# THE ROLE OF DESMETHYLIMIPRAMINE IN THE REVERSAL OF IMIPRAMINE-INDUCED LOCOMOTOR ACTIVITY BY TETRABENAZINE IN RATS

by

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# DEDICATION

TO

"Junior"

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# INTRODUCTION

The etiology of most mental disorders is poorly understood, and as a result therapeutic measures have been directed by necessity toward control of symptomatology rather than alleviation of cause. Fortunately, however, pharmacological screening programs have produced a number of chemical agents which are of some value in the treatment of the associated symptomatology. Included within the armamentarium of useful psychopharmacologic agents is the compound imipramine, which is of clinical value in the therapy of endogenous depression. The present studies were directed toward a better understanding of the relationship between the biological disposition of this compound and its therapeutic activity.

## A. Historical Background

Imipramine hydrochloride, N-(gamma-diethylamino-propyl)iminodibenzyl hydrochloride (Figure 2), was synthesized initially as one member of a series of 40 iminodibenzyl derivatives which were evaluated for potential anti-histaminic, sedative-hypnotic, and analgesic activities (1). Following preliminary screening, imipramine was selected for therapeutic trial on the basis of its pronounced sedative properties.

The ensuing clinical investigations by Kuhn (2,3) indicated that imipramine was relatively ineffective in sedating agitated psychotic patients. In fact, hyperactive, agitated, and anxious depression appeared to be aggravated in many patients subjected to imipramine therapy. Contrary to initial expectations, imipramine exhibited remarkable therapeutic efficacy in certain types of inactive or regressive depression.

B. Endogenous Depression

The anti-depressant properties of imipramine are of greatest value in therapy of patients suffering from endogenous depression. This form of depression afflicts 10 to 15 percent of patients broadly classified as depressed (4). Endogenous depression may be subdivided into the following four types: 1) manic-depressive depression, which is identified by the history of prior episodes of mania or hypomania; 2) recurrent depression which lacks a manic phase, but retains the hypomanic phase characterized by psychic retardation and loss of initiative; 3) non-recurrent depression which is distinguished from the former two subgroups by the absence of prior history of mania or depression; and 4) involutional depression which is distinguished by the presence of greater agitation, feelings or delusions of guilt, and paranoid delusions in middle-aged patients (4).

In 42 controlled studies on the anti-depressant effects of imipramine including approximately 2,705 patients, the reported incidence of improvement averaged 66.7 percent with a range of 19 to 100 percent (5). In contrast, electro-convulsive therapy is claimed by most investigators to result in improvement in 85 percent of depressed patients. However, this type of therapy is more difficult to administer, and often impairs memory, a side effect which does not accompany drug treatment. Thus, in the past decade, imipramine has rapidly been established as an effective therapeutic agent in the treatment of the endogenously depressed patient.

## C. Chemistry and Structure-Activity Relationships of Imipramine and Analogues

Crystalline imipramine hydrochloride, M.W. 316.5, is water soluble and melts at 173°C (6). The chemical structure of imipramine includes two benzene rings which are connected by an ethylene bridge and a nitrogen atom to which is bound a dimethylaminopropyl side chain (Figure 2). Structure-activity studies have shown that various imipramine analogues also exhibit anti-depressant activity. Anti-depressant activity remains with the presence of a two carbon side chain, an unsubstituted terminal nitrogen group, and a saturated ethylene bridge (7). Anti-depressant activity is also retained

when the central ring nitrogen atom is replaced by a carbon atom. It is of interest to note that exchange of the ethylene bridge of imipramine for a sulfur atom, and chlorination of the carbon in position 3 would yield chlorpromazine, a compound with very different pharmacological activity.

### D. Absorption, Distribution, Metabolism, and Excretion of Imipramine

Imipramine is readily absorbed from the gastro-intestinal tract or parenteral sites, and readily diffuses across the peritoneal membrane and blood-brain barrier (8, 9). Fifteen minutes after intraperitoneal administration, imipramine can be detected in liver, lung, and brain (9). This suggests rapid distribution and uptake by larger parenchymal organs. Plasma imipramine levels remain low with tissue-plasma ratios ranging from 10/1 to 100/1 (9). Imipramine has a high affinity for plasma proteins, and particularly for the alpha-globulin fraction. Binding studies in various tissues have demonstrated that imipramine is bound primarily to the particulate matter of cells (10,11).

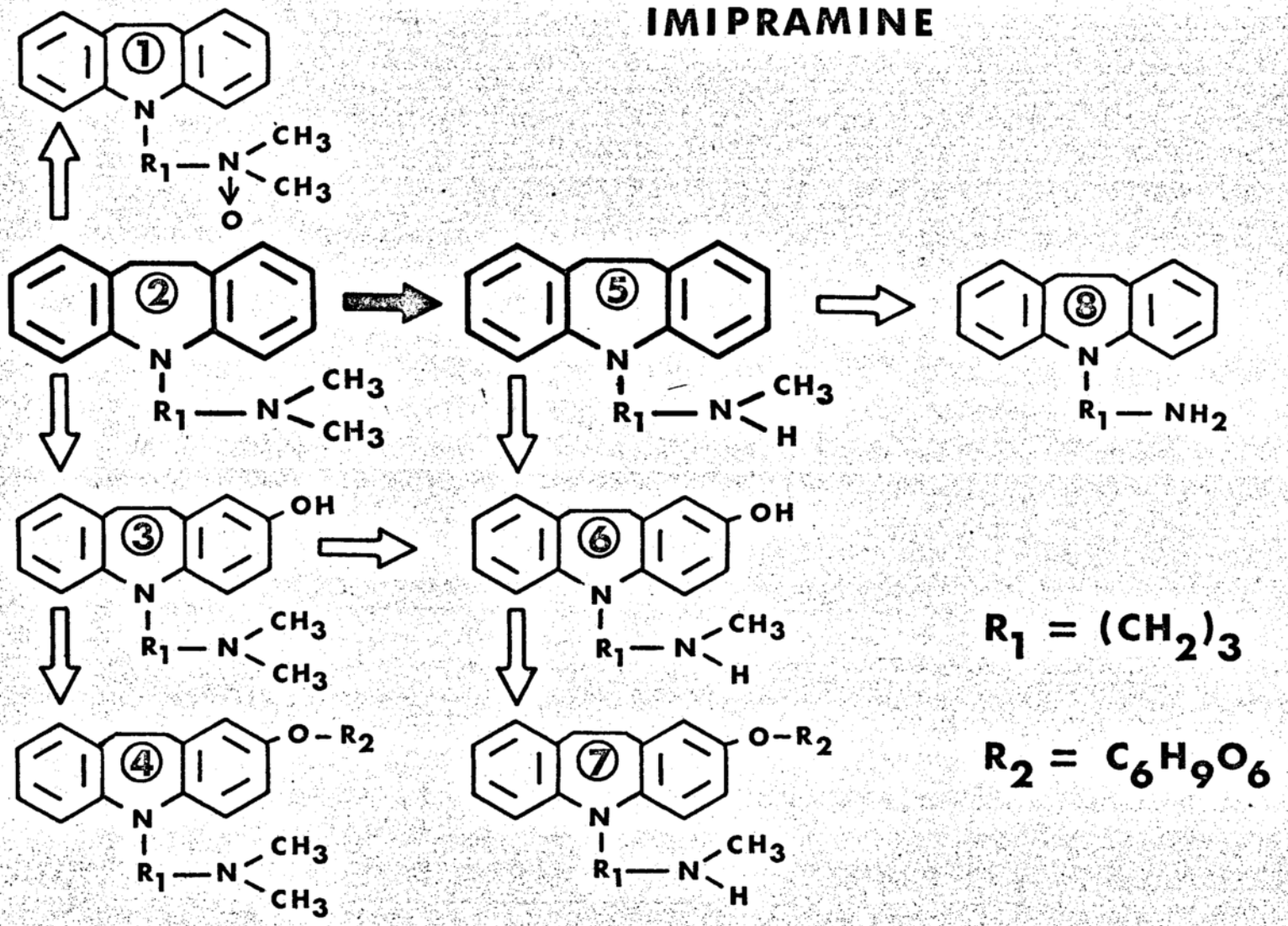
Imipramine is metabolized exclusively in the liver by a microsomal enzyme system which requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen (12). Pathways of imipramine metabolism include

hydroxylation in the 2-position, conjugation with glucuronic acid, and demethylation of the amino nitrogen of the side chain (13) (Figure 1). In addition to desmethylimipramine (DMI), hydroxylimipramine, and the glucuronide conjugate formed by these reactions, DMI can be further demethylated to desdimethylimipramine (DDMI). Hydroxylation can follow demethylation to form hydroxy-desmethylimipramine. Glucuronic acid conjugation can then result in the corresponding glucuronide (14). Imipramine is also metabolized in part to the N-oxide (15).

Little imipramine is eliminated unchanged (16). However, species differences exist with respect to the relative importance of the various metabolic pathways. Following imipramine administration, DMI accumulates in the tissues of rat and man, but not in tissues of rabbit or mouse (12). Rabbit liver microsomes rapidly metabolize both imipramine and DMI to hydroxydesmethylimipramine (12, 17). Rat liver microsomes rapidly convert imipramine to DMI, but slowly transform this metabolite to other products. Thus, DMI accumulates in this species.

After imipramine administration, two-thirds of the total drug is excreted via the kidney, and one-third is excreted by the intestinal tract (9). More than one-half of the administered dose is excreted in the bile, and undergoes entero-hepatic circulation. Large amounts of imipramine and its metabolites are excreted in the bile

# MAJOR METABOLIC PATHWAYS OF IMIPRAMINE




$R_1 = (CH_2)_3$

$R_2 = C_6H_9O_6$

during the first hour after drug administration, and a portion of imipramine and its nonpolar metabolites are reabsorbed from the gut. As a consequence of biliary excretion, imipramine and its nonpolar metabolites are sequestered for hours in the intestine (9).

## E. Pharmacology of Imipramine

Imipramine, like most chemotherapeutic agents, exerts a multitude of pharmacological activities, and exhaustive reviews have appeared which compile the diverse and sometimes ambiguous effects of imipramine in various species (18-23). A complete review of imipramine pharmacology would be far too lengthy. Therefore, the following text will be limited to pharmacological activities associated with proposed mechanisms of imipramine action.

1. Cellular Effects on Amine Metabolism - Numerous investigations have been conducted in vivo to study the effects of imipramine on synthesis, metabolism, transport, uptake, binding, and release of cellular monoamines in central and peripheral tissues.

Initial studies by Axelrod and coworkers (24), using $^{3}$H-norepinephrine, showed that imipramine interferes with the uptake of circulating norepinephrine in the isolated heart. In addition, other investigators reported that imipramine reduced the transport of labeled catecholamines

into the brain and spleen (25,26).

In recent investigations, Carlson and Waldeck (27) employed $^{3}$H-metaraminol to study the mechanism of amine uptake in heart. These studies demonstrated that the uptake mechanism for metaraminol, and probably norepinephrine, consists of two major components. These include active transport through the cell membrane (cell membrane pump), and subsequent uptake into specific intraneuronal storage sites. Imipramine was shown to inhibit the uptake of $^{3}$H-metaraminol by blocking the cell membrane pump. Hamberger and Masuoka (28) used a histochemical fluorescence method to study the uptake of norepinephrine into rat brain. They demonstrated that axons and synaptic terminals of central adrenergic neurons have an efficient concentration mechanism for norepinephrine which appears to be localized at the cell membrane. Thus, central adrenergic neurons would appear to be similar to peripheral adrenergic neurons in this respect.

From these investigations, it has been postulated that imipramine might block the uptake of extracellular norepinephrine. This effect might be achieved by inactivation or competitive inhibition of an active transport system in central and peripheral, adrenergic, neuronal membranes.

2. Peripheral Effects - Early studies showed that imipramine does not potentiate the response of the nictitating membrane to cervical sympathetic nerve stimulation (29-32). Subsequent studies, however, demonstrated that imipramine over a wide range of doses actually enhanced and prolonged adrenergic responses elicited by pre- or postganglionic sympathetic nerve stimulation, e.g., at the nictitating membrane (33-35). While low concentrations of imipramine increased the response elicited by nerve stimulation, higher concentrations appeared to reduce the intensity of the effects. Imipramine exhibits a similar pattern of activity in the isolated perfused spleen. Perfusion with 3 micrograms of imipramine per minute was shown to cause an increase of contraction amplitude and norepinephrine output following stimulation of the intact sympathetic neuron, while perfusion with 30 micrograms per minute abolished changes in volume and vascular resistance of the spleen, and caused a decrease in norepinephrine output (36).

Numerous studies designed to assess the influence of imipramine on sympathetic effectors have been carried out with injected catecholamines rather than with sympathetic nerve stimulation. Imipramine potentiates the effect of exogenous norepinephrine in the intact or denervated

nictitating membrane (37), the isolated vas deferens (38), and the seminal vesicle (39).

Low doses of imipramine (100-300 mcg/kg) potentiate the decrease in arterial blood pressure and bradycardia following acetylcholine administration or peripheral vagal stimulation (37,40). However, larger doses of imipramine exhibit cholinolytic activity. The hypotensive response to exogenous acetylcholine and vagal stimulation, as well as pilocarpine-induced salivation are diminished by large doses of imipramine (37).

Imipramine inhibits acetylcholine-induced contractions in isolated intestinal smooth muscle (39,41). Salivation in response to stimulation of the chorda tympani nerve is not modified by imipramine (42).

There is little evidence that imipramine has any effect on ganglionic transmission. Post-ganglionic potentials and response of the nictitating membrane to pre-ganglionic stimulation are not modified by imipramine (32,33,43).

3. Central Effects - Central nervous system (CNS) effects of imipramine include induction of hypothermia in laboratory animals (41,44,45), weak anti-emetic activity in dogs (46), and partial inhibition of electro-shock convulsions in the rat (47,48). Electrophysiological studies with imipramine are numerous. However, the findings from these studies are difficult to interpret due

to the complex effects of imipramine, and the difficulties encountered when attempting to correlate electrophysiological phenomena and behavior.

4. Interaction with Autocoids - Potentiation by imipramine of serotonin-induced contraction of the denervated cat nictitating membrane has been reported (32, 33,42,49). In contrast, other actions of serotonin are diminished by large doses of imipramine. Serotonin-induced edema and increased capillary permeability are partially blocked by imipramine (39,50). Similarly, the incidence of serotonin-induced gastric ulcers is reduced by imipramine. Imipramine has been shown to antagonize the action of serotonin on isolated guinea pig ileum and rat uterus preparations (39,51).

Antihistaminic properties of imipramine have been demonstrated by inhibition of histamine aerosol induced broncho-spasm in guinea pigs, and antagonism of histamine induced contractions in isolated guinea pig ileum preparations (39,41). Furthermore, imipramine has been shown to protect guinea pigs from a fatal dose of histamine (39).

5. Interaction with CNS Drugs - The foregoing discussion has dealt primarily with the pharmacological effects of imipramine. These effects may be attributed to imipramine itself, or possibly to an active metabolite. Because

pharmacological interaction of drugs is of great theoretical and therapeutic importance, it is not surprising that a great deal of information has accumulated on the interaction of imipramine with other agents, and particularly other agents affecting the CNS. A variety of interaction studies have been conducted in an attempt to better understand the pharmacological effects and mechanism of action of imipramine.

