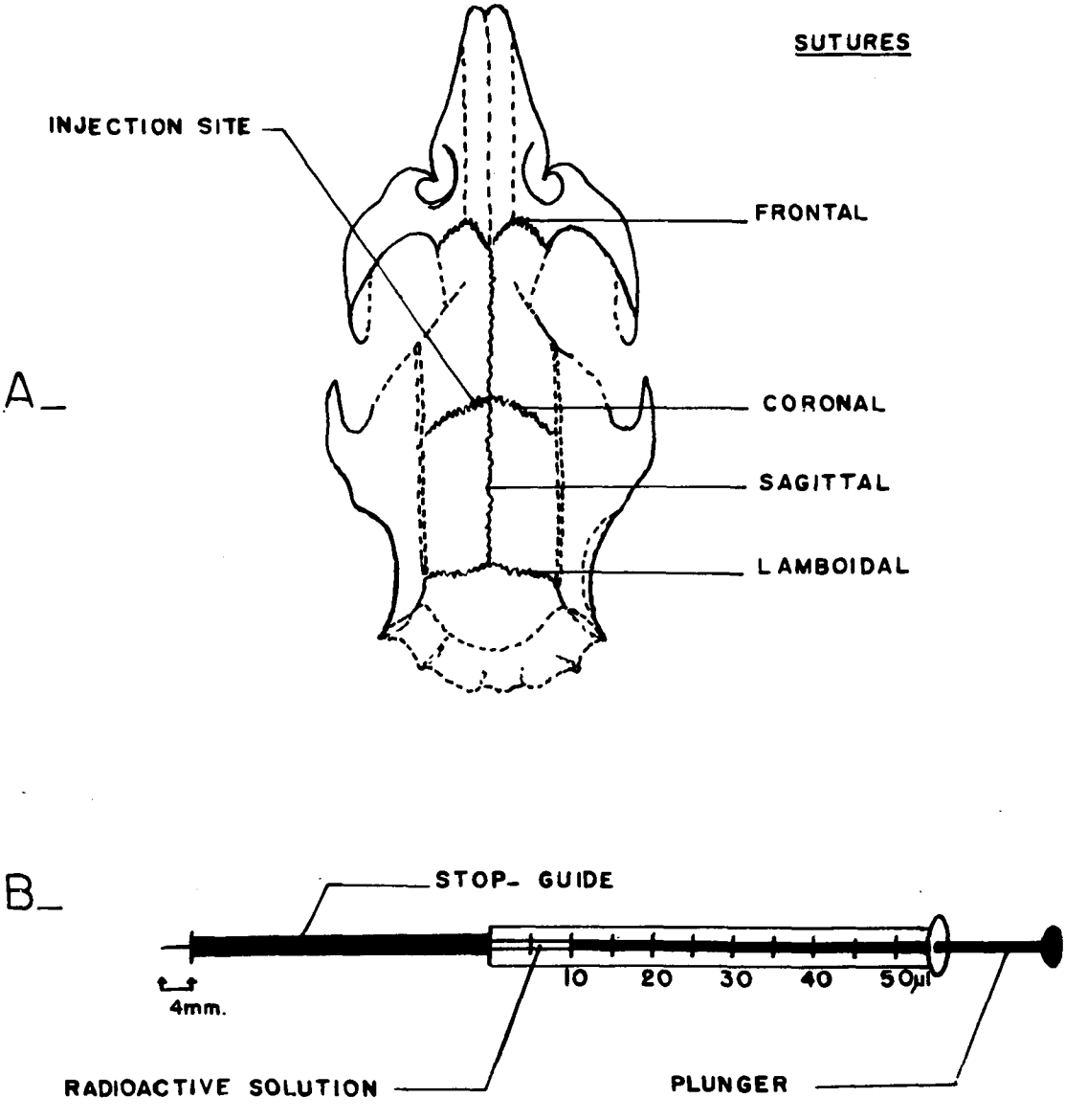


Fig.9 A- Rat skull indicating the suture lines and the injection site for intraventricular injections. B- Hamilton syringe used for cerebroventricular injections.