In certain doses, imipramine increases the duration of barbiturate hypnosis (37,39,44,48,52,53). Potentiating doses of imipramine lie within the range of 10-50 mg/kg i.p. (37,44). In contrast, lower doses of imipramine have been shown to reduce hexobarbital sleeping time. With respect to phenobarbital, intermediate doses of imipramine (25-50 mg/kg s.c.) prolong sleeping time in guinea pigs (41,54). However, doses of imipramine below or above this range have been shown to shorten phenobarbital hypnosis. Thiopental sleep is also prolonged by 30 mg/kg doses of imipramine, but is shortened by 10 mg/kg doses of the drug (41,54). In contrast, the hypnotic effect of a non-barbiturate, ethanol, is potentiated only by high doses of imipramine (37,44,53,55,56).

Imipramine has in some studies been shown to inhibit, and in others to potentiate, the central actions of amphetamine (46,54,57,58). However, the quality of the interaction is again dose dependent. Theobold and

coworkers (53) found amphetamine hyperactivity to be enhanced after intraperitoneal administration of 2-5 mg/kg imipramine, but decreased by a 20 mg/kg dose. This tendency for amphetamine potentiation to decay as imipramine dosage is increased from 5-10 mg/kg to 20-40 mg/kg has also been noted by other investigators (59,60). From these findings, it is apparent that imipramine tends to potentiate amphetamine hyperactivity at low doses, while antagonism prevails at high doses. Intermediate doses of imipramine have been most commonly employed in animal studies. This may partially explain the often reported lack of modification of the CNS effects of amphetamine by imipramine (54,61).

Imipramine has been shown to protect against electroconvulsive shock (ECS), and to diminish the tonic-extensor convulsions and coma associated with this treatment (47,48). No significant influence could be noted, however, on metrazol-induced seizures, or on the fatal outcome of seizures induced by strychnine (48,55,62). In addition to the protective effect of imipramine against convulsions from ECS, significant reduction in mortality after convulsant doses of caffeine has been reported (55). In contrast, picrotoxin convulsions in mice are potentiated by imipramine (39).

Domenzoz and Theobold (39) noted that imipramine prevents certain symptoms accompanying reserpine

administration in rats. A number of more recent studies have verified that imipramine can antagonize or prevent various autonomic effects of reserpine such as ptosis, hypothermia, lachrymation, diarrhea, and bradycardia (39,42,46,51,53,55,58,63-69). In some cases, antagonism of behavioral changes was noted. However, reserpine-like behavioral changes induced by the more rapid acting, synthetic benzoquinolazines (RC 4-1284 and tetrabenazine) can be modified by imipramine (70,71).

The administration of tetrabenazine to rats pretreated with imipramine results in a syndrome characterized by exophthalmus and persistent, pronounced hyperactivity quite unlike that induced by amphetamine. The animals exhibit increased locomotor activity, but do not show the erratic haste observed with amphetamine. In a cage, they ignore food, water, and external stimuli, and pay no attention to other rats caged with them. The overall condition is one of endogenous excitation rather than increased responsiveness or irritability (70,71). This phenomenon is known as "tetrabenazine-reversal," because imipramine completely reverses the reserpine-like behavior elicited by tetrabenazine in many animals.

## F. Mechanism of Imipramine Action

1. Adrenergic Mechanisms - A number of experimental observations suggest that the biological activity of

imipramine is intimately associated with peripheral and central adrenergic mechanisms. These observations include: a) potentiation of the peripheral effects of exogenous catecholamines; b) potentiation of the adrenergic responses elicited by electrical stimulation of sympathetic ganglia; c) potentiation or antagonism of the various central effects elicited by amphetamine; and d) antagonism of the reserpine-like syndrome in rodents.

The dose dependent potentiation of exogenous and endogenous catecholamines at peripheral adrenergic sites by imipramine was reported by Sigg, et al. (33). These investigators interpreted these data as being suggestive of a duality of biological activities which include a) an indirect adrenergic effect or sensitization, most apparent at low doses, and dependent upon the blockade of norepinephrine uptake into adrenergic nerve terminals; and b) an adrenolytic effect, most apparent at high doses, and dependent upon an affinity of imipramine for adrenergic receptor sites. They further proposed that the relative importance of the two mechanisms is a function of imipramine concentration and the regional importance of norepinephrine uptake into storage sites for the termination of peripheral pharmacological action. It has also been suggested, by Haefly and coworkers, that the paradoxical peripheral effects of imipramine are due to a) preferential affinity for norepinephrine-binding sites

in sympathetic nerve endings which leads to norepinephrine potentiation, and b) the affinity of imipramine for adrenergic receptor sites which is apparent at higher doses, and results in norepinephrine antagonism (72).

Central interactions of drugs with adrenergic neuromediators are difficult to study due to the complexity of the neuronal pathways involved, and to the lack of precise information concerning the physiological role of adrenergic transmitters in the central nervous system. Therefore, experimental approaches to the study of central adrenergic effects of imipramine must necessarily be indirect. However, several compounds have proven to be valuable research tools for the study of central adrenergic processes. Included among these agents are amphetamine and reserpine.

Sulser, et al. (73) have shown that imipramine inhibits amphetamine hydroxylation, and have suggested that imipramine potentiation of the CNS effects of low doses of amphetamine reported by Theobald, et al. (53) might be explained on this basis. Antagonism of amphetamine induced CNS stimulation by large doses of imipramine might be due to competition for central adrenergic receptors. This would imply that the latter effect is capable of overcoming the former.

The ability of imipramine to reverse the signs of CNS depression produced by reserpine has been

demonstrated by Sulser, et al. (66). This phenomenon is characterized by extreme hyperactivity, and is generally known as reserpine reversal. Reserpine reversal by imipramine can be antagonized by chlorpromazine, a centrally acting alpha adrenergic blocker (74).

The compound, alpha-methyl-metatyrosine (alpha-MMT), an agent with no intrinsic adrenergic activity, and which is not metabolized by catechol-o-methyltransferase (COMT) or monoamineoxidase (MAO), has served as an additional tool for elucidation of the mechanism of the central adrenergic action of imipramine (70,75). Alpha-MMT is capable of stoichiometrically replacing norepinephrine in central storage granules (76). Subsequent treatment with reserpine causes release of this false transmitter substance, accompanied by lack of the typical reserpine syndrome. Administration of alpha-MMT prior to imipramine and reserpine abolishes reserpine reversal (77). These data suggest that norepinephrine release is a required component of reserpine reversal by imipramine.

Imipramine does not prevent amine depletion by reserpine in the brain, indicating that it does not interfere with reserpine-induced incapacity to store norepinephrine in intraneuronal sites (69,70,71,78). Thus, it seems apparent that imipramine potentiation of norepinephrine released by reserpine might be due primarily to blockade of the active cellular uptake mechanism by imipramine.

Investigation of the pharmacological interaction between imipramine and agents such as amphetamine and reserpine, coupled with biochemical studies, have provided the basis for the "biogenic amine theory of depression" (79,80). This theory proposes that some, if not all, forms of depression are associated with an absolute or relative decrease in central catecholamines, particularly norepinephrine, available to central adrenergic receptor sites. Elation, conversely, may be associated with an excess of such amines.

The previous findings with amphetamine and reserpine are compatible with the hypothesis that an increased availability of norepinephrine at critical central sites may be the mechanism by which the anti-depressant effect of imipramine is achieved. For the present, however, the possibility that other pharmacological actions of imipramine might modify or contribute to anti-depressant activity cannot be ruled out.

2. Cholinergic Mechanisms - Excessive cholinergic activity appears to be present in some types of depression (43,81). This has led several investigators to attempt to relate the cholinolytic activity of imipramine to its anti-depressant effect (39,53). Conclusive supporting evidence for these hypotheses is also lacking due to technical limitations accompanying direct study of the CNS.

Reserpine has been shown to cause accumulation of norepinephrine in the vicinity of certain central parasympathetic nuclei of the brain, e.g., Edinger-Westphal and certain vagal nuclei (82). Depletion of central amines by reserpine enhances parasympathetic outflow; however, this outflow is diminished by accumulation of norepinephrine (83). These findings suggest that an adrenergic inhibitory "brake" might normally exist which modulates parasympathetic outflow from the CNS. Thus, by interfering with central amine uptake mechanisms, imipramine might activate an "adrenergic brake" which in turn results in a central "cholinolytic" effect.

Fekete (84) has also proposed a possible cholinergic mechanism in norepinephrine potentiation of imipramine. He suggested that sensitivity of the adrenergic receptor to norepinephrine increases as the concentration of acetylcholine increases at the adrenergic receptor site. Thus, the increased adrenergic activity caused by the inhibition of norepinephrine uptake by imipramine might be enhanced further by this mechanism.

Recent investigations by Scheelkŭger and Randrup (85) on tetrabenazine-reversal by imipramine have resulted in a proposed "combination" mechanism of action for imipramine. Although they agree that the blockade

of uptake of catecholamines is implicated in the reversal of tetrabenazine sedation by imipramine, they conclude from their studies that this reversal is closely connected with the depression of central norepinephrine mediated transmission. They propose that the net result of these phenomena is central cholinergic predominance. Thus, both central adrenergic and cholinergic mechanisms seem to be involved in the reversal of tetrabenazine by imipramine.

Neither the site of action nor the mechanism of action of imipramine with respect to anti-depressant activity are precisely known. However, the foregoing pharmacological studies have elucidated interactions of imipramine with central adrenergic and/or cholinergic mechanisms, and suggest that these interactions might be related to the clinical effect of imipramine. Nevertheless, adequate clinical correlation between changes in central adrenergic or cholinergic activity, and reduction of endogenous depression are too few at the present time to allow an unequivocal scientific judgement.

## G. Present Studies

In 1961, Brodie and coworkers presented a new hypothesis regarding the mechanism of action of imipramine (86,87). They proposed that although imipramine exerts a mild sedative action in normal animals and man,

the anti-depressant activity is due to the metabolite, desmethylimipramine (DMI). This proposal was based primarily on the following data: 1) the anti-depressant effect of imipramine in rats is delayed in onset, while that of DMI is rapid in onset (42); 2) the imipramine/DMI ratio in rat brain is inversely proportional to the degree to which benzoquinolazine reversal by imipramine can be achieved (42,64); 3) DMI is a more potent anti-depressant than imipramine in rats (42); and 4) DMI is a metabolic product of imipramine resulting from N-demethylation, and can be detected in rat brain after imipramine administration (13). Therefore, it seemed possible that imipramine per se may not be the anti-depressant, but is a "pro-drug," and that DMI is responsible for benzoquinolazine reversal in rats.

As a consequence of this theory, many investigations were hastily conducted in an attempt to support or disprove the hypothesis that DMI mediates the anti-depressant effect of imipramine. Numerous papers comparing imipramine with DMI were published in the early 1960's. However, significant findings were few and inconclusive. The net result of these studies was simply that the question of whether imipramine or its metabolite, DMI, was responsible for improvement of endogenous depression remained unresolved, and required further attention.

Studies conducted by Sulser, Bickel and Brodie (70) have indicated that reversal of benzoquinolazine-induced reduction of spontaneous motor activity by DMI appears to be dependent on the concentration of DMI in the brain. However, the degree of tetrabenazine reversal following imipramine administration has been shown to not correlate with DMI levels in the brain. For this reason, a detailed time-response study of the interaction between imipramine and the benzoquinolazine, tetrabenazine, on spontaneous motor activity in rats was undertaken. The disposition of imipramine and/or its demethylated metabolite in whole blood and brain were monitored over a 24 hour period, and spontaneous motor activity was used as a pharmacological endpoint to quantitate tetrabenazine reversal. In addition, DMI dose versus tetrabenazine-reversal studies were conducted, as well as investigations on the effect of SKF-525A on the *in vivo* metabolism of imipramine, in an attempt to elucidate the relative importance of imipramine and DMI on tetrabenazine-reversal. It was anticipated that these findings would help clarify the present "imipramine or DMI" controversy, and possibly extend our knowledge as to the pharmacological action of anti-depressants in the treatment of endogenous depression.

## EXPERIMENTAL

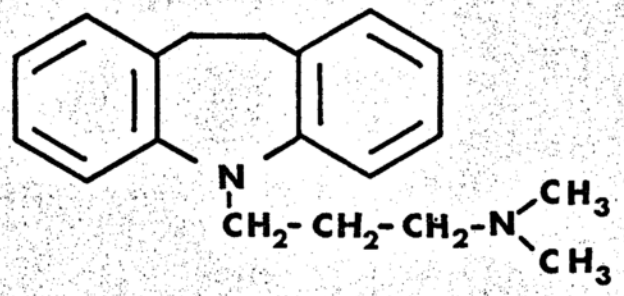
### A. Materials

1. Chemicals - The chemicals used in this study (Figure 2) and their sources were as follows: imipramine and desmethylimipramine hydrochloride (Geigy Pharmaceuticals); Tetrabenazine methanesulfonate (Hoffmann-La Roche, Inc.); and SKF-525A (Smith Kline and French Laboratories). Heptane (Phillips 66 Pure Grade) was redistilled and the 97-98°C distillate was used for extraction purposes. All other materials used in this study were obtained from standard sources.

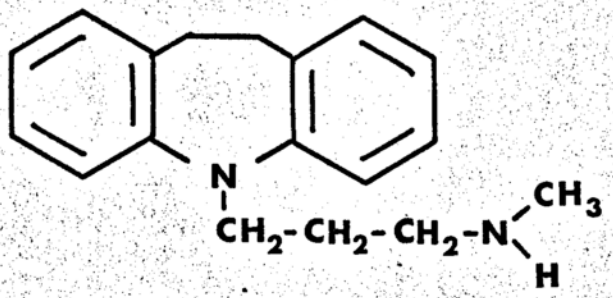
NOMENCLATURE

| Common name | Brand name | Chemical name |
|---|---|---|
| Imipramine (IMI) | Tofranil$^{R}$ | 5-(3-Dimethylamino-propyl)-10,11-dihydro-5H-dibenz[b,f]azepine hydrochloride |
| Desmethyl-imipramine (DMI) | Pertofrane$^{R}$ | 5-(Methylaminopropyl)-10,11-dihydro-5H-dibenz[b,f]azepine hydrochloride |
| Tetrabenazine (TBZ) | Nitoman$^{R}$ | 2-Oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]-quinolizine |
| SKF-525A | Proadiphen$^{R}$ | Diethylaminoethyl diphenylpropylacetate |

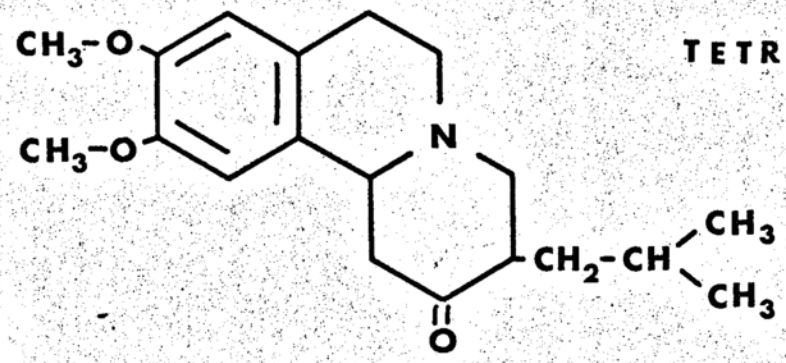
IMIPRAMINE
(IMI)



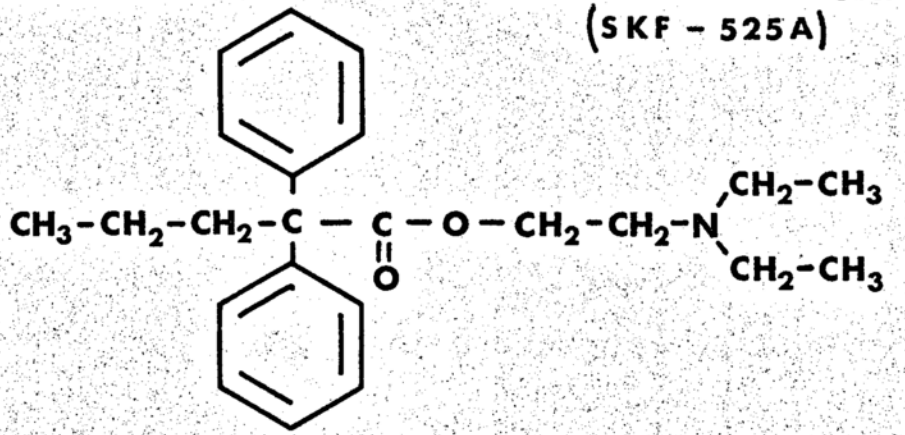
DESMETHYL-
IMIPRAMINE
(DMI)



TETRABENAZINE
(TBZ)



DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE
(SKF - 525A)



2. Animals - White, male, Holtzman rats weighing between 250 and 350 grams were employed throughout this study. All animals were maintained in our animal quarters for at least 24 hours prior to use in order to allow them to equilibrate with their new environment. Rockland Complete Mouse/Rat Diet and tap water were available ad libitum.

3. Activity Cages - Activity cages obtained from the Woodard Research Corporation were used in these studies (Plates 1 and 2). The dimensions of this instrument are 14.5 inches on each side, and 9.5 inches in height. Each unit consists of a large cylindrical cage, the center of which is occluded by a smaller cylinder placed in the exact center of the cage. This central cylinder houses an infrared generator (light bulb plus infrared filters) which emits six equally spaced infrared light beams toward independently operating photocells situated on the inner aspect of the outer cylinder. Each photocell is directly opposite an infrared light beam. As the animal moves about the circular raceway, the infrared beams are "broken" and electrical impulses are initiated, amplified, and transmitted to an impulse counter mounted in a standard cabinet (8 x 6 x 3.5 inches). Each "break" is registered as one count. The total number of counts are summated by the counter.

Plate 1

Woodard Research Activity Cage (front view)

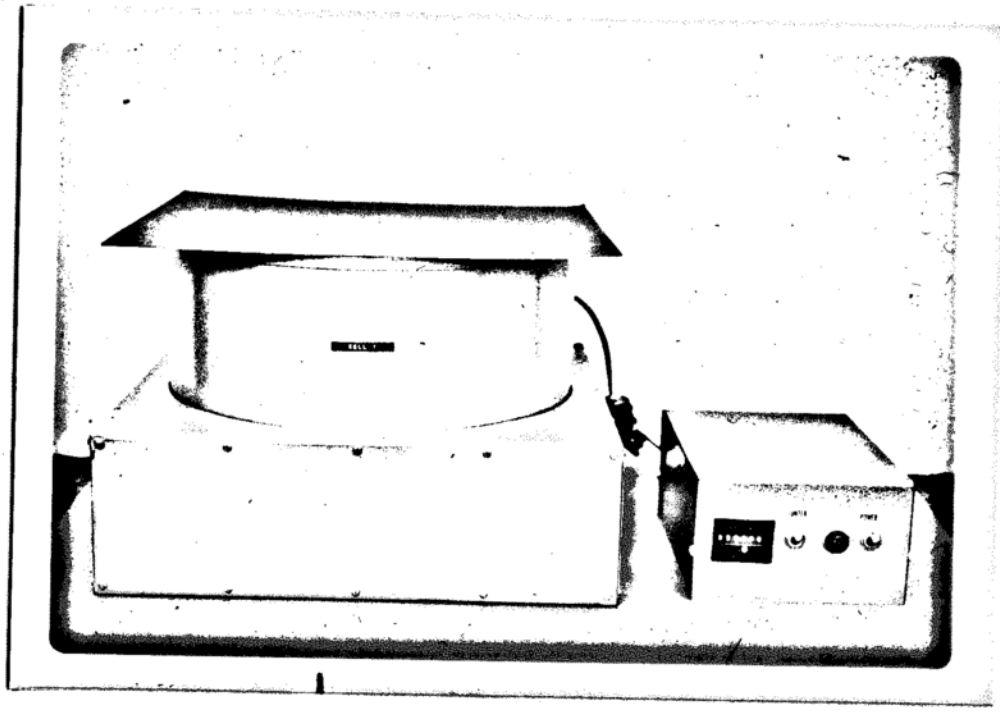
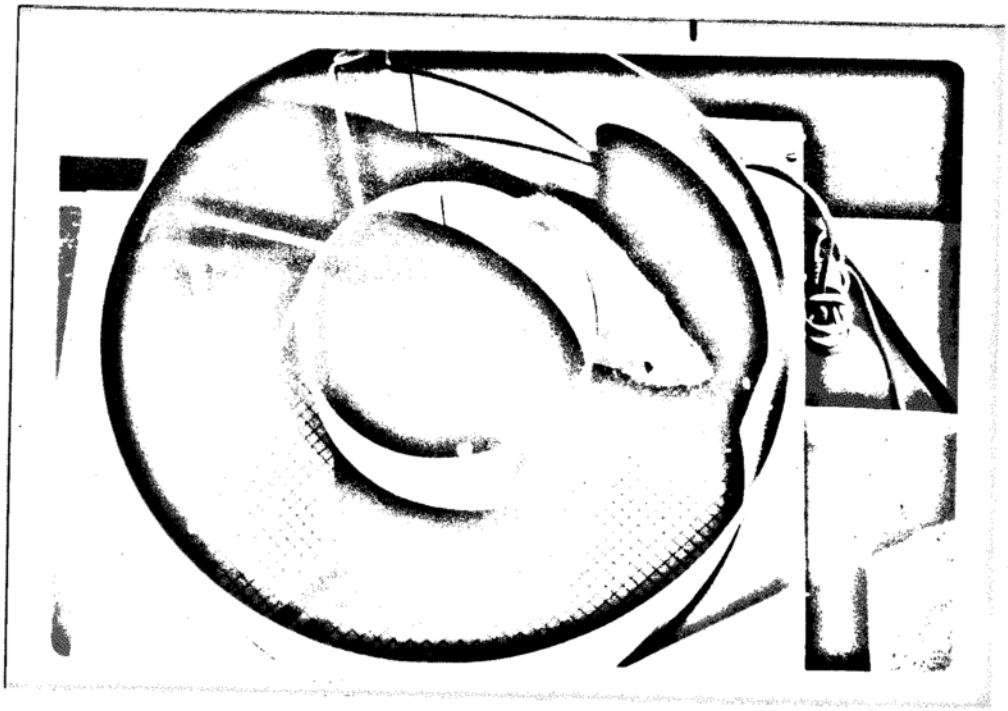


Plate 2

Woodard Research Activity Cage (top view)



## B. Methods

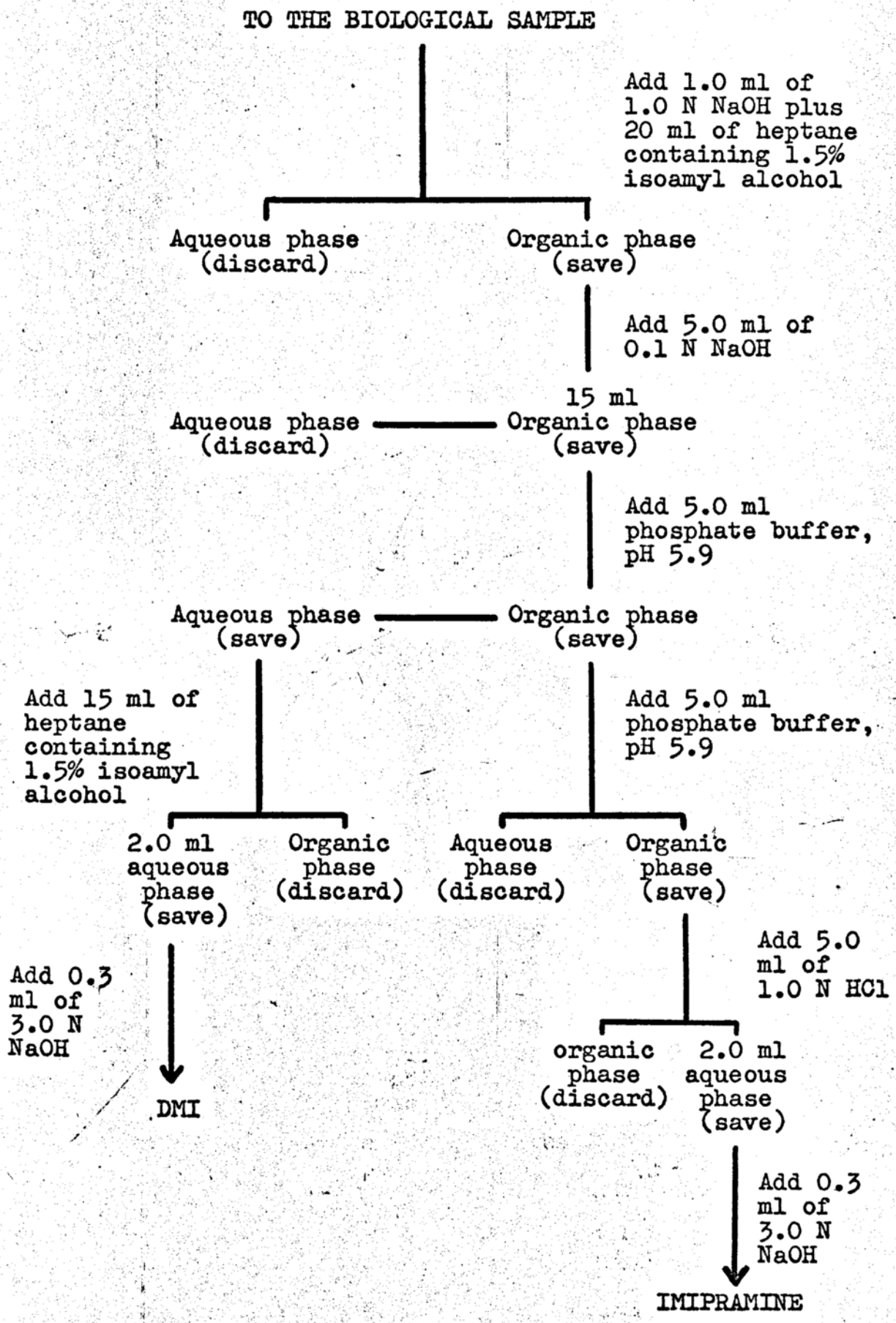
1. Drug Administration - Aqueous solutions of imipramine, DMI, tetrabenazine, and sodium chloride (0.9 percent) were administered to rats intraperitoneally using 3 cc Tomac Disposable Syringes with 21 gauge, 1½ inch needles. Aqueous solutions of SKF-525A were administered orally using 3 cc Tomac Disposable Syringes with a Yale B-D 16 gauge, 3 inch oral needle.

2. Preparation of Biological Materials - For those experiments in which blood and brain levels of imipramine and DMI were measured, control and drug treated animals were sacrificed by decapitation. Blood samples were collected immediately, and the whole brain was rapidly dissected from the skull. Blood samples (approximately 2.0 ml) were collected in tared 45 ml glass-stoppered centrifuge tubes containing 0.1 ml of saturated sodium oxalate. The tubes were weighed, and the net blood weights calculated. Division of sample net blood weight by previously determined whole blood density produced a dividend equal to the total volume of whole blood contained in the collection sample. Whole brains were homogenized in 3 volumes of distilled water with a motor driven, ground glass, Potter-Elvehjem tissue homogenizer. Four ml aliquots of the whole brain homogenate were pipetted into 45 ml glass-stoppered centrifuge tubes.

Blood samples and whole brain homogenates were stored at 2°C prior to solvent extraction.

3. Imipramine and Desmethylimipramine Solvent Extraction and Assay - Blood samples and whole brain homogenates were extracted and assayed for imipramine and DMI content by the technique of Dingell, Sulser and Gillette (12).

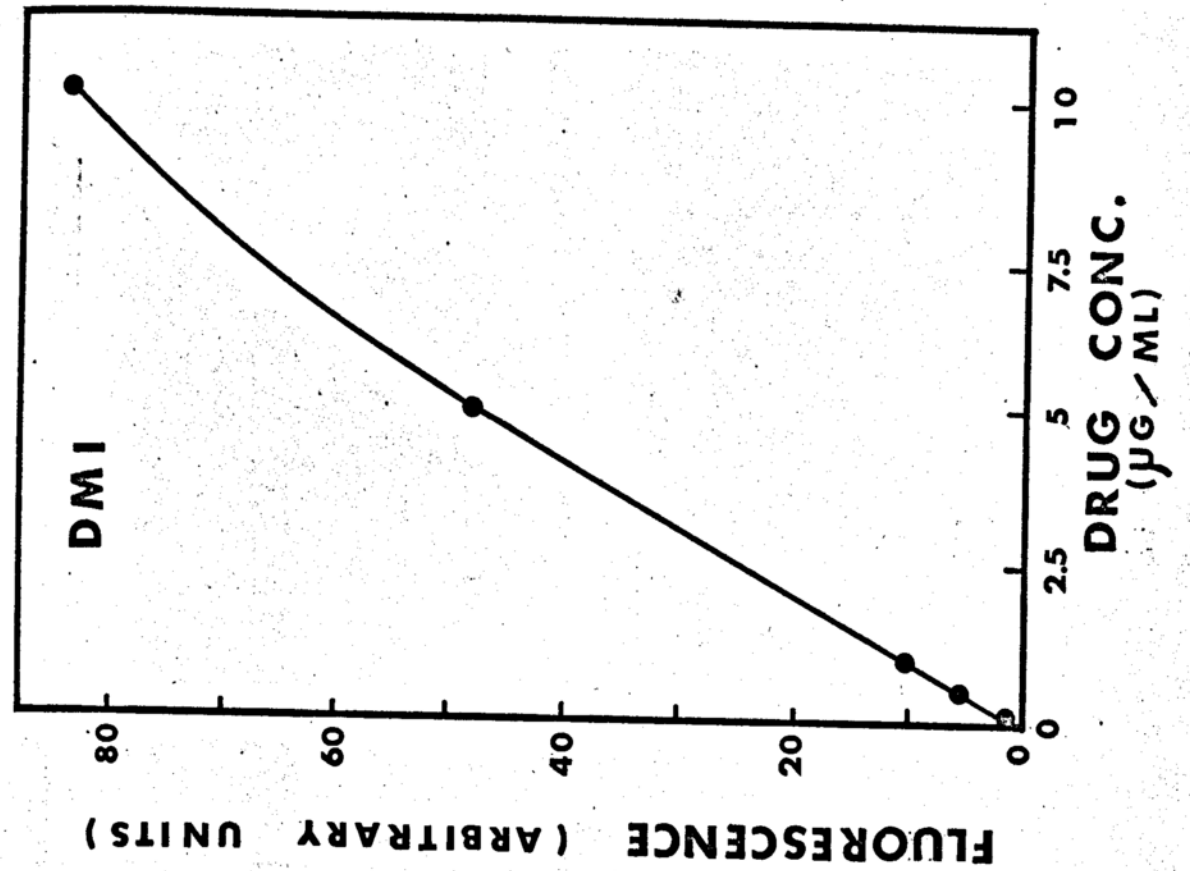
The procedure was as follows (Figure 3). Twenty ml of redistilled heptane containing 1.5% isoamyl alcohol and 1 ml of 1.0 N sodium hydroxide were added to a biological sample contained in a 45 ml glass-stoppered centrifuge tube. The tube was shaken for 45 minutes on an International Bottle Shaker, and centrifuged for 10 minutes on an International, Model K - Size 2, Centrifuge. The aqueous layer was aspirated and discarded. Five ml of 0.1 N sodium hydroxide was added to the organic phase of each sample. The tube was shaken for 10 minutes, and centrifuged for 10 minutes. Fifteen ml of the organic phase was transferred to a glass-stoppered centrifuge tube containing 5.0 ml of 0.2 M phosphate buffer, pH 5.9. The tube was shaken for 10 minutes, and centrifuged for 10 minutes. The organic phase was transferred to a glass-stoppered centrifuge tube containing 5.0 ml of 0.2 M phosphate buffer, pH 5.9. The tube was shaken for 10 minutes, and centrifuged for 10 minutes. The aqueous phase was