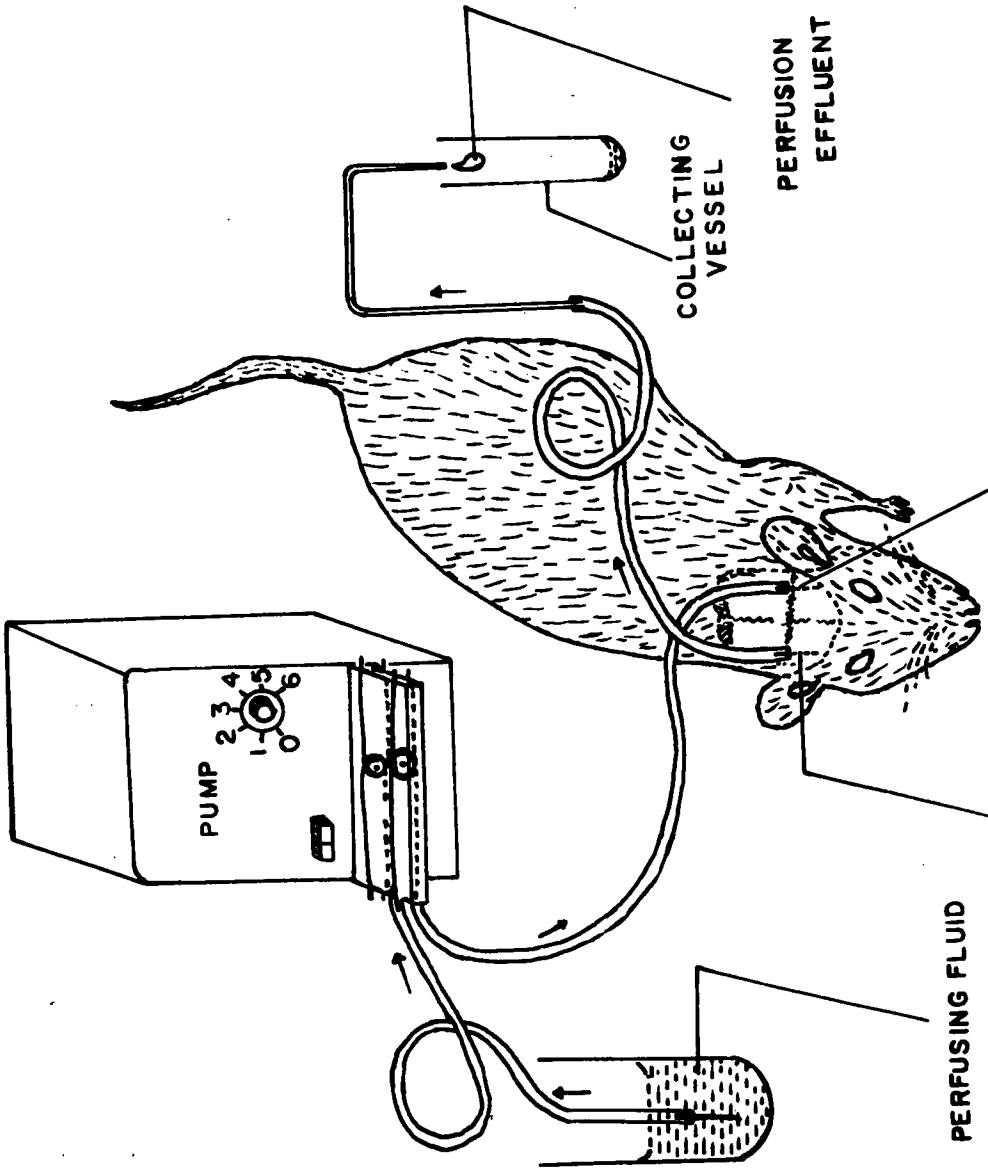


Fig.10 - Diagram showing position of the cannulae in the rat skull during cerebroventricular perfusion.

Fig.11 - Subcellular distribution of radioactivity in various areas of the rat brain after intravenous injection of ^{14}C-mescaline. Results are expressed in mean CPM per mg synaptosomal protein $\pm$ standard error of the mean.

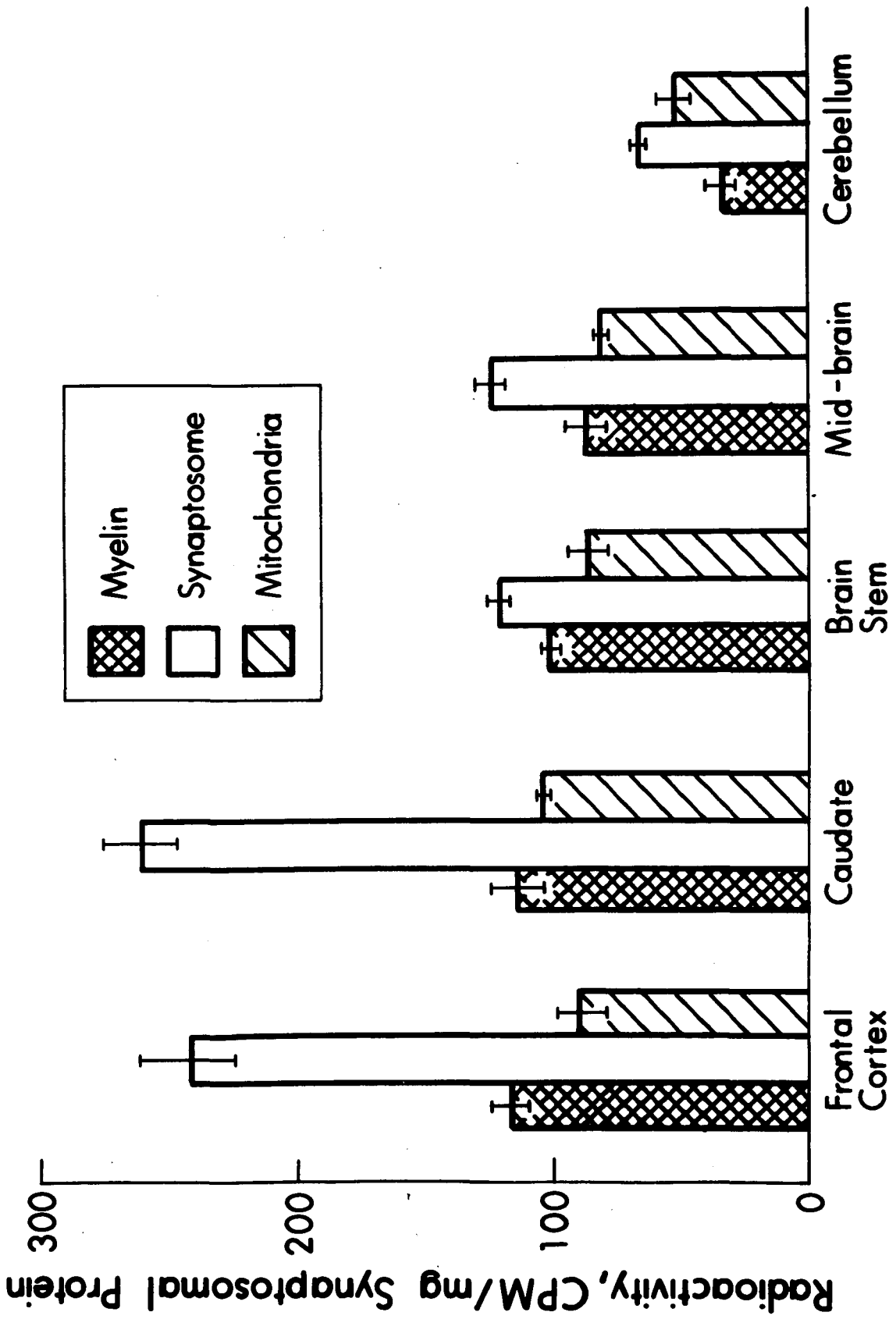

Radioactivity, CPM/mg Synaptosomal Protein
300
200
100
0
Myelin
Synaptosome
Mitochondria
Frontal Cortex
Caudate
Brain Stem
Mid-brain
Cerebellum

Fig.11

Fig.12 - Time course of the concentration of ^{3}H-dopamine and its metabolites in the perfusion effluent during a control experiment. Open bar represents the number of samples collected at 1 minute interval.