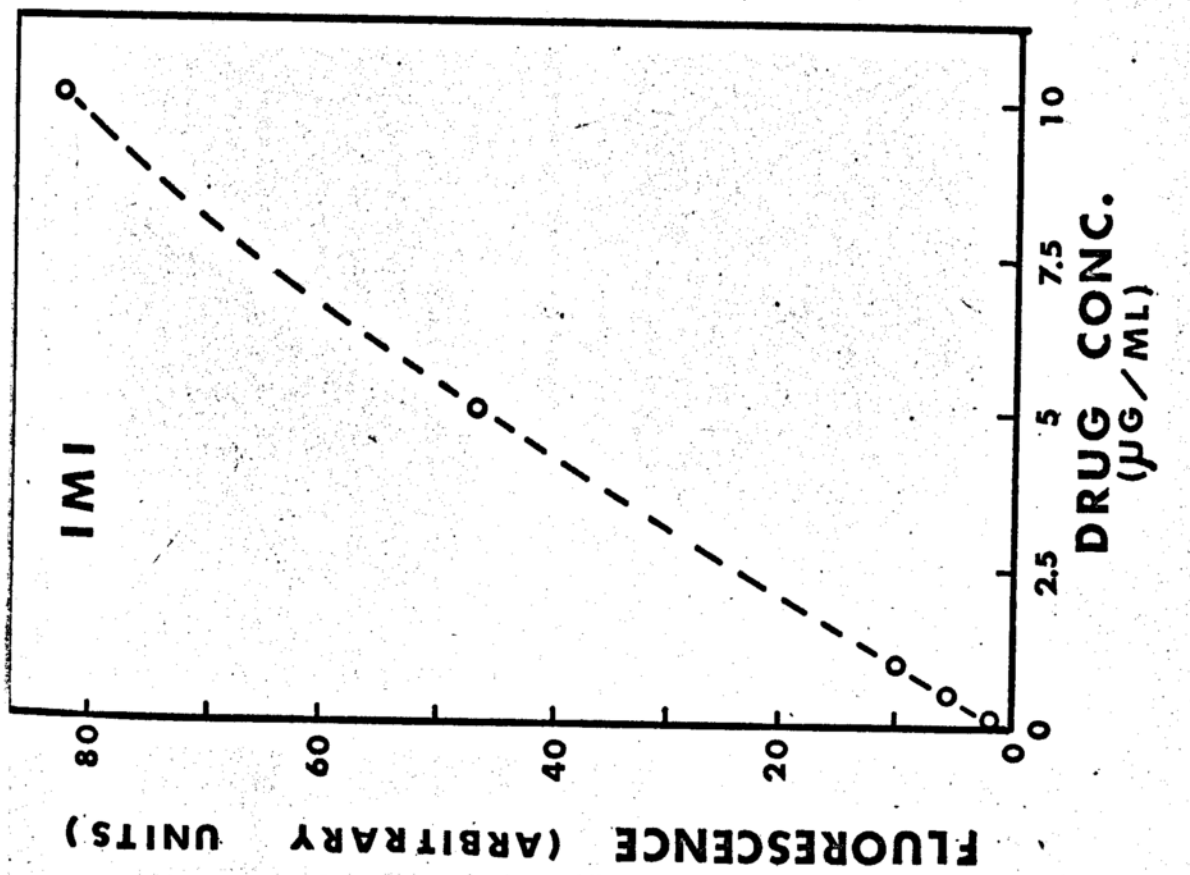

TO THE BIOLOGICAL SAMPLE
Add 1.0 ml of 1.0 N NaOH plus 20 ml of heptane containing 1.5% isoamyl alcohol
Aqueous phase (discard)
Organic phase (save)
Add 5.0 ml of 0.1 N NaOH
15 ml
Aqueous phase (discard)
Organic phase (save)
Add 5.0 ml phosphate buffer, pH 5.9
Aqueous phase (save)
Organic phase (save)
Add 15 ml of heptane containing 1.5% isoamyl alcohol
Add 5.0 ml phosphate buffer, pH 5.9
2.0 ml aqueous phase (save)
Organic phase (discard)
Aqueous phase (discard)
Organic phase (save)
Add 0.3 ml of 3.0 N NaOH
DMI
Add 5.0 ml of 1.0 N HCl
organic phase (discard)
2.0 ml aqueous phase (save)
Add 0.3 ml of 3.0 N NaOH
IMIPRAMINE

aspirated, and 5.0 ml of 0.1 N hydrochloric acid was added. The tube was shaken for 10 minutes, and centrifuged for 10 minutes. The organic phase was aspirated, and the acidic phase reserved for the measurement of imipramine. Fifteen ml of heptane containing 1.5% isoamyl alcohol was added to the pH 5.9 buffer extract which contained DMI. The tube was shaken for 10 minutes, and centrifuged for 10 minutes. The organic phase was aspirated, and the buffer phase reserved for the measurement of DMI. Two ml of either the acidic or the buffer phase were transferred to a quartz cuvette, and 0.3 ml of 3.0 N sodium hydroxide was added. The contents were mixed, and the fluorescence measured in an Aminco-Bowman Spectrophotofluorimeter (Model 4-8202).

Both imipramine and DMI were found to have identical fluorescence spectra with activation maxima at 290 mμ and fluorescence maxima at 410 mμ, uncorrected. Beer's Law was found to apply in a concentration range of 0.1 μg/ml to 7.5 μg/ml (Figure 4). At higher concentrations deviation from Beer's Law was noted due to a "quenching" phenomenon common to fluorescent assays.

4. Locomotor Activity - Spontaneous motor activity was quantitated by placing a single rat into an activity cage immediately after drug administration. Summated


DMI
FLUORESCENCE (ARBITRARY UNITS)
0
20
40
60
80
0
2.5
5
7.5
10
DRUG CONC. (µG/ML)


IMI
FLUORESCENCE (ARBITRARY UNITS)
0
20
40
60
80
0
2.5
5
7.5
10
DRUG CONC. (µG/ML)

spontaneous motor activity counts were recorded periodically over one hour. Animals used in these studies were unconditioned to the activity cage environment prior to their use. All activity studies were carried out between the hours of 12:00 and 6:00 P.M. at an ambient temperature of approximately 27°C.

## RESULTS AND DISCUSSION

### A. Recovery of Imipramine and Desmethylimipramine from Biological Samples

Preliminary experimentation was required to validate the techniques of extraction, separation, and assay of imipramine and desmethylimipramine, DMI (12). Recovery of imipramine and DMI from rat plasma, whole blood, and brain homogenates was determined. The steps in the solvent extraction technique are summarized in Figure 3 and included: 1) phosphate buffer extract; 2) phosphate buffer wash; 3) hydrochloric acid extract; and 4) the initial phosphate buffer extract after washing with n-heptane plus isoamyl alcohol. The percent recovery of a known amount of added drug was determined for each extraction phase, and a cumulative recovery total was calculated by summation of drug contained in each of the individual phases. These data are summarized in Table I. Values denoted by a double asterisk were calculated from partition coefficients equal to percent drug recovery in the phosphate buffer washes divided by one hundred.

Plasma recovery studies of imipramine (Table I) disclosed that 73.6 percent of the added imipramine accumulated in the hydrochloric acid extract, while only

TABLE I

PERCENT RECOVERY OF IMIPRAMINE (IMI) AND DESMETHYLIMIPRAMINE (DMI) FROM RAT PLASMA, WHOLE BLOOD, AND WHOLE BRAIN HOMOGENATES USING THE SOLVENT EXTRACTION AND ESTIMATION METHOD OF DINGELL, SULSER, AND GILLETTE (12)

A. Recovery of imipramine (IMI) from rat plasma (12.5 micrograms IMI to 1.0 milliliter plasma):

| Extraction phase quantitated | Number of trials | Percent IMI recovery* | Cumulative IMI recovery |
|---|---|---|---|
| Phosphate buffer extract | - | 11.2** | 11.2%** |
| Phosphate buffer wash | 8 | 10.0±0.9 | 21.2% |
| Hydrochloric acid extract | 8 | 73.6±3.2 | 94.8% |
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 8 | 0.3±0.2 | 95.1% |

B. Recovery of desmethylimipramine (DMI) from rat plasma (12.5 micrograms DMI to 1.0 milliliter plasma):

| Extraction phase quantitated | Number of trials | Percent DMI recovery* | Cumulative DMI recovery |
|---|---|---|---|
| Phosphate buffer extract | 3 | 82.1<br>86.7** | 82.1% |
| Phosphate buffer wash | 9 | 11.5±1.0 | 93.6% |
| Hydrochloric acid extract | 9 | 1.7±0.2 | 95.3% |
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 6 | 47.9±5.0 | -- |

(continued)

TABLE I - Cont.

C. Recovery of imipramine (IMI) plus desmethylimipramine (DMI) from rat plasma (12.5 micrograms IMI plus 12.5 micrograms DMI to 1.0 milliliter of plasma):

| Extraction phase quantitated | Number of trials | Percent IMI & DMI recovery* | Cumulative IMI & DMI recovery |
|---|---|---|---|
| Phosphate buffer extract | 3 | 89.0<br>73.2** | 89.0% |
| Phosphate buffer wash | 6 | 19.6±0.4 | 108.6% |
| Hydrochloric acid extract | 6 | 74.3±0.4 | 182.9% |
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 3 | 61.4 | -- |

D. Recovery of imipramine (IMI) from rat whole blood (12.5 micrograms IMI to 1.0 milliliter whole blood):

| Extraction phase quantitated | Number of trials | Percent IMI recovery* | Cumulative IMI recovery |
|---|---|---|---|
| Phosphate buffer extract | 3 | 9.4<br>8.0** | 9.4% |
| Phosphate buffer wash | 4 | 7.4±0.3 | 16.8% |
| Hydrochloric acid extract | 12 | 67.4±1.5 | 84.2% |
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 5 | 1.9±0.5 | 86.1% |

(continued)

TABLE I - Cont.

E. Recovery of desmethylimipramine (DMI) from rat whole blood (12.5 micrograms DMI to 1.0 milliliter whole blood):

| Extraction phase quantitated | Number of trials | Percent DMI recovery | Cumulative DMI recovery |
|---|---|---|---|
| Phosphate buffer extract | - | 90.0** | 90.0%** |
| Phosphate buffer wash | 5 | 9.1±0.3 | 99.1% |
| Hydrochloric acid extract | 9 | 5.2±0.3 | 104.3% |
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 12 | 56.8±3.2 | -- |

F. Recovery of imipramine (IMI) from rat whole brain homogenate (12.5 micrograms IMI to 1.0 milliliter whole brain homogenate):

| Extraction phase quantitated | Number of trials | Percent IMI recovery |
|---|---|---|
| Hydrochloric acid extract | 6 | 70.6±2.9 |

G. Recovery of desmethylimipramine (DMI) from rat whole brain homogenate (12.5 micrograms DMI to 1.0 milliliter whole brain homogenate):

| Extraction phase quantitated | Number of trials | Percent DMI recovery |
|---|---|---|
| Phosphate buffer extract after heptane plus isoamyl alcohol wash | 6 | 42.5±3.9 |

*Percent drug recovery $\pm s_{\bar{x}}$ (standard error of the mean).

**Calculated.

0.3 percent could be found in the phosphate buffer extract after heptane plus isoamyl alcohol wash. In contrast, recovery studies of DMI revealed that 82.1 percent appeared in the phosphate buffer extract, while 1.7 percent was detected in the hydrochloric acid extract. The calculated value of 86.7 percent for the accumulation of DMI in the phosphate buffer extract was in agreement with experimental findings. Cumulative recovery totals of imipramine and DMI were 95.1 and 95.3 percent, respectively.

The addition of equal concentration of imipramine and DMI resulted in recovery of 74.3 percent total drug in the hydrochloric acid extract with 89.0 percent in the phosphate buffer extract. These values are in agreement with corresponding 73.6 and 82.1 percent values obtained when imipramine and DMI were extracted separately. The cumulative recovery total of combined imipramine and DMI equaled 182.9 percent (see Table I, Part C).

Recovery studies from whole blood agreed with initial plasma findings in that 67.4 percent of added imipramine was present in the hydrochloric acid extract while 1.9 percent appeared in the phosphate buffer extract after washing with heptane plus isoamyl alcohol. The calculated recovery of added DMI from whole blood was 90.0 percent in the phosphate buffer extract, and

5.2 percent in the hydrochloric acid phase. Cumulative recovery totals of imipramine and DMI in whole blood were 86.1 and 104.3 percent, respectively.

The study of recovery of added imipramine and DMI from whole brain homogenates was restricted to phosphate buffer wash and to the hydrochloric acid extract. After the addition of imipramine or DMI to whole brain homogenates, recovery of imipramine in the acidic extract was 70.6 percent, and the recovery of DMI in the phosphate buffer wash after heptane plus isoamyl alcohol was 42.5 percent. Additional extraction phases were not measured, nor were cumulative recoveries calculated.

These recovery studies in plasma, whole blood, and brain homogenates indicate that the solvent extraction and estimation techniques used (12) adequately differentiate between imipramine and DMI, and yield reproducible quantitation of these compounds in plasma, whole blood, and brain homogenates. These results are in agreement with Gillette and coworkers who have shown significant separation of imipramine and DMI with this solvent extraction technique (64).

B. Imipramine Time Course Studies

Imipramine hydrochloride, 40 mg/kg free base, was administered intraperitoneally to rats weighing

250-350 grams. At various times the animals were sacrificed, and whole blood and brain samples were taken for measurement of imipramine and DMI content. A compilation of the blood and brain imipramine time course data appears in Table II.