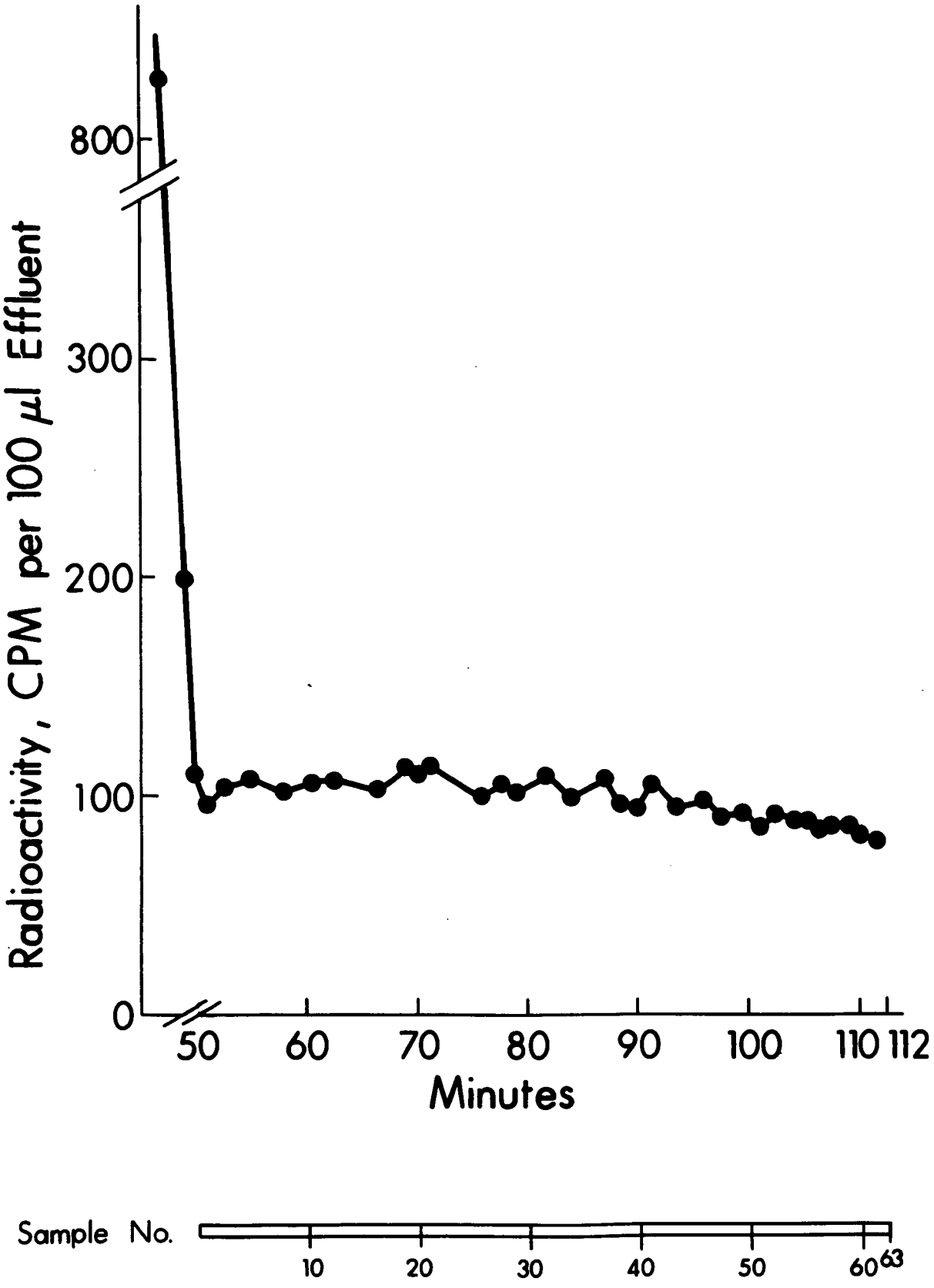

Radioactivity, CPM per 100 µl Effluent
800
300
200
100
0
50
60
70
80
90
100
110
112
Minutes
Sample No.
10
20
30
40
50
60
63

Fig.12

Fig. 13 - Effect of mescaline at low doses on the concentration of ^{3}H-dopamine and its metabolites in the perfusion effluent. The solid horizontal bars represent the time period during mescaline perfusion at the concentrations indicated. The data are for a representative experiment.