In both whole blood and brain, a triphasic course of imipramine concentration is apparent (Figures 5 and 6). In the first phase, from 0 to 1 hour, there is a sharp increase in imipramine concentration with a peak at 1.15 micrograms per milliliter in whole blood, and 20.9 micrograms per gram in brain. This initial phase is followed by a rapid decrease during the second phase which occurs between 1 and 3 hours. The final phase is characterized by the appearance of a slow, nearly exponential decline after 3 hours. This time course pattern of imipramine was previously noted by Bickel and Weder (9), and they attribute the respective three phases to invasion (absorption), distribution, and elimination of imipramine. Half-life calculations for the distribution phase of imipramine in brain and blood equal 1.3 and 1.2 hours, respectively, while the elimination half-lives in brain and blood were 3.4 and 9.5 hours (Figure 7). Blood and brain imipramine concentrations of 0.07 and 1.5 micrograms per milliliter or gram, respectively, were detectable 24 hours after intraperitoneal injection of imipramine. The ratio of

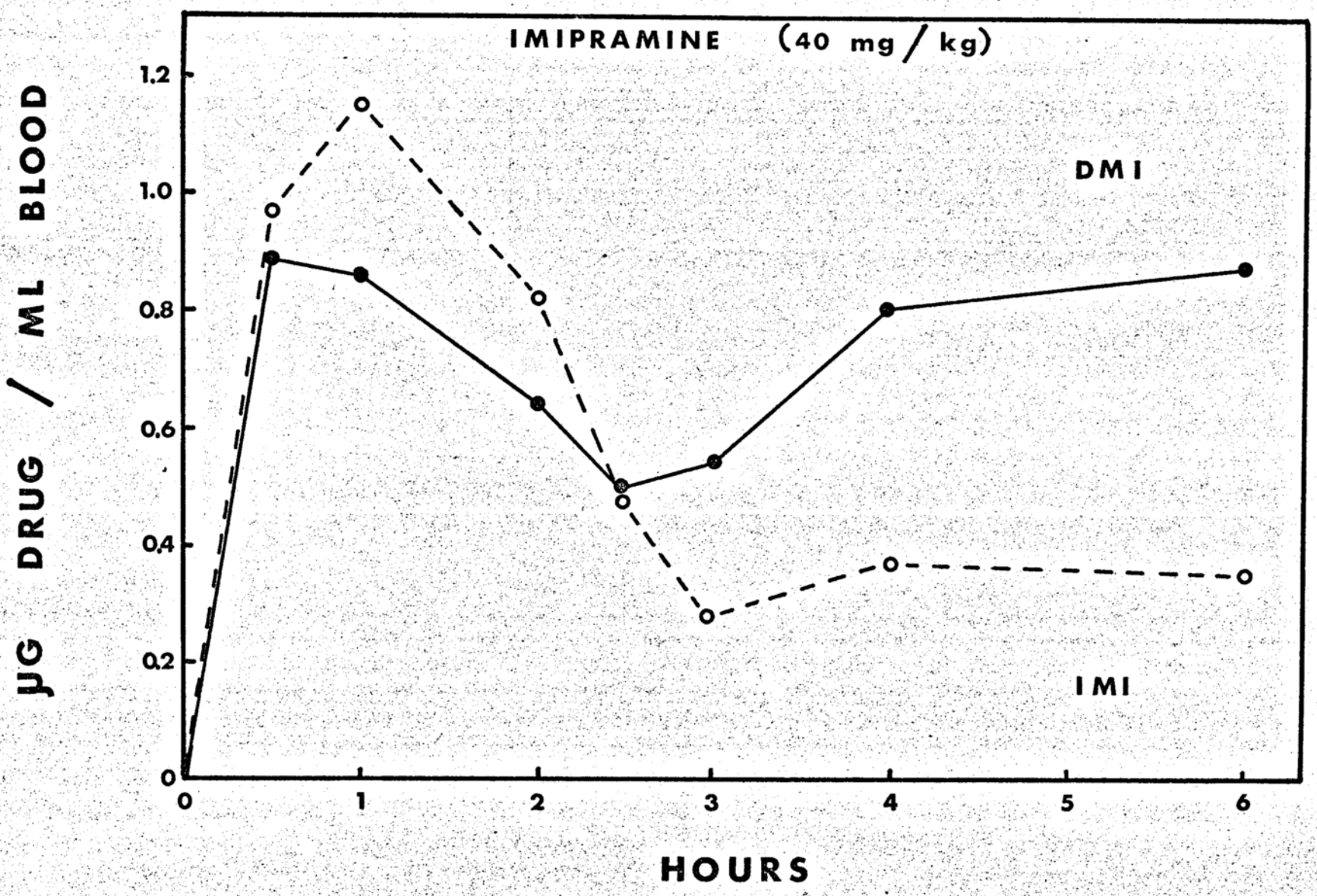
## TABLE II

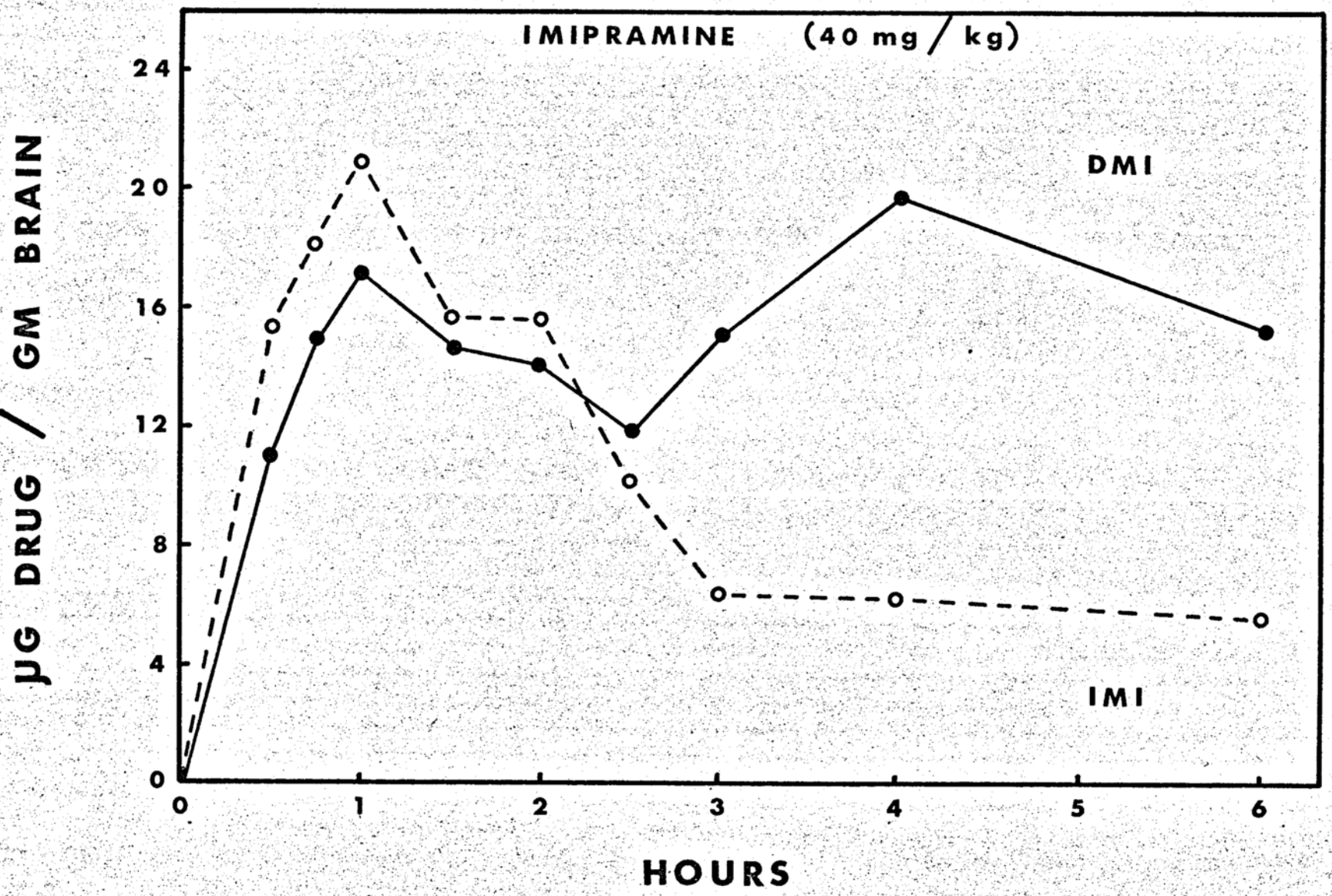
LEVELS OF IMIPRAMINE (IMI) AND DESMETHYLIMIPRAMINE (DMI) IN WHOLE BLOOD AND BRAIN AFTER ADMINISTRATION OF IMIPRAMINE[+] TO RATS

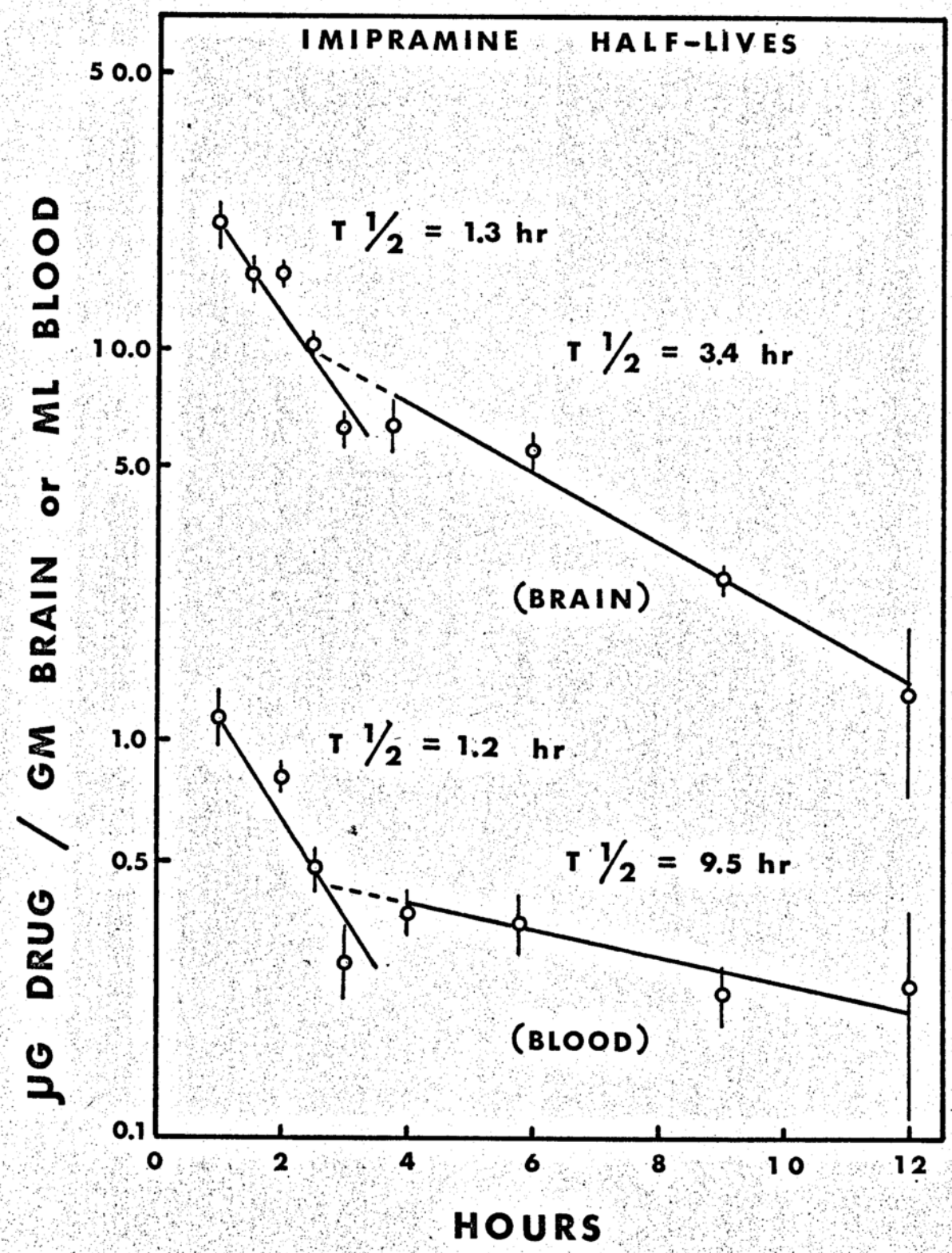
| Time (min) | Whole Blood (μg/ml)* | | Whole Brain (μg/gm)* | |
|---|---|---|---|---|
| | IMI | DMI | IMI | DMI |
| 30 | 0.97±0.17 (5) | 0.89±0.12 (5) | 17.3±3.5 (5) | 11.0±0.5 (4) |
| 45 | --- | --- | 18.1±2.4 (8) | 14.8±1.8 (12) |
| 60 | 1.15±0.18 (8) | 0.86±0.18 (8) | 20.9±2.7 (10) | 17.1±2.9 (10) |
| 90 | --- | --- | 15.7±1.5 (6) | 14.7±1.9 (6) |
| 120 | 0.82±0.06 (10) | 0.64±0.11 (10) | 15.7±0.9 (10) | 14.1±1.2 (10) |
| 150 | 0.48±0.06 (12) | 0.49±0.05 (12) | 10.1±0.8 (11) | 12.0±1.7 (11) |
| 180 | 0.28±0.06 (6) | 0.54±0.07 (6) | 6.3±0.9 (7) | 14.5±1.9 (7) |
| 240 | 0.37±0.05 (8) | 0.80±0.12 (8) | 6.4±0.9 (8) | 19.8±1.6 (8) |
| 360 | 0.35±0.07 (6) | 0.87±0.15 (6) | 5.6±0.6 (6) | 15.2±1.8 (6) |
| 540 | 0.23±0.04 (4) | 0.85±0.14 (4) | 2.6±0.2 (4) | 12.6±1.8 (4) |
| 720 | 0.24±0.14 (4) | 0.77±0.06 (4) | 1.3±0.6 (4) | 10.3±0.8 (4) |
| 1140 | 0.09±0.03 (4) | 0.56±0.09 (4) | 2.2±0.3 (4) | 9.0±0.9 (4) |
| 1440 | 0.07±0.03 (4) | 0.66±0.17 (4) | 1.5±0.4 (4) | 10.1±1.1 (4) |

*Expressed as the mean values obtained with the number of animals in parentheses ±$s_{\bar{x}}$ (standard error of the mean).

[+]40 ml/kg.


IMIPRAMINE (40 mg / kg)
DMI
IMI
µG DRUG / ML BLOOD
HOURS
0
0.2
0.4
0.6
0.8
1.0
1.2
0
1
2
3
4
5
6


IMIPRAMINE (40 mg / kg)
DMI
IMI
µG DRUG / GM BRAIN
HOURS
0
4
8
12
16
20
24
0
1
2
3
4
5
6


IMIPRAMINE HALF-LIVES
50.0
10.0
5.0
1.0
0.5
0.1
T 1/2 = 1.3 hr
T 1/2 = 3.4 hr
(BRAIN)
T 1/2 = 1.2 hr
T 1/2 = 9.5 hr
(BLOOD)
0
2
4
6
8
10
12
HOURS
µG DRUG / GM BRAIN or ML BLOOD

brain imipramine to blood imipramine was approximately 20/1 at all time points studied.

In the rat, imipramine is rapidly metabolized by demethylation to DMI in liver microsomes (12), and microgram quantities of imipramine and DMI may be detected in plasma or tissues within 15 minutes after intraperitoneal administration of imipramine (9). Following administration of imipramine, 40 mg/kg free base, the demethylated metabolite, DMI, exhibited two peak concentrations in both whole blood and brain (Figures 5 and 6). Peak blood DMI concentrations of 0.86 and 0.87 microgram per milliliter appeared at 1 and 6 hours, respectively. Brain DMI levels peaked at 17.1 and 19.8 micrograms per gram at 1 and 4 hours, respectively. Statistical comparison of peak DMI levels with DMI levels at 2.5 hours verified the existence of a significant difference, thus suggesting that these are true peaks, and not simply a reflection of biological variation. Standard error of the mean was used as the statistical criterion for significance. Blood and brain DMI concentrations of 1.5 and 10.1 micrograms per milliliter or gram, respectively, were detectable 24 hours after intraperitoneal injection of imipramine. The ratio of brain DMI to blood DMI was approximately 20/1 at all time points studied.

The appearance of the double DMI peak in the blood and brain after imipramine administration was reported in 1969 by Anderson, Morris, and Finger (88). Data reported by other investigators concerning DMI levels versus time after imipramine injection lacked sufficient replication, and failed to uncover the development of this unusual blood and brain level pattern due to the number and spacing of time points chosen (9,12).

C. Desmethylimipramine Time Course Studies

The observation of a double peaked disappearance curve of DMI in blood and brain after imipramine administration prompted investigations into the time course of DMI concentrations in the blood and brain after the administration of DMI. Desmethylimipramine hydrochloride, 40 mg/kg free base, was administered intraperitoneally to rats. At succeeding time points, the animals were sacrificed and whole blood and brain samples were taken for quantitation of DMI. The results of this study appear in Table III and Figures 8 and 9.