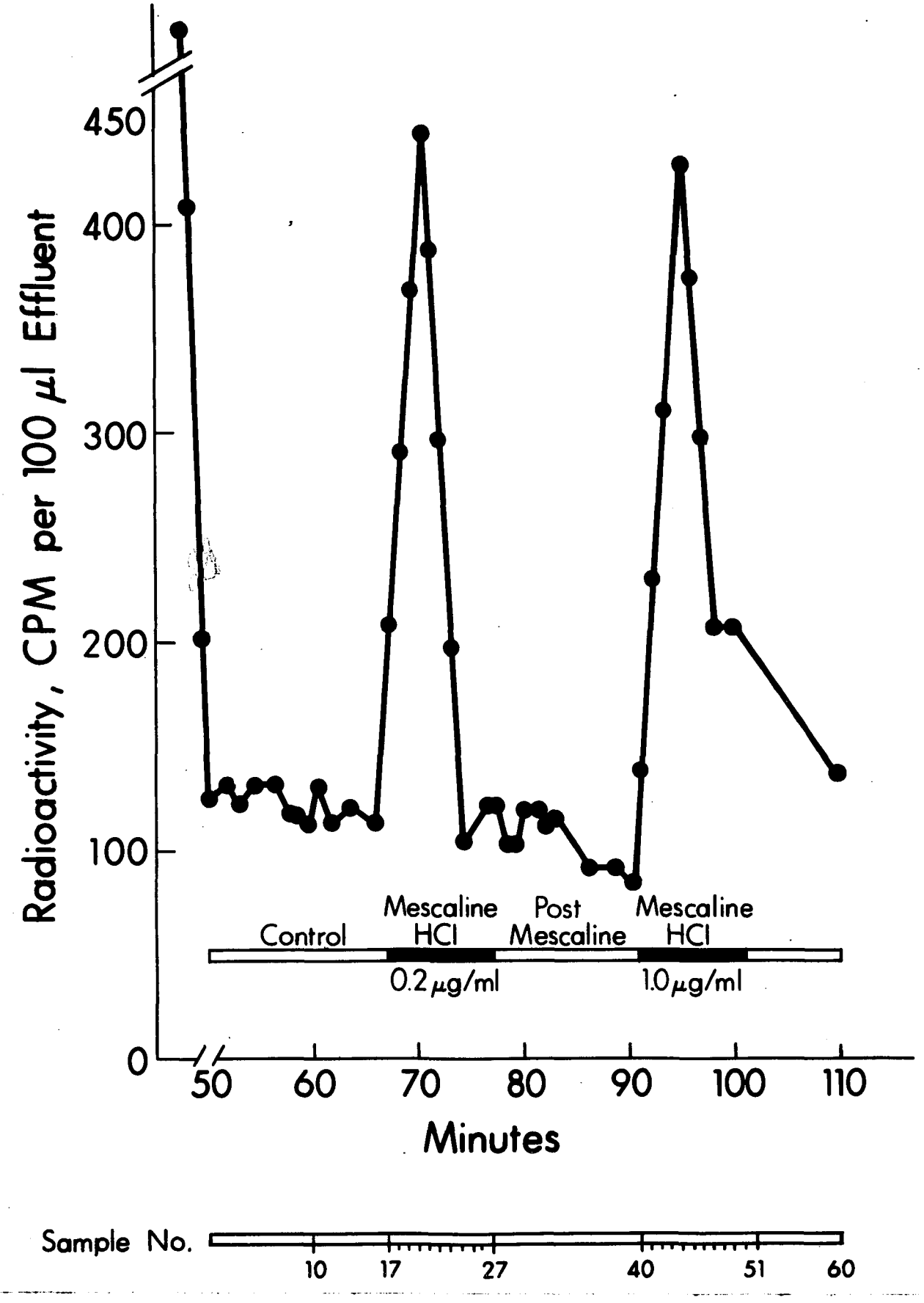

Radioactivity, CPM per 100 µl Effluent
450
400
300
200
100
0
50
60
70
80
90
100
110
Minutes
Control
Mescaline HCl
0.2 µg/ml
Post Mescaline
Mescaline HCl
1.0 µg/ml
Sample No.
10
17
27
40
51
60

Fig. 13

Fig. 14 - Effects of mescaline at low and high doses on the efflux of ^{3}H-dopamine and its metabolites from the rat cerebral ventricle. Solid horizontal bars represent the time period during mescaline perfusion, at the concentrations indicated.

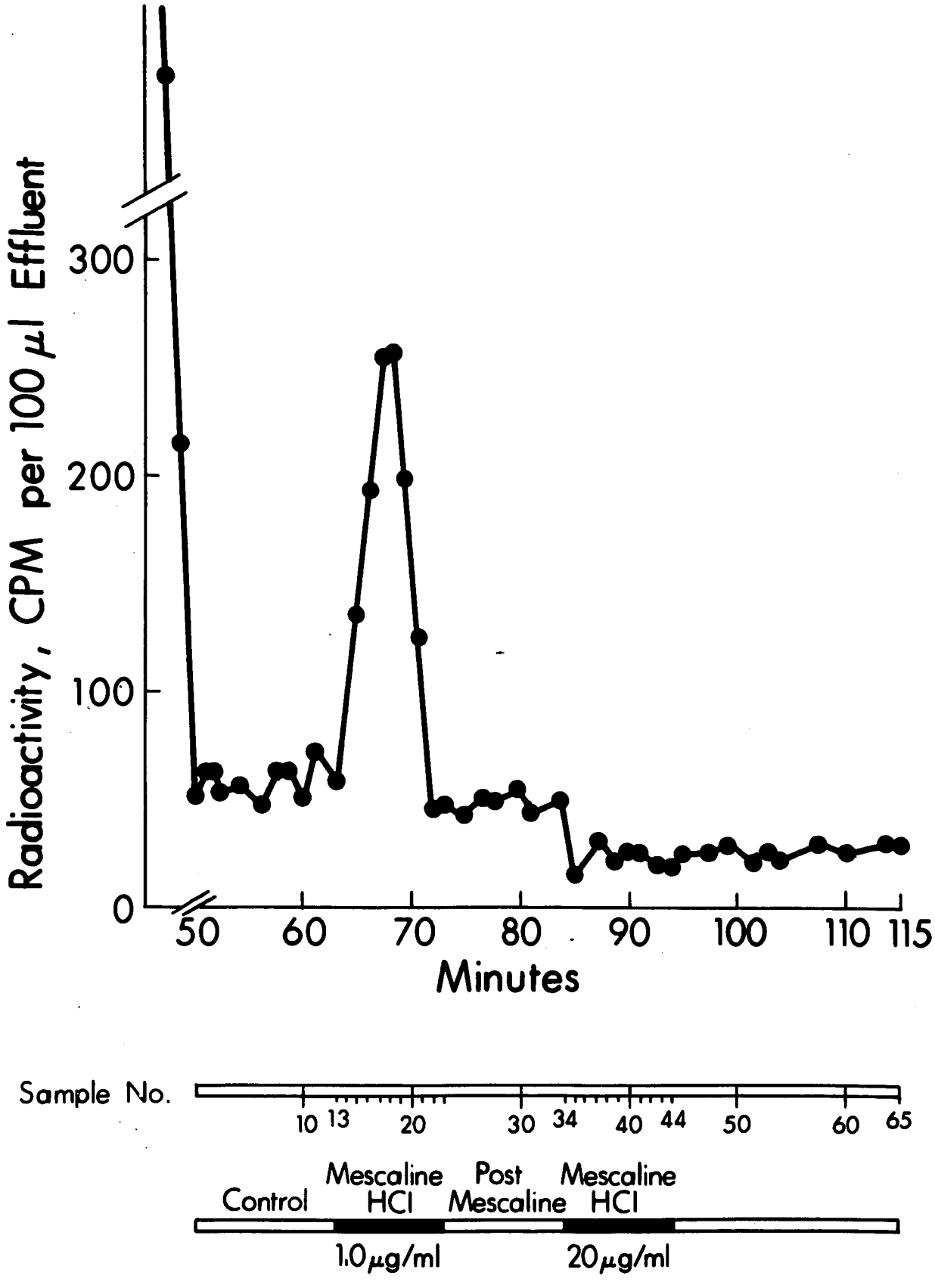

Radioactivity, CPM per 100 µl Effluent
300
200
100
0
50
60
70
80
90
100
110
115
Minutes
Sample No.
10
13
20
30
34
40
44
50
60
65
Control
Mescaline HCl
Post Mescaline
Mescaline HCl
1.0µg/ml
20µg/ml

Fig.14

Fig.15 - The effects of mescaline at high dose followed by low dose on the efflux of ^{3}H-dopamine and its metabolites from the rat cerebral ventricle. The time period during mescaline perfusion is indicated by the solid horizontal bars.

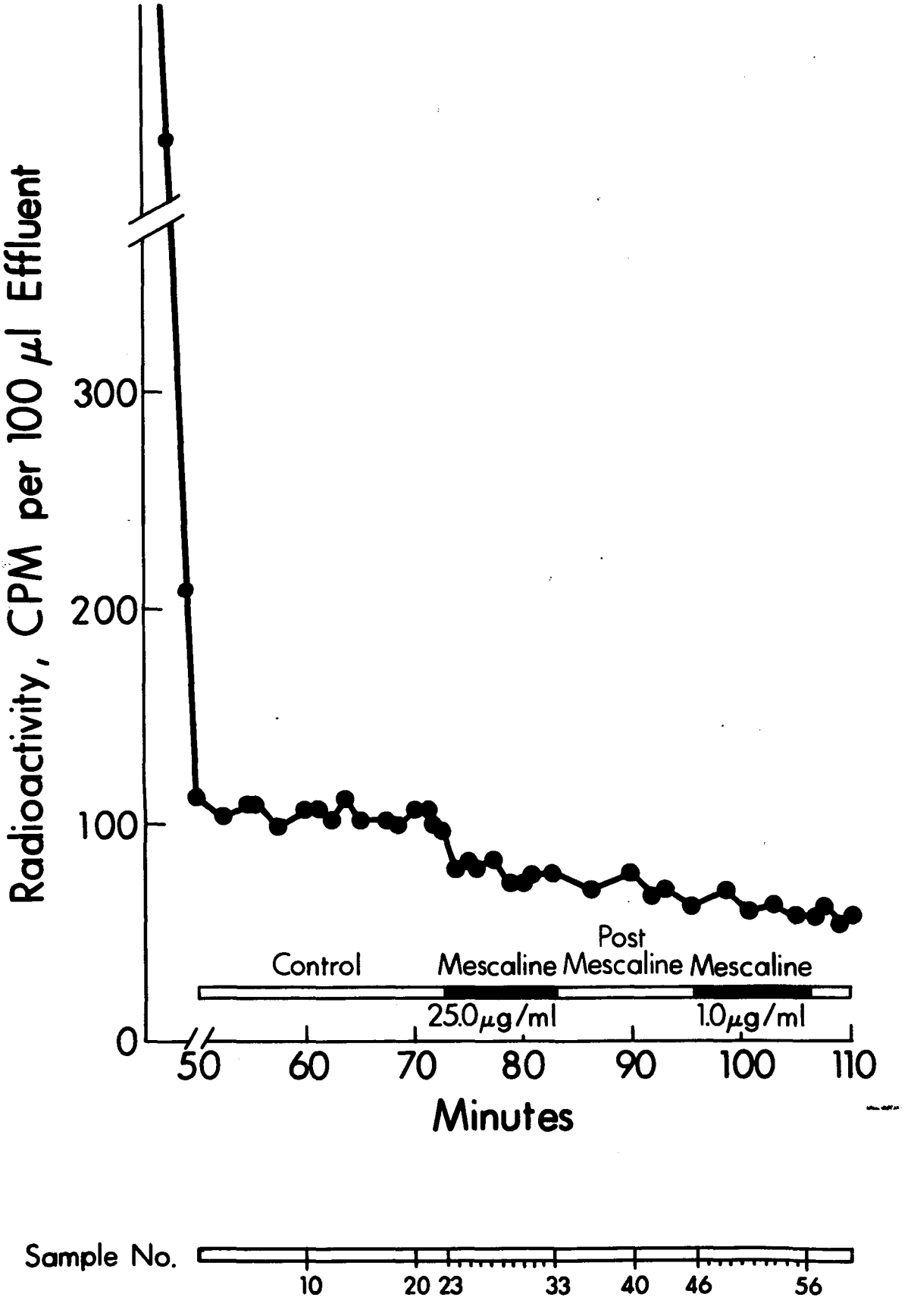

Radioactivity, CPM per 100 µl Effluent
300
200
100
0
50
60
70
80
90
100
110
Minutes
Control
Mescaline
Post Mescaline
Mescaline
25.0µg/ml
1.0µg/ml
Sample No.
10
20 23
33
40
46
56

Fig. 15

Fig. 16 - Effect of mescaline at various concentrations on the efflux of Urea-^{14}C during perfusion of the rat lateral ventricle. Mescaline was perfused during the time period indicated by the solid horizontal bars.