Desmethylimipramine concentrations in the blood and brain reached 2.28 and 27.2 micrograms per milliliter or gram, respectively, within 30 minutes, and proceeded to attain peak levels of 2.28 micrograms per milliliter in the blood and 39.4 micrograms per gram

TABLE III

LEVELS OF DESMETHYLIMIPRAMINE IN WHOLE BLOOD AND BRAIN ATER ADMINISTRATION OF DESMETHYLIMIPRAMINE TO RATS

| Time (min) | Dose (mg/kg) | Whole Blood DMI (μg/ml)* | | Whole Brain DMI (μg/gm)* | |
|---|---|---|---|---|---|
| 30 | 40 | 2.28 | (2) | 27.2±13.6 | (3) |
| 60 | 40 | 2.03±0.32 | (6) | 30.5± 6.1 | (6) |
| 120 | 40 | 1.45±0.33 | (5) | 39.4±11.6 | (6) |
| 150 | 40 | 1.41±0.34 | (4) | 34.1± 7.7 | (4) |
| 180 | 40 | 1.08±0.26 | (3) | 19.8± 3.1 | (6) |
| 240 | 40 | 1.24±0.26 | (5) | 17.0± 3.1 | (7) |
| 360 | 40 | 0.67±0.13 | (6) | 13.2± 2.2 | (6) |
| 720 | 40 | 0.64 | (2) | 6.8± 1.5 | (3) |
| 1440 | 40 | 0.40 | (2) | 5.9± 4.0 | (3) |
| 60 | 20 | 1.11±0.10 | (3) | 14.2± 1.1 | (4) |
| 120 | 20 | 0.73±0.06 | (4) | 9.2± 0.8 | (4) |
| 150 | 20 | 0.70-0.04 | (4) | 11.4± 1.1 | (4) |
| 240 | 20 | --- | | 5.3± 0.2 | (4) |
| 360 | 20 | 0.49±0.07 | (4) | 8.0± 1.0 | (4) |
| 540 | 20 | 0.51 | (2) | 4.4 | (2) |

*Expressed in the mean value obtained with the number of animals in parentheses $\pm s_{\bar{x}}$ (standard error of the mean).

DESMETHYLIMIPRAMINE
(40 mg/kg)
(20 mg/kg)
μG DRUG / ML BLOOD
HOURS
0
0.4
0.8
1.2
1.6
2.0
2.4
1
2
3
4
5
6

DESMETHYLIMIPRAMINE
(40 mg/kg)
(20 mg/kg)
μG DRUG / GM BRAIN
HOURS
0
10
20
30
40
1
2
3
4
5
6

in the brain. The appearance of the double peak was not noted at this dose. However, it was apparent that the blood and brain levels attained after an intraperitoneal dose of 40 mg/kg DMI greatly exceeded the DMI concentrations observed after the administration of the same dose of imipramine, and suggested that the biphasic peak might be masked. Thus, the DMI dose was reduced to 20 mg/kg and the experiment was repeated (Table III).

After DMI administration, 20 mg/kg intraperitoneally, peak DMI concentration was observed in the blood and brain within 1 hour. Blood DMI peaked at 1.11 micrograms per milliliter while the DMI concentration in the brain attained a peak level of 14.2 micrograms per gram (Figures 8 and 9). These blood and brain DMI levels coincide closely with DMI concentrations noted after administration of imipramine, 40 mg/kg. However, the double peak again was not apparent at a DMI dose of 20 mg/kg.

Blood and brain levels of DMI following a dose of 40 mg/kg exhibited a triphasic time course in a manner similar to that observed with imipramine (Figures 8 and 9). This triphasic time course includes invasion (absorption), distribution, and elimination phases (9). Half-life calculations for the distribution phase of DMI in brain and blood equal 1.1 and 2.1 hours,

respectively, while the elimination phase half-lives of DMI in brain and blood equaled 15.8 and 9.8 hours (Figure 10). Blood and brain concentrations of 0.4 and 5.9 micrograms per milliliter or gram, respectively, were detectable 24 hours after DMI administration, 40 mg/kg.

Half-life, t½, values for imipramine and DMI have been compiled in Table IV. Values signified by an asterisk were determined from the approximately exponential elimination of metabolically formed DMI after the administration of imipramine, 40 mg/kg. These calculated t½ values are in agreement with the experimentally determined elimination half-life values for DMI after the intraperitoneal administration of DMI, 40 mg/kg.

The absence of the double peaked DMI levels in whole blood and brain after DMI administration, in contrast with the presence of this phenomenon after administration of imipramine, indicates the possibility of an interaction between imipramine and DMI that might alter the disposition of metabolically formed DMI in comparison with exogenously administered DMI. Several experimental observations by other investigators suggest possible explanations for this unusual pattern.

Experiments reported by Bickel (89) on the <u>in vitro</u> metabolism of imipramine and its metabolites by

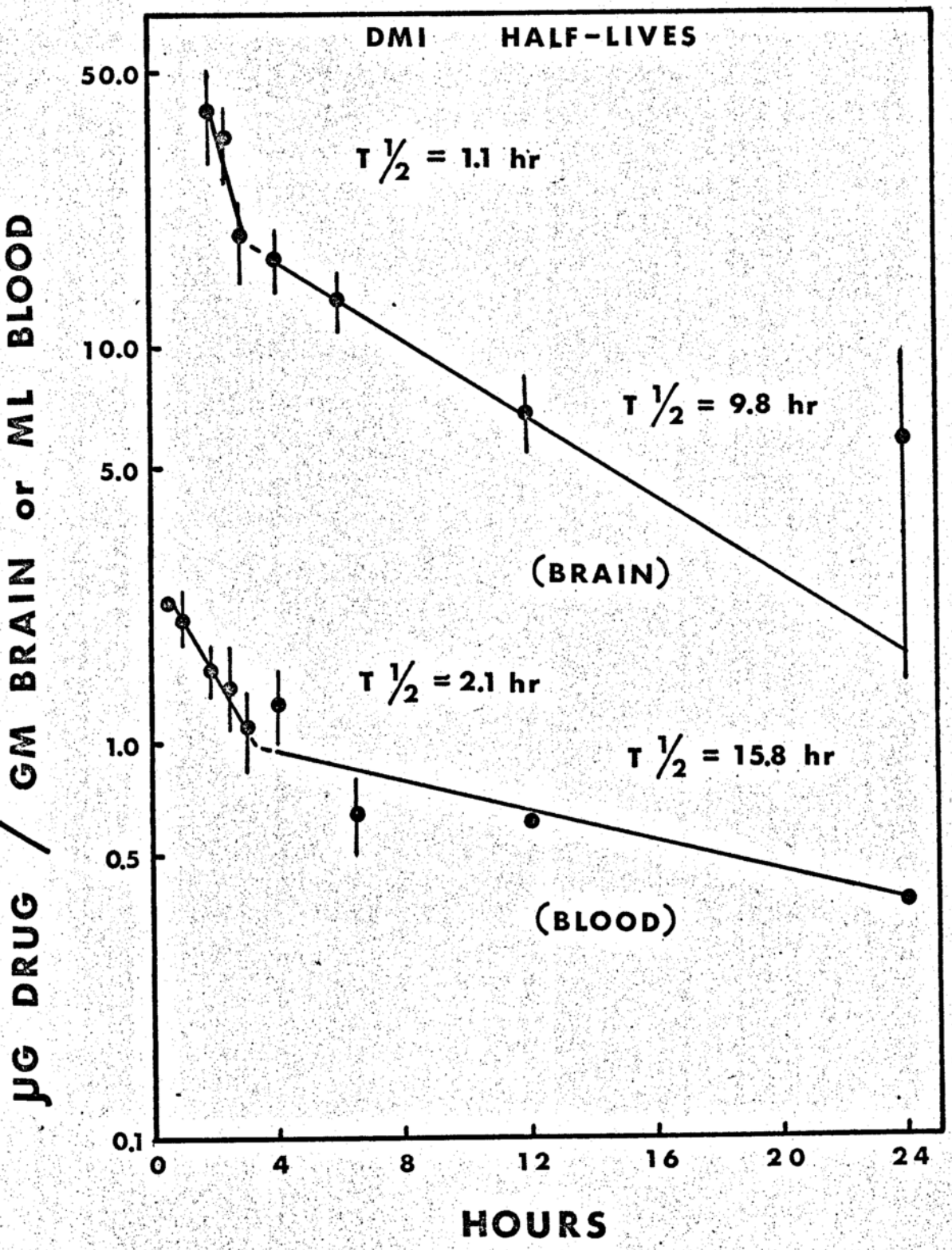

DMI HALF-LIVES
T ½ = 1.1 hr
T ½ = 9.8 hr
(BRAIN)
T ½ = 2.1 hr
T ½ = 15.8 hr
(BLOOD)
50.0
10.0
5.0
1.0
0.5
0.1
0
4
8
12
16
20
24
HOURS
µG DRUG / GM BRAIN or ML BLOOD

TABLE IV

HALF-LIFE OF IMIPRAMINE AND DESMETHYLIMIPRAMINE IN RATS

| Treatment | Distribution (hours) | | Elimination (hours) | |
|---|---|---|---|---|
| | IMI | DMI | IMI | DMI |
| Imipramine (blood) | 1.2 | - | 9.5 | 25.6* |
| Desmethylimipramine (blood) | - | 2.1 | - | 15.8 |
| Imipramine (brain) | 1.3 | - | 3.4 | 9.3* |
| Desmethylimipramine (brain) | - | 1.1 | - | 9.8 |

*Determined from exponential elimination of metabolically formed DMI after imipramine administration.

rat liver microsomes indicated that the rate of imipramine desmethylation to DMI decreases when the imipramine concentration exceeds 2-3 micromoles per 5 milliliters of a standardized rat liver microsomal preparation. This decrease in the rate of formation of DMI is apparently due to enzyme inhibition by excess substrate (imipramine). Bickel (89) has also shown that imipramine is extensively bound to rat liver microsomes, and has suggested that this might decrease the concentration of free imipramine, and thus may limit the rate of conversion to DMI. These data confirm the original report of Dingell (11) which showed that imipramine is bound to the extent of 1.1 micrograms per microgram microsomal protein.

The occurrence of two peak DMI levels in time following imipramine administration might be related to the phenomenon of substrate inhibition and microsomal protein binding. As the amount of imipramine presented to the liver increases during the invasion phase, saturation of the N-demethylation reaction might occur, and be followed by substrate inhibition. This phenomenon might be accompanied by extensive microsomal binding of imipramine. The net result of these two mechanisms would be a decrease in the rate of formation of DMI. In the presence of this decrease in the rate of formation of DMI, distribution to other large

parenchymous organs and metabolism of the initially formed DMI by other routes might contribute to the decline in blood DMI. Eventually, as the imipramine levels decrease in the liver during the imipramine distribution phase, the rate of enzymatic demethylation of imipramine might increase. Thus, the concentration of imipramine presented to the liver might determine the rate of formation of DMI. This initial inhibition of imipramine metabolism to DMI followed by the restoration of demethylation could account for the double peaked time course of DMI in the blood and brain after the administration of imipramine.

An alternative possible explanation for the existence of the dual DMI peaks following imipramine administration might be based on the fact that both imipramine and DMI are excreted in the bile, and undergo enterohepatic circulation (9). In most species, large amounts of imipramine and DMI are excreted as glucuronides in the bile during the first hour after imipramine administration. At least part of the imipramine and its nonpolar metabolites are reabsorbed from the gut, and are available for recycling. As a consequence of biliary excretion, a percentage of the metabolically formed DMI is sequestered for varying periods of time in the intestine prior to reabsorption. This unavailability of DMI might correlate in time with

the interval between the two peak concentrations in the blood (Figure 5). Whether biliary excretion and enterohepatic circulation, indeed, are responsible for the double peaked time course of DMI in the blood after imipramine administration requires further investigation.

D. Effect of SKF-525A on Imipramine Demethylation

It previously has been reported that SKF-525A (diethylaminoethyl diphenylpropylacetate) inhibits a variety of microsomal oxidative reactions (90). Recently, SKF-525A has been observed to inhibit the N-dealkylation of imipramine to DMI in rats (8). Inhibition of imipramine demethylation by SKF-525A provides a useful tool for the elucidation of the respective pharmacological roles of imipramine and DMI. For this reason, the effect of SKF-525A pretreatment on the demethylation of exogenously administered imipramine in rats was studied.

SKF-525A, 80 mg/kg, was administered orally to rats one hour prior to the intraperitoneal injection of imipramine, 40 mg/kg, and the animals were sacrificed at various time points thereafter. Whole blood and whole brain homogenates were collected and prepared for quantitation of imipramine and DMI content. Results of this study are presented in Table V and depicted in Figures 11 and 12.

TABLE V

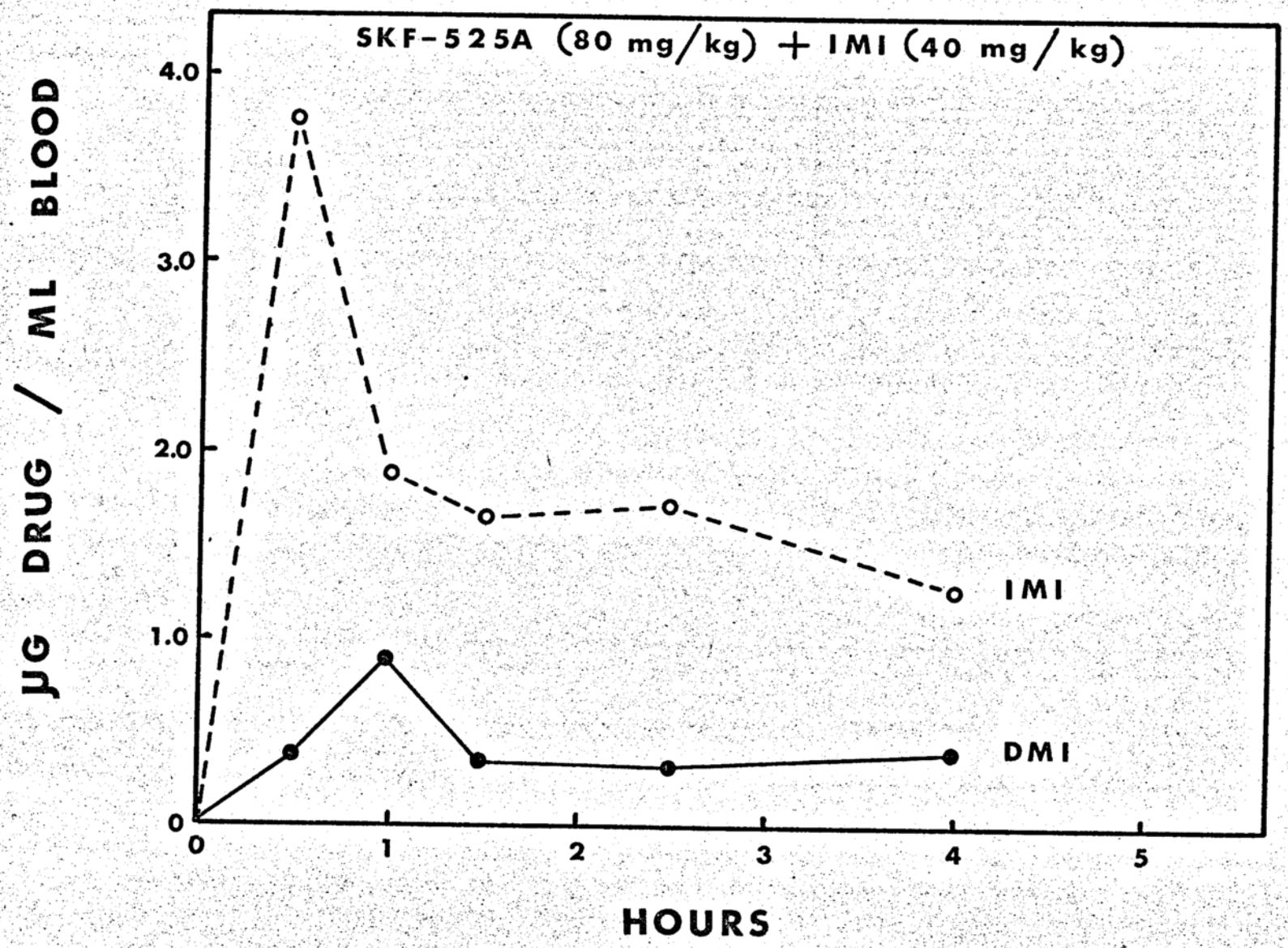
LEVELS OF IMIPRAMINE (IMI) AND DESMETHYLIMIPRAMINE (DMI) IN WHOLE BLOOD AND BRAIN WITH OR WITHOUT ADMINISTRATION OF SKF-525A[+] FOLLOWED BY ADMINISTRATION OF IMIPRAMINE[°] TO RATS