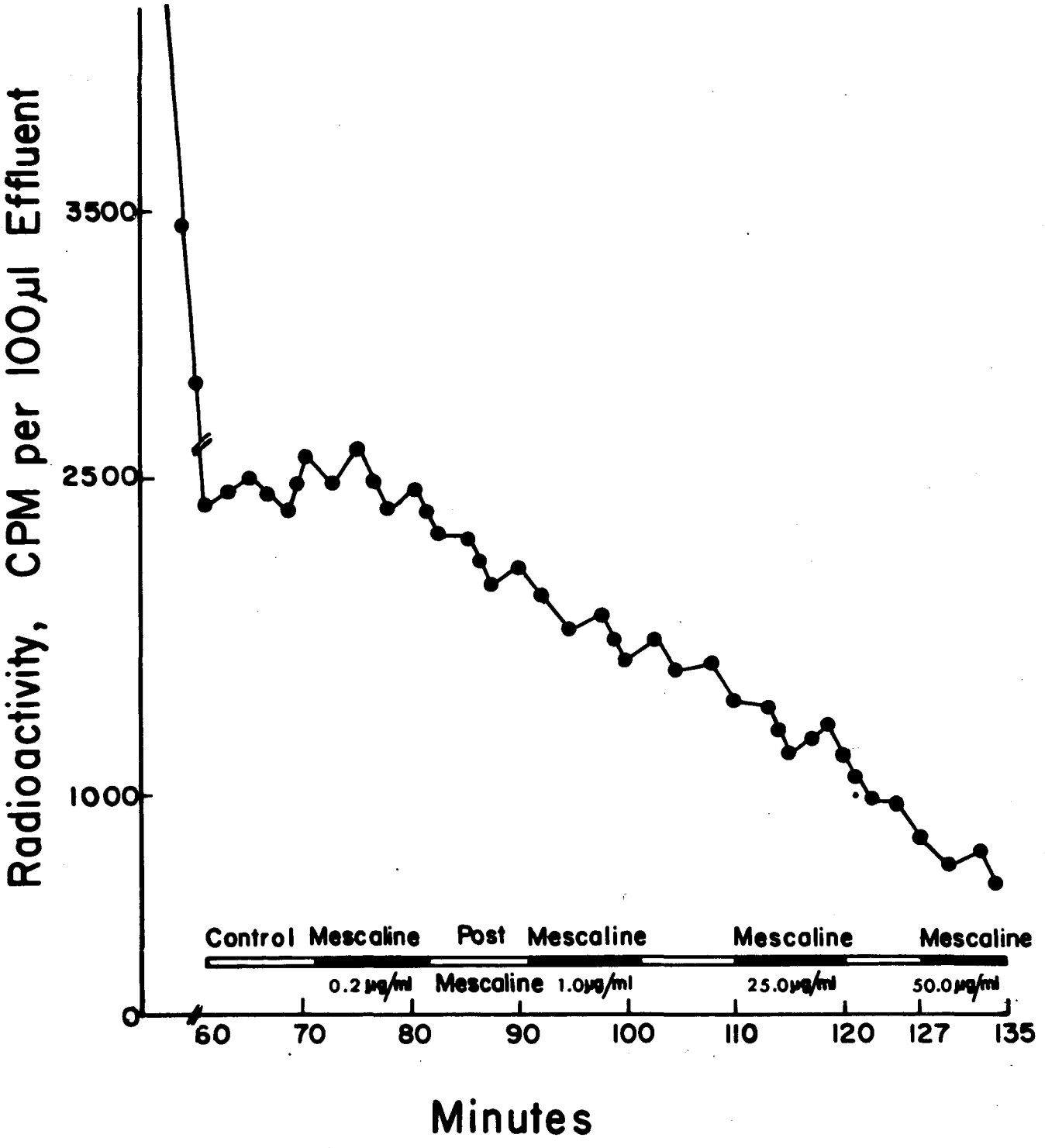

Radioactivity, CPM per 100µl Effluent
3500
2500
1000
0
Control
Mescaline
Post Mescaline
Mescaline
Mescaline
0.2 µg/ml Mescaline 1.0µg/ml
25.0µg/ml
50.0µg/ml
60
70
80
90
100
110
120
127
135
Minutes

Fig. 16

APPENDIX I

Table 9A

Rat # 1	Time of Collection	Treatment	CPM per 100 µl Effluent
"	50th Min.	Artificial CSF	239
"	51st "	"	221
"	52nd "	"	223
"	53rd "	"	179
"	54th "	"	154
"	55th "	"	162
"	56th "	"	137
"	57th "	"	130
"	58th "	"	122
"	59th "	"	130
"	60th "	"	123
"	61st "	"	128
"	62nd "	"	125
"	63rd "	"	142
"	64th "	"	128
"	65th "	"	121
"	66th "	"	104
"	67th "	"	106
"	68th "	"	109
"	69th "	"	116
"	70th "	"	119
"	71st "	"	121
"	72nd "	"	113
"	73rd "	"	106
"	74th "	"	100
"	75th "	"	102
"	76th "	"	105
"	77th "	"	100
"	78th "	"	95
"	79th "	"	98
"	80th "	"	96
"	81st "	"	94
"	82nd "	"	90
"	83rd "	"	93
"	84th "	"	101
"	85th "	"	91
"	86th "	"	90
"	87th "	"	89
"	88th "	"	87
"	89th "	"	85
"	90th "	"	91

Table 9A (continued)

Rat #2	Time of Collection	Treatment	CPM per 100 µl Effluent
"	11th Min.	Artificial CSF	626
"	12th "	"	620
"	13th "	"	596
"	14th "	"	561
"	15th "	"	529
"	16th "	"	513
"	17th "	"	488
"	18th "	"	487
"	19th "	"	483
"	20th "	"	456
"	21st "	"	460
"	22nd "	"	486
"	23rd "	"	426
"	24th "	"	416
"	25th "	"	413
"	26th "	"	391
"	27th "	"	386
"	28th "	"	367
"	29th "	"	368
"	30th "	"	370
"	31st "	"	346
"	32nd "	"	291
"	33rd "	"	292
"	34th "	"	250
"	35th "	"	232
"	36th "	"	216
"	37th "	"	226
"	38th "	"	230
"	39th "	"	234
"	40th "	"	260
"	41st "	"	238
"	42nd "	"	288
"	43rd "	"	293
"	44th "	"	230
"	45th "	"	210
"	46th "	"	205
"	47th "	"	200
"	48th "	"	198
"	49th "	"	170
"	50th "	"	128
"	51st "	"	133

Table 9A (continued)

Rat #2	Time of Collection	Treatment	CPM per 100 µl Effluent
"	52nd Min.	Artificial CSF	130
"	53rd "	"	128
"	54th "	"	130
"	55th "	"	129
"	56th "	"	128
"	57th "	"	122
"	58th "	"	131
"	59th "	"	115
"	60th "	"	117
"	61st "	"	117
"	62nd "	"	119
"	63rd "	Mescaline (0.2 µg/ml)	178
"	64th "	"	240
"	65th "	"	378
"	66th "	"	432
"	67th "	"	321
"	68th "	"	330
"	69th "	"	210
"	70th "	"	138
"	71st "	"	124
"	72nd "	"	113
"	73rd "	"	114
"	74th "	Artificial CSF	116
"	75th "	"	115
"	76th "	"	113
"	77th "	"	116
"	78th "	"	114
"	79th "	"	117
"	80th "	"	116
"	81st "	"	119
"	82nd "	"	112
"	83rd "	"	109
"	84th "	"	105
"	85th "	"	113
"	86th "	"	100
"	87th "	"	103
"	88th "	"	105
"	89th "	"	109
"	90th "	"	98
"	91st "	"	103
"	92nd "	"	97
"	93rd "	"	98

Table 9A (continued)

Rat #2	Time of Collection	Treatment	CPM per 100 µl Effluent
"	94th Min.	Artificial CSF	96
"	95th "	"	98
"	96th "	"	94
"	97th "	"	90
"	98th "	"	88
"	99th "	"	85
"	100th "	"	89
"	101st "	"	85
Rat #3	45th Min.	Artificial CSF	213
"	46th "	"	226
"	47th "	"	197
"	48th "	"	192
"	49th "	"	215
"	50th "	"	206
"	51st "	"	219
"	52nd "	"	192
"	53rd "	"	200
"	54th "	"	190
"	55th "	"	186
"	56th "	"	180
"	57th "	"	177
"	58th "	"	160
"	59th "	"	179
"	60th "	"	171
"	61st "	"	160
"	62nd "	Mescaline (20 µg/ml)	115
"	63rd "	"	75
"	64th "	"	59
"	65th "	"	53
"	66th "	"	52
"	67th "	"	45
"	68th "	"	39
"	69th "	"	40
"	70th "	"	39
"	71st "	"	36
"	72nd "	"	41
"	73rd "	Artificial CSF	41
"	74th "	"	41
"	75th "	"	40
"	76th "	"	44

Table 9A (continued)

Rat #3	Time of Collection	Treatment	CPM per 100 µl Effluent
"	77th Min.	Artificial CSF	58
"	78th "	"	63
"	79th "	"	58
"	80th "	"	64
"	81st "	"	57
Rat #4	50th Min.	Artificial CSF	97
"	51st "	"	94
"	52nd "	"	100
"	53rd "	"	97
"	54th "	"	98
"	55th "	"	89
"	56th "	"	98
"	57th "	"	82
"	58th "	"	90
"	59th "	"	80
"	60th "	"	75
"	61st "	Mescaline (0.2 µg/ml)	89
"	62nd "	"	138
"	63rd "	"	145
"	64th "	"	165
"	65th "	"	135
"	66th "	"	100
"	67th "	"	93
"	68th "	"	85
"	69th "	"	80
"	70th "	"	75
"	71st "	"	70
"	72nd "	"	75
"	73rd "	"	80
"	74th "	"	65
"	75th "	"	72
"	76th "	"	58
"	77th "	"	65
"	78th "	Artificial CSF	70
"	79th "	"	72
"	80th "	"	75
"	81st "	"	64
"	82nd "	"	65
"	83rd "	"	72
"	84th "	"	60

Table 9A (continued)

Rat #4	Time of Collection	Treatment	CPM per 100 µl Effluent
"	85th Min.	Artificial CSF	65
"	86th "	"	75
"	87th "	"	70
"	88th "	"	68
"	89th "	"	65
"	90th "	"	61
"	91st "	"	65
"	92nd "	"	70
"	93rd "	"	65
"	94th "	"	58
"	95th "	"	50
"	96th "	"	55
"	97th "	"	58
"	98th "	"	55
"	99th "	"	59
"	100th "	"	53
"	101st "	"	59
"	102nd "	Mescaline(0.2µg/ml)	70
"	103rd "	"	82
"	104th "	"	90
"	105th "	"	102
"	106th "	"	85
"	107th "	"	75
"	108th "	"	65
"	109th "	"	70
"	110th "	"	67
"	111th "	"	68
"	112th "	"	60
Rat #5	50th Min.	Artificial CSF	125
"	51st "	"	130
"	52nd "	"	125
"	53rd "	"	130
"	54th "	"	125
"	55th "	"	115
"	56th "	"	117
"	57th "	"	120
"	58th "	"	118
"	59th "	"	117
"	60th "	"	122

Table 9A (continued)

Rat #5	Time of Collection	Treatment	CPM per 100 µl Effluent
"	61st Min.	Artificial CSF	125
"	62nd "	"	114
"	63rd "	"	120
"	64th "	"	121
"	65th "	"	115
"	66th "	Mescaline (0.2 µg/ml)	117
"	67th "	"	210
"	68th "	"	292
"	69th "	"	370
"	70th "	"	445
"	71st "	"	385
"	72nd "	"	295
"	73rd "	"	195
"	74th "	"	105
"	75th "	"	110
"	76th "	Artificial CSF	120
"	77th "	"	120
"	78th "	"	115
"	79th "	"	105
"	80th "	"	105
"	81st "	"	120
"	82nd "	"	120
"	83rd "	"	115
"	84th "	"	117
"	85th "	"	119
"	86th "	"	119
"	87th "	"	105
"	88th "	"	98
"	89th "	"	92
"	90th "	"	93
"	91st "	Mescaline (1.0.µg/ml)	137
"	92nd "	"	230
"	93rd "	"	310
"	94th "	"	430
"	95th "	"	375
"	96th "	"	300
"	97th "	"	210
"	98th "	"	210
"	99th "	"	195
"	100th "	"	185
"	101st "	"	175
"	102nd "	Artificial CSF	165
"	103rd "	"	160

Table 9A (continued)

Rat #6	Time of Collection	Treatment	CPM per 100 µl Effluent
"	50th Min.	Artificial CSF	104
"	51st "	"	110
"	52nd "	"	124
"	53rd "	"	124
"	54th "	"	113
"	55th "	"	101
"	56th "	"	120
"	57th "	"	124
"	58th "	"	100
"	59th "	"	109
"	60th "	"	114
"	61st "	"	100
"	62nd "	"	125
"	63rd "	"	120
"	64th "	Mescaline (1.0 µg/ml)	165
"	65th "	"	260
"	66th "	"	380
"	67th "	"	450
"	68th "	"	360
"	69th "	"	250
"	70th "	"	160
"	71st "	"	135
"	72nd "	"	100
"	73rd "	"	101
"	74th "	Artificial CSF	105
"	75th "	"	115
"	76th "	"	120
"	77th "	"	118
"	78th "	"	115
"	79th "	"	114
"	80th "	"	109
"	81st "	"	100
"	82nd "	"	102
"	83rd "	"	105
"	84th "	Mescaline (50 µg/ml)	33
"	85th "	"	45
"	86th "	"	60
"	87th "	"	50
"	88th "	"	48
"	89th "	"	50
"	90th "	"	45

Table 9A (continued)

Rat #6	Time of Collection	Treatment	CPM per 100 µl Effluent
		Mescaline (50 µg/ml)	
"	91st Min.	"	49
"	92nd "	"	40
"	93rd "	"	47
"	94th "	Artificial CSF	54
"	95th "	"	49
"	96th "	"	58
"	97th "	"	60
Rat #7	70th Min.	Artificial CSF	98
"	71st "	"	102
"	72nd "	"	102
"	73rd "	Mescaline (1.0 µg/ml)	97
"	74th "	"	112
"	75th "	"	147
"	76th "	"	162
"	77th "	"	120
"	78th "	"	105
"	79th "	"	108
"	80th "	Artificial CSF	96
"	81st "	"	112
"	82nd "	"	90
"	83rd "	"	99
"	84th "	"	112
"	85th "	"	95
"	86th "	"	88
"	87th "	"	85
"	88th "	"	80
"	89th "	"	75
"	90th "	"	77
"	91st "	"	75
"	92nd "	"	79
Rat #8	70th Min.	Artificial CSF	99
"	71st "	"	105
"	72nd "	"	80
"	73rd "	"	83
"	74th "	"	81
"	75th "	"	75
"	76th "	"	82

Table 9A (continued)