| Time (min) | SKF-525A | Whole Blood (µg/ml)* IMI | Whole Blood (µg/ml)* DMI | Whole Brain (µg/gm)* IMI | Whole Brain (µg/gm)* DMI |
|---|---|---|---|---|---|
| 30 | 0 | 0.97±0.17 (5) | 0.89±0.12 (5) | 17.3±3.5 (5) | 11.0±0.5 (4) |
| | + | 3.75±1.0 (5) | 0.39±0.04 (5) | 56.1±4.4 (5) | 4.3±1.1 (5) |
| 60 | 0 | 1.15±0.18 (8) | 0.86±0.18 (8) | 20.9±2.7 (10) | 17.1±2.9 (10) |
| | + | 1.88±0.85 (3) | 0.89±0.14 (3) | 33.2±8.7 (4) | 2.6±1.6 (5) |
| 90 | 0 | 0.63±0.08 (6) | 0.75±0.10 (6) | 15.7±1.5 (6) | 14.7±1.9 (6) |
| | + | 1.65±0.36 (6) | 0.37±0.16 (6) | 35.1±7.0 (6) | 4.3±1.1 (6) |
| 150 | 0 | 0.48±0.06 (12) | 0.49±0.05 (12) | 10.1±0.8 (11) | 12.0±1.7 (11) |
| | + | 1.72±0.24 (5) | 0.30±0.05 (5) | 29.3±2.2 (5) | 6.1±0.5 (5) |
| 240 | 0 | 0.37±0.05 (8) | 0.80±0.12 (8) | 6.4±0.9 (8) | 19.8±1.6 (8) |
| | + | 1.28±0.33 (4) | 0.41±0.11 (4) | 19.8±5.0 (4) | 11.1±2.7 (4) |

*Expressed as the mean values obtained with the number of animals in parentheses ± $s_{\bar{x}}$ (standard error of the mean).

[+]80 mg/kg.

[°]40 mg/kg.


SKF-525A (80 mg/kg) + IMI (40 mg/kg)
μG DRUG / ML BLOOD
4.0
3.0
2.0
1.0
0
0
1
2
3
4
5
HOURS
IMI
DMI

SKF-525A (80 mg/kg) + IMI (40 mg/kg)
IMI
DMI
HOURS
μg DRUG / GM BRAIN
0
10
20
30
40
50
60
1
2
3
4
5

In both blood and brain, levels of imipramine exceeded those of DMI from one-half to four hours. In blood and brain, imipramine concentrations peaked at 3.75 micrograms per milliliter and 56.1 micrograms per gram, respectively, at one-half hour. In contrast, DMI concentrations of 0.39 micrograms per milliliter and 4.3 micrograms per gram were observed at this time point. The blood imipramine to DMI ratio remained relatively constant from 1 to 4 hours. However, the brain imipramine to DMI ratio was significantly less at the 4 hour time point. Table V compares the blood and brain concentrations of imipramine and DMI observed after imipramine administration to rats with or without SKF-525A pretreatment. From these data, it is apparent that SKF-525A significantly inhibits the metabolic conversion of imipramine to DMI, and that this is due in large part to inhibition of microsomal N-dealkylation by SKF-525A. Anders (91) has suggested that SKF-525A produces this inhibitory effect by competitive combination with the active site of a microsomal N-demethylase.

### E. Effect of Varying the Dose of Desmethylimipramine on the Whole Blood and Brain Concentrations of Desmethylimipramine

The degree of pharmacological efficacy of a drug is generally proportional to drug concentration at an active

site (receptor). Dose-response relationships have been established for numerous pharmacological agents. However, the relationship between DMI dosage and the tetrabenazine reversal response has not been established. For this reason, the concentration of DMI administered was varied, and the subsequent whole blood and brain concentrations of DMI quantitated.

Desmethylimipramine doses, ranging from 1.0 to 60.0 mg/kg were administered intraperitoneally to rats, and the animals were sacrificed 4 hours later. Whole blood and brain samples were collected and assayed for DMI content. The results of this study appear in Table VI.

As the dosage of DMI was increased from 1.0 to 50.0 mg/kg, the whole blood levels of DMI rose linearly to approximately 1.0 microgram per milliliter (Figure 13). In whole brain, as the dosage of DMI was increased from 1.0 to 30.0 mg/kg, the DMI levels rose linearly to approximately 7.5 micrograms per gram (Figure 14). However, as DMI doses greater than 30.0 mg/kg were administered, a steeper linear rise in DMI levels was observed.

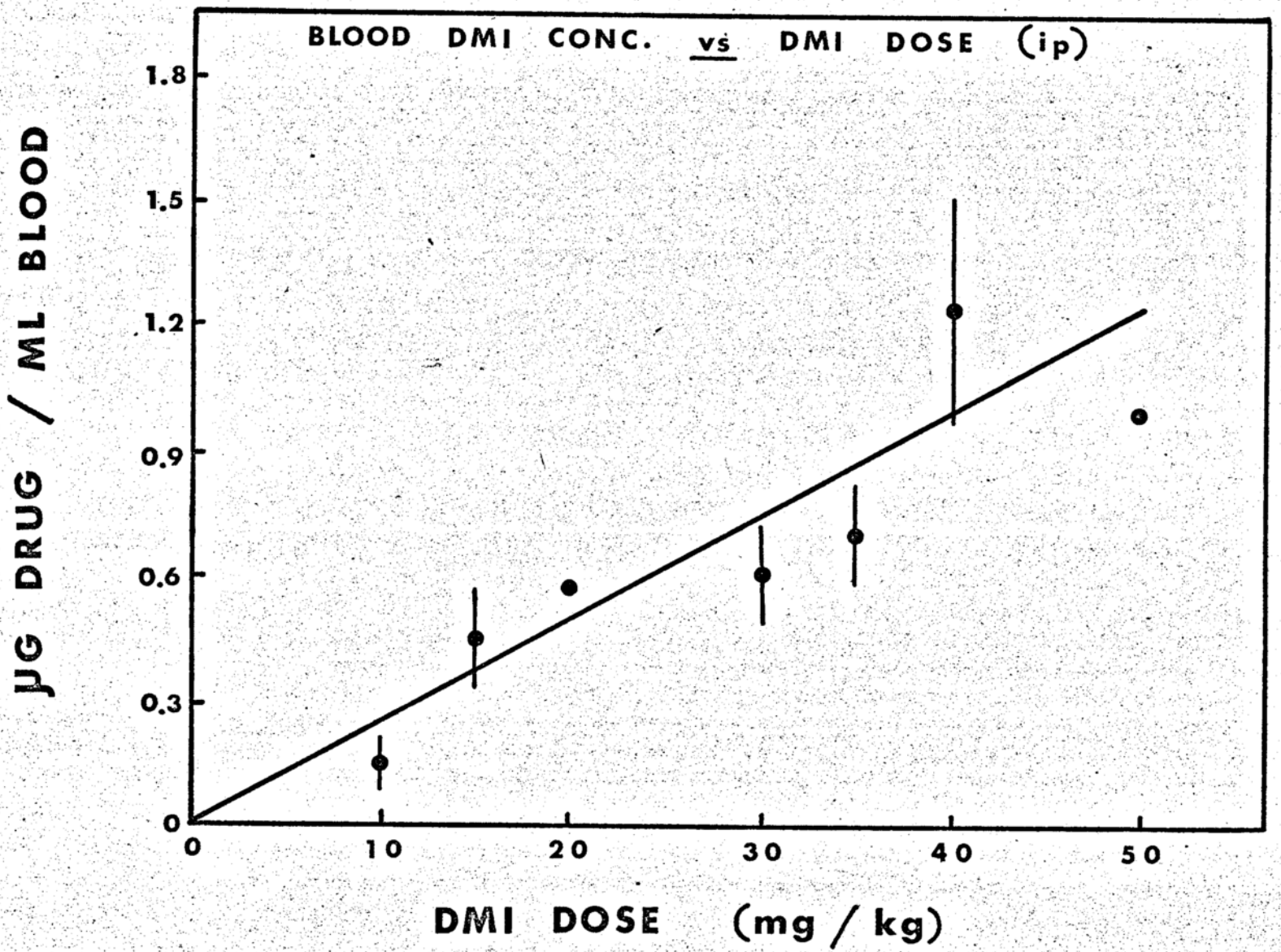
The biphasic increase in brain DMI concentrations after varying intraperitoneal doses of DMI suggests that more than one uptake mechanism plays a role in the entry of DMI into the brain. It is possible that DMI

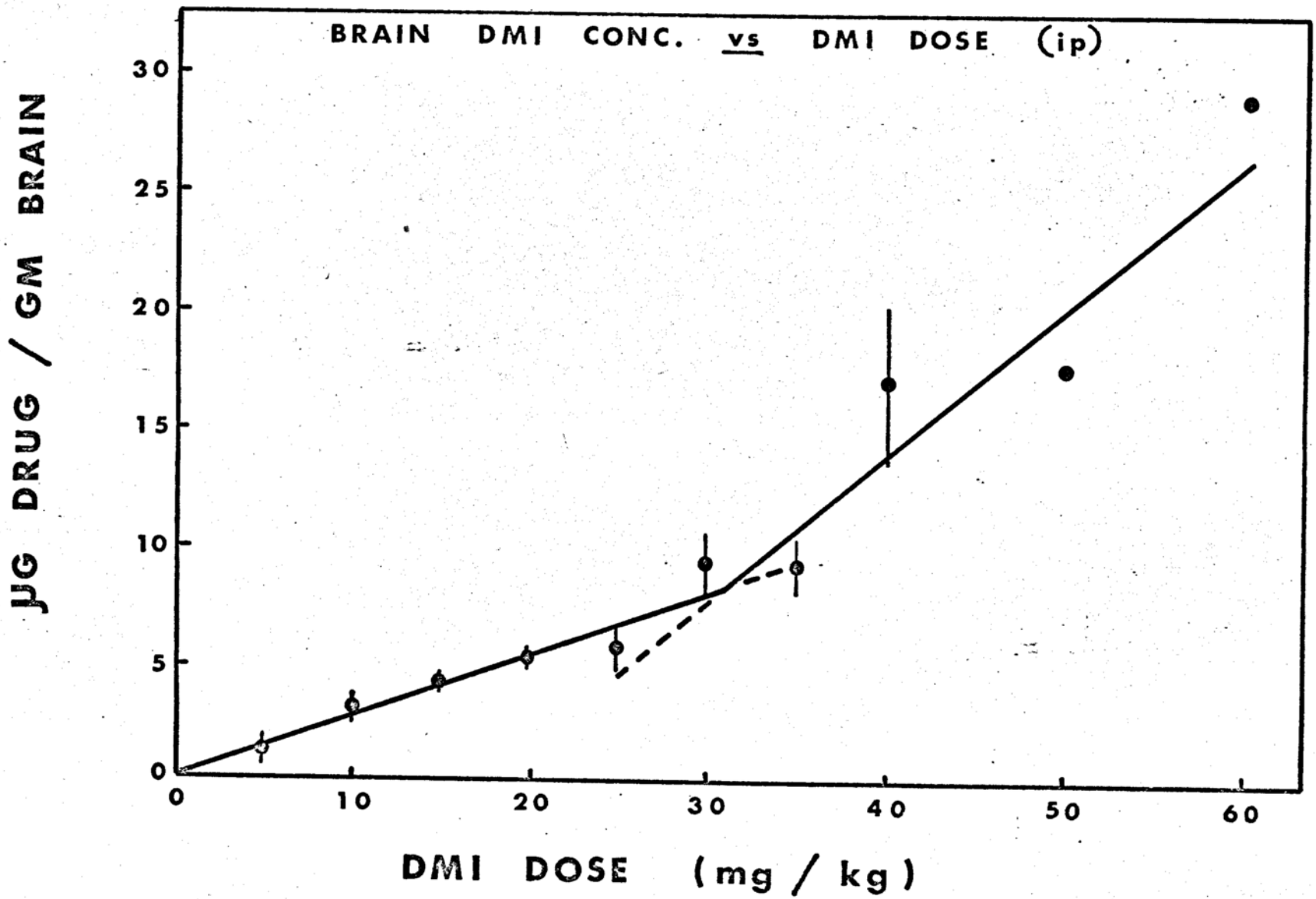
## TABLE VI

LEVELS OF DESMETHYLIMIPRAMINE (DMI) IN WHOLE BLOOD AND BRAIN AFTER ADMINISTRATION OF VARIOUS DOSES OF DESMETHYLIMIPRAMINE TO RATS

| DMI Dose (mg/kg) | Whole Blood DMI (μg/ml)* | | Whole Brain DMI (μg/gm)* | |
|---|---|---|---|---|
| 1 | ---- | (2) | 0.1 | (2) |
| 5 | ---- | (2) | 1.4±0.6 | (5) |
| 10 | 0.14±0.06 | (4) | 3.2±0.6 | (4) |
| 15 | 0.45±0.13 | (4) | 4.3±0.2 | (6) |
| 20 | ---- | | 5.3±0.2 | (4) |
| 25 | 0.56 | (1) | 5.6±1.0 | (4) |
| 30 | 0.60±0.12 | (6) | 9.4±1.2 | (6) |
| 35 | 0.69±0.12 | (4) | 9.2±1.2 | (4) |
| 40 | 1.24±0.26 | (5) | 17.0±3.1 | (7) |
| 50 | 0.99 | (2) | 15.0 | (2) |
| 60 | ---- | | 29.0 | (1) |

*Expressed as the mean value obtained with the number of animals in parentheses ± $s_{\bar{x}}$ (standard error of the mean).


BLOOD DMI CONC. vs DMI DOSE (ip)
µG DRUG / ML BLOOD
1.8
1.5
1.2
0.9
0.6
0.3
0
0
10
20
30
40
50
DMI DOSE (mg / kg)


BRAIN DMI CONC. vs DMI DOSE (ip)
μG DRUG / GM BRAIN
0
5
10
15
20
25
30
0
10
20
30
40
50
60
DMI DOSE (mg / kg)

may enter the brain by simple diffusion, and/or by active transport.

### F. Effect of Imipramine, Desmethylimipramine, Tetrabenazine, and SKF-525A on Locomotor Activity

The preceeding studies have dealt primarily with the disposition of imipramine and desmethylimipramine (DMI) in whole blood and brain. These concentration versus time studies were carried out in order to provide a basis for investigations attempting to correlate pharmacological activity with blood and brain levels of imipramine and its major metabolite, DMI.