Rat #8	Time of Collection	Treatment	CPM per 100 µl Effluent
		Mescaline (0.2 µg/ml)	
"	77th Min.	"	85
"	78th "	"	171
"	79th "	"	234
"	80th "	"	160
"	81st "	"	127
"	82nd "	"	98
"	83rd "	"	85
"	84th "	"	75
"	85th "	"	82
"	86th "	"	94
"	87th "	Artificial CSF	87
"	88th "	"	81
"	89th "	"	83
"	90th "	"	85
"	91st "	"	79
"	92nd "	"	77
"	93rd "	"	76
"	94th "	"	85
"	95th "	"	75
"	96th "	"	74
"	97th "	"	72
"	98th "	"	71
"	99th "	Mescaline (10.0 µg/ml)	30
"	100th "	"	25
"	101st "	"	20
"	102nd "	"	19
"	103rd "	"	18
"	104th "	"	15
"	105th "	"	16
"	106th "	"	17
"	107th "	"	19
Rat #9	50th Min.	Artificial CSF	206
"	51st "	"	205
"	52nd "	"	192
"	53rd "	"	200
"	54th "	"	186
"	55th "	"	207
"	56th "	"	190
"	57th "	"	179
"	58th "	"	171
"	59th "	"	175

Table 9A (continued)

Rat #9	Time of Collection	Treatment	CPM per 100 µl Effluent
"	60th Min.	Artificial CSF	170
"	61st "	"	171
"	62nd "	"	172
"	63rd "	"	167
"	64th "	Mescaline (25 µg/ml)	59
"	65th "	"	53
"	66th "	"	52
"	67th "	"	49
"	68th "	"	45
"	69th "	"	39
"	70th "	"	40
"	71st "	"	41
"	72nd "	"	42
"	73rd "	"	38
"	74th "	"	41
"	75th "	"	45
"	76th "	Artificial CSF	52
"	77th "	"	55
"	78th "	"	65
"	79th "	"	78
"	80th "	"	81
"	81st "	"	90
"	82nd "	"	94
"	83rd "	"	99
"	84th "	"	95
"	85th "	"	91
"	86th "	"	100
"	87th "	"	90
"	88th "	Mescaline (1.0 µg/ml)	93
"	89th "	"	85
"	90th "	"	92
"	91st "	"	89
"	92nd "	"	86
"	93rd "	"	95
"	94th "	"	99
"	95th "	"	86
"	96th "	"	75
"	97th "	"	79
"	98th "	"	83
"	99th "	Artificial CSF	85

Table 9A (continued)

Rat #9	Time of Collection	Treatment	CPM per 100 µl Effluent
"	100th Min.	Artificial CSF	78
"	101st "	"	79
"	102nd "	"	69
"	103rd "	"	70
"	104th "	"	73
"			

Table 9B

Rat #1	Time of Collection	Treatment	CPM per 100 µl Effluent
"	50th Min.	Artificial CSF	295
"	51st "	"	248
"	52nd "	"	220
"	53rd "	"	162
"	54th "	"	190
"	55th "	"	170
"	56th "	Amphetamine (0.2 µg/ml)	163
"	57th "	"	156
"	58th "	"	162
"	59th "	"	161
"	60th "	"	161
"	61st "	"	145
"	62nd "	"	150
"	63rd "	"	152
"	64th "	"	144
"	65th "	"	154
"	66th "	Artificial CSF	146
"	67th "	"	155
"	68th "	"	157
"	69th "	"	147
"	70th "	"	144
"	71st "	"	156
"	72nd "	"	143
"	73rd "	"	139
"	74th "	"	140
"	75th "	"	140
"	76th "	Amphetamine (0.4µg/ml)	140
"	77th "	"	144
"	78th "	"	148
"	79th "	"	143
"	80th "	"	143
"	81st "	"	147
"	82nd "	"	152
"	83rd "	"	152
"	84th "	"	158

Table 9B (continued)

Rat #2	Time of Collection	Treatment	CPM per 100 µl Effluent
"	50th Min.	Artificial CSF	267
"	51st "	"	220
"	52nd "	"	193
"	53rd "	"	160
"	54th "	"	134
"	55th "	"	132
"	56th "	"	130
"	57th "	"	132
"	58th "	"	134
"	59th "	"	128
"	60th "	"	131
"	61st "	Amphetamine (1.0µg/ml)	130
"	62nd "	"	128
"	63rd "	"	129
"	64th "	"	127
"	65th "	"	127
"	66th "	"	125
"	67th "	"	122
"	68th "	"	114
"	69th "	"	117
"	70th "	"	116
"	71st "	Artificial CSF	110
"	72nd "	"	110
"	73rd "	"	119
"	74th "	"	111
"	75th "	"	116
"	76th "	"	115
"	77th "	"	112
"	78th	Amphetamine (50 µg/ml)	143
"	79th "	"	152
"	80th "	"	203
"	81st "	"	332
"	82nd "	"	336
"	83rd "	"	232
"	84th "	"	203
"	85th "	"	178
"	86th "	"	160

APPENDIX II

Table 11

Time of collection	Treatment	CPM per 100 µl effluent
	Artificial	
60th Min.	CSF	2425
61st "	"	2575
62nd "	"	2501
63rd "	"	2475
64th "	"	2420
65th "	"	2510
66th "	"	2559
67th "	"	2613
68th "	"	2691
69th "	"	2410
70th "	Mescaline (0.2 µg/ml)	2550
71st "	"	2493
72nd "	"	2530
73rd "	"	2440
74th "	"	2690
75th "	"	2450
76th "	"	2329
77th "	"	2064
78th "	"	1865
79th "	"	1887
80th "	"	2029
81st "	Artificial CSF	1764
82nd "	"	1790
83rd "	"	1860
84th "	"	1955
85th "	"	1849
86th "	"	1849
87th "	"	1879
88th "	"	1926
89th "	Mescaline (1.0 µg/ml)	1927
90th "	"	2100
91st "	"	1994
92nd "	"	2029
93rd "	"	1784
94th "	"	1669
95th "	"	1681
96th "	"	1651
97th "	Artificial CSF	1277
98th "	"	1292
99th "	"	1160

Table 11 (continued)

Time of collection	Treatment	CPM per 100 µl effluent
100th Min.	Artificial CSF	1100
101st "	"	1138
102nd "	"	992
103rd "	Mescaline (10.0 µg/ml)	857
104th "	"	901
105th "	"	884
106th "	"	960
107th "	"	857
108th "	"	732
109th "	"	638
110th "	"	632