The pharmacological endpoint chosen for further investigation was locomotor activity, e.g., horizontal movement from one location to another. The locomotor activity measured in the following studies was spontaneous and not induced by external stimuli. Therefore, it should not be confused with the forced motor activity associated with the use of rotarods and motor-driven rotating drums. Thus, the term spontaneous motor activity will be used in the following text to describe locomotor activity.

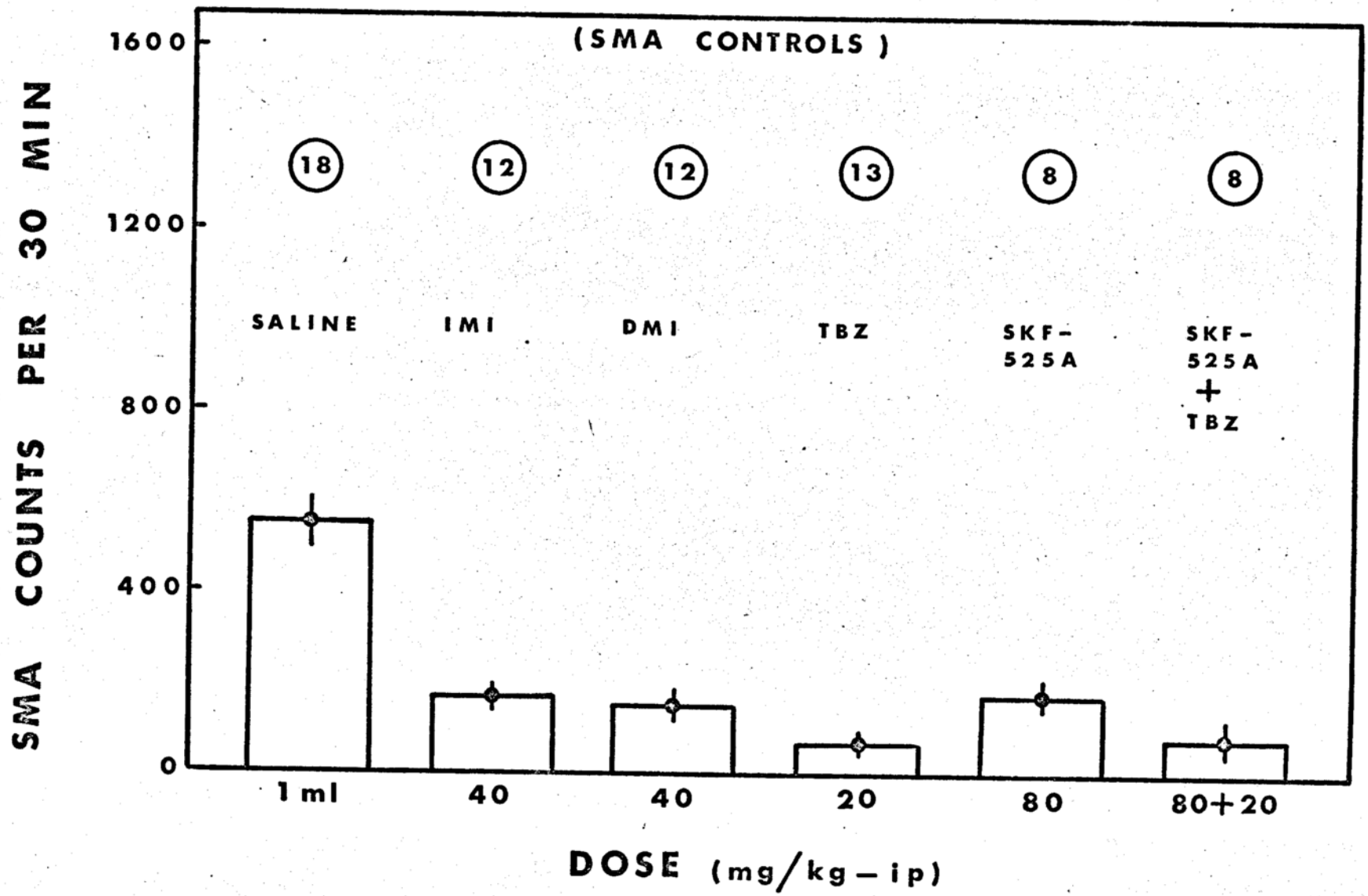
The spontaneous motor activity (SMA) induced by the administration of the benzoquinolizine tetrabenazine methanesulfonate after imipramine and DMI pretreatment is dramatically increased (70,71). This phenomenon,

known as "tetrabenazine reversal," was the pharmacological parameter measured in the following studies. Spontaneous motor activity was quantitated in photoelectric SMA cages (92). The results obtained by this method are in agreement with those reported by other investigators using jiggle cage or rotating drum methods (93).

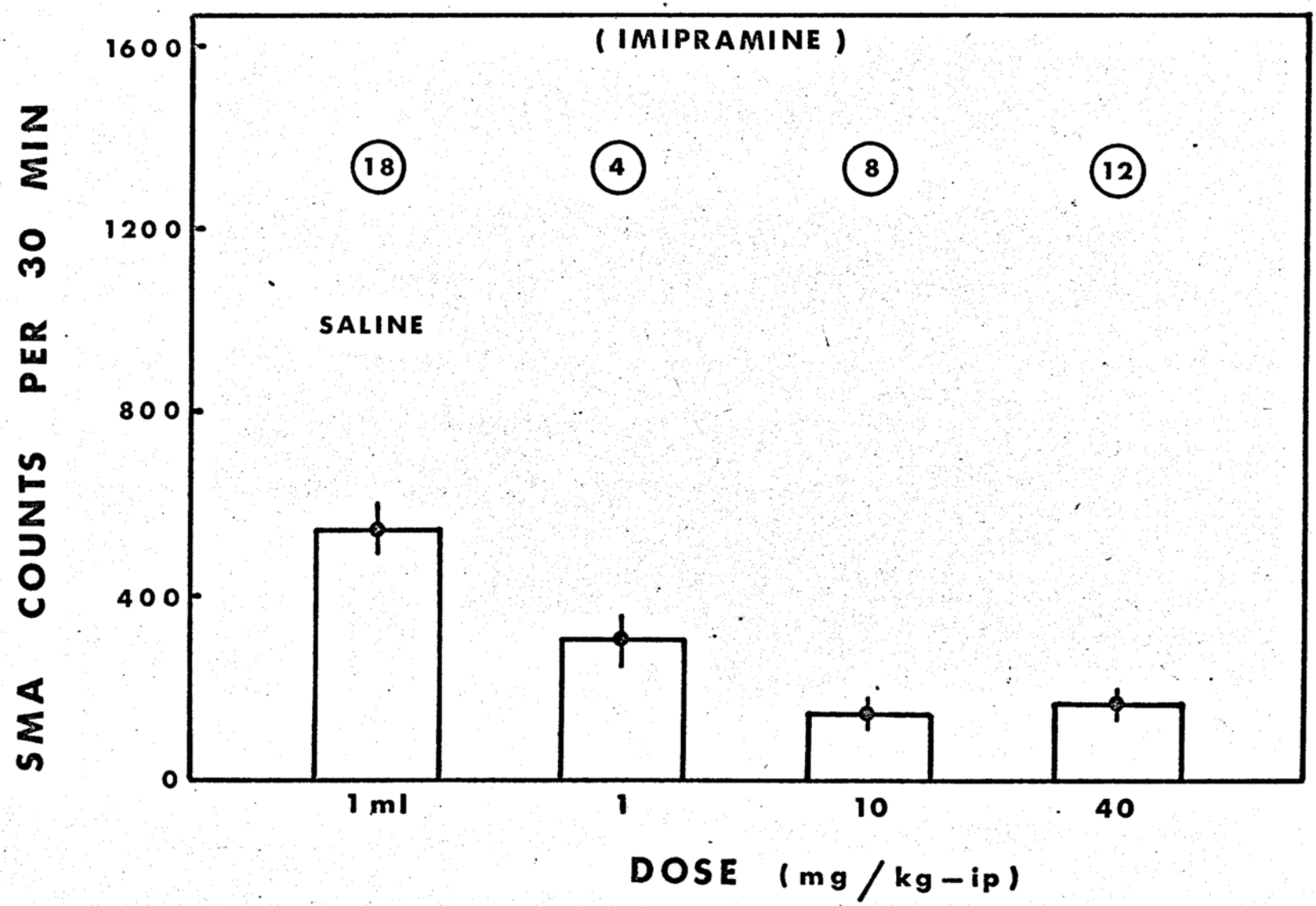
The effects of saline, imipramine, DMI, tetrabenazine (TBZ), SKF-525A, and SKF-525A plus TBZ on rat SMA are presented in Figure 15. Spontaneous motor activity values for saline, imipramine, DMI, and TBZ were summated for 30 minutes during the 20-50 minute interval following drug administration. Thirty minute summated SMA values were obtained for SKF-525A alone and SKF-525A plus TBZ during the 80-110 minute interval following oral administration of SKF-525A.

Saline (control) SMA was found to be approximately 590 counts per 30 minutes. The SMA elicited after imipramine, DMI, TBZ, SKF-525A, and SKF-525A plus TBZ administration was markedly reduced as compared to saline (control) SMA. Tetrabenazine was the most potent agent in reducing SMA, and this reduction was not influenced by SKF-525A pretreatment. The decreased SMA noted with imipramine, DMI, and SKF-525A was approximately equal.

Quantitation of SMA was also conducted using different doses of imipramine and DMI, and the results


(SMA CONTROLS)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
18
12
12
13
8
8
SALINE
IMI
DMI
TBZ
SKF-525A
SKF-525A + TBZ
1 ml
40
40
20
80
80+20
DOSE (mg/kg – ip)

obtained were compared to saline (control) SMA. Imipramine in doses as low as 1.0 mg/kg caused a significant reduction in SMA (Figure 16). This SMA reduction by imipramine appeared to reach a maximum at a dose of 10.0 mg/kg. Furguielle and coworkers (59) have reported that imipramine at doses of 7.5 and 15.0 mg/kg caused marked depression of SMA, while lower or higher doses did not alter SMA. These observations are not consistent with the results noted in the present study, although experimental conditions in both cases were somewhat similar. Both studies used naive rats, circular photocell activity cages, and 30 minute SMA recording periods. However, Furguielle, et al., recorded SMA for 30 minute periods ending 30, 150, and 270 minutes after imipramine injection. The rats were removed from the photocell cage during the intervals between SMA measurement. It has been observed in the present investigations that animals are subjected to trauma ranging from slight to extreme during intraperitoneal injection of chemical agents, initial placement into the unfamiliar environment of the SMA cage, and repeated handling. For these reasons, the present studies allowed a 20 minute period for injection recovery during cage acclimation, and the experimental use of each animal was limited to a single SMA counting period. Control animals were subjected to the same


( IMIPRAMINE )
1600
1200
800
400
0
18
4
8
12
SALINE
SMA COUNTS PER 30 MIN
1 ml
1
10
40
DOSE ( mg / kg – ip )

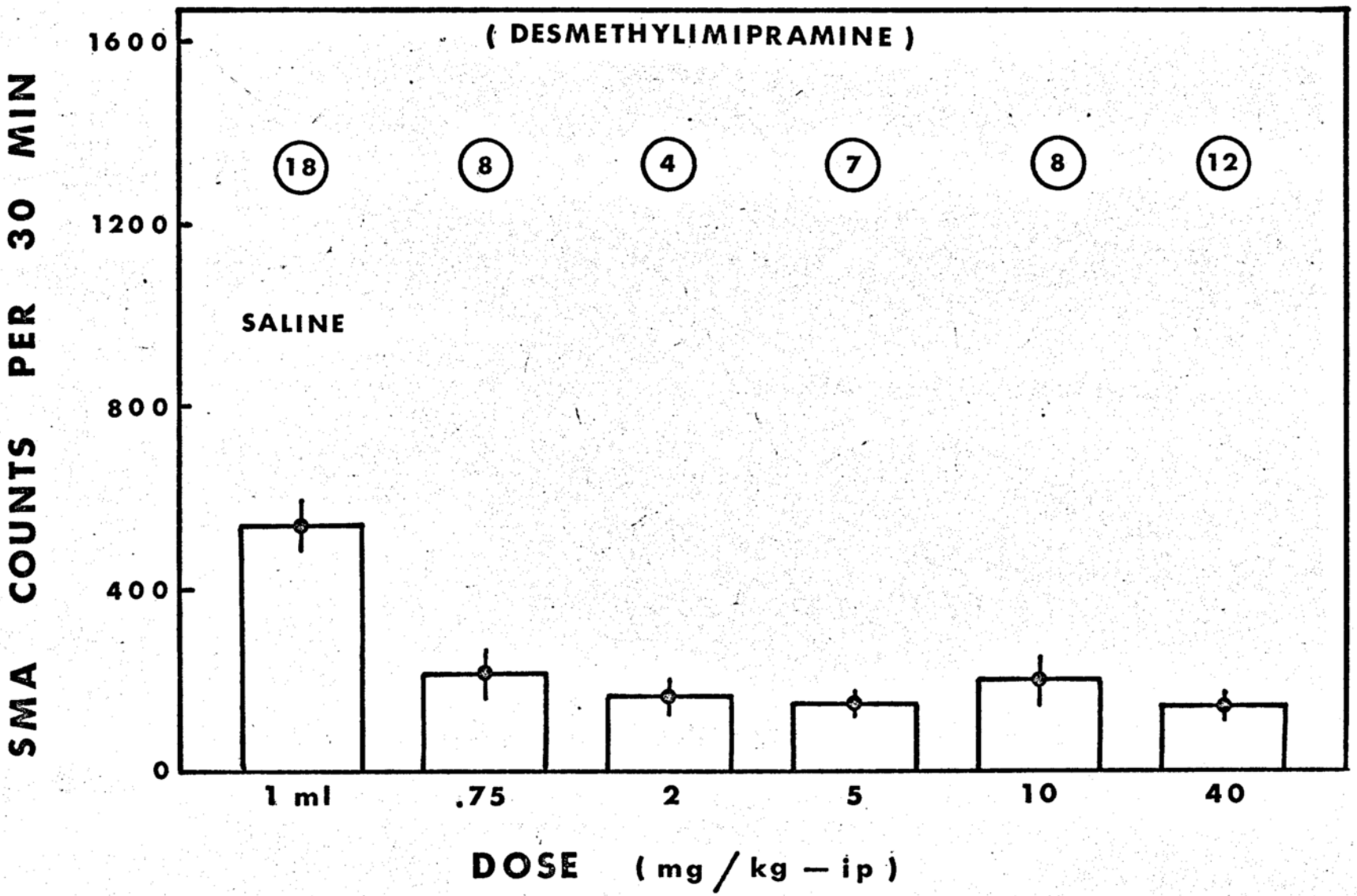
experimental conditions as drug-treated animals. These differences in technique probably account for the previously mentioned disagreement in SMA reduction after imipramine injection.

Desmethylimipramine, in doses ranging from 0.75 to 40.0 mg/kg also produced a significant depression in SMA as compared to the control (Figure 17). The observation that DMI decreases SMA at a dose of 10.0 mg/kg contradicts the finding of Meduna, et al. (94), who reported a significant increase in rat SMA with comparable doses of DMI. This discrepancy may also be due to differences in experimental techniques used in SMA quantitation.

The reduction of SMA by DMI in doses ranging from 0.75 to 40.0 mg/kg seemed to approximate the reduced SMA values obtained after 10.0 and 40.0 mg/kg doses of imipramine. Previous reports (59,95) have claimed that reduction of SMA by imipramine is significantly greater than SMA reduction by DMI. The present investigations do not confirm these findings.

### G. Effect of Tetrabenazine Administration on Imipramine-induced Spontaneous Motor Activity in Rats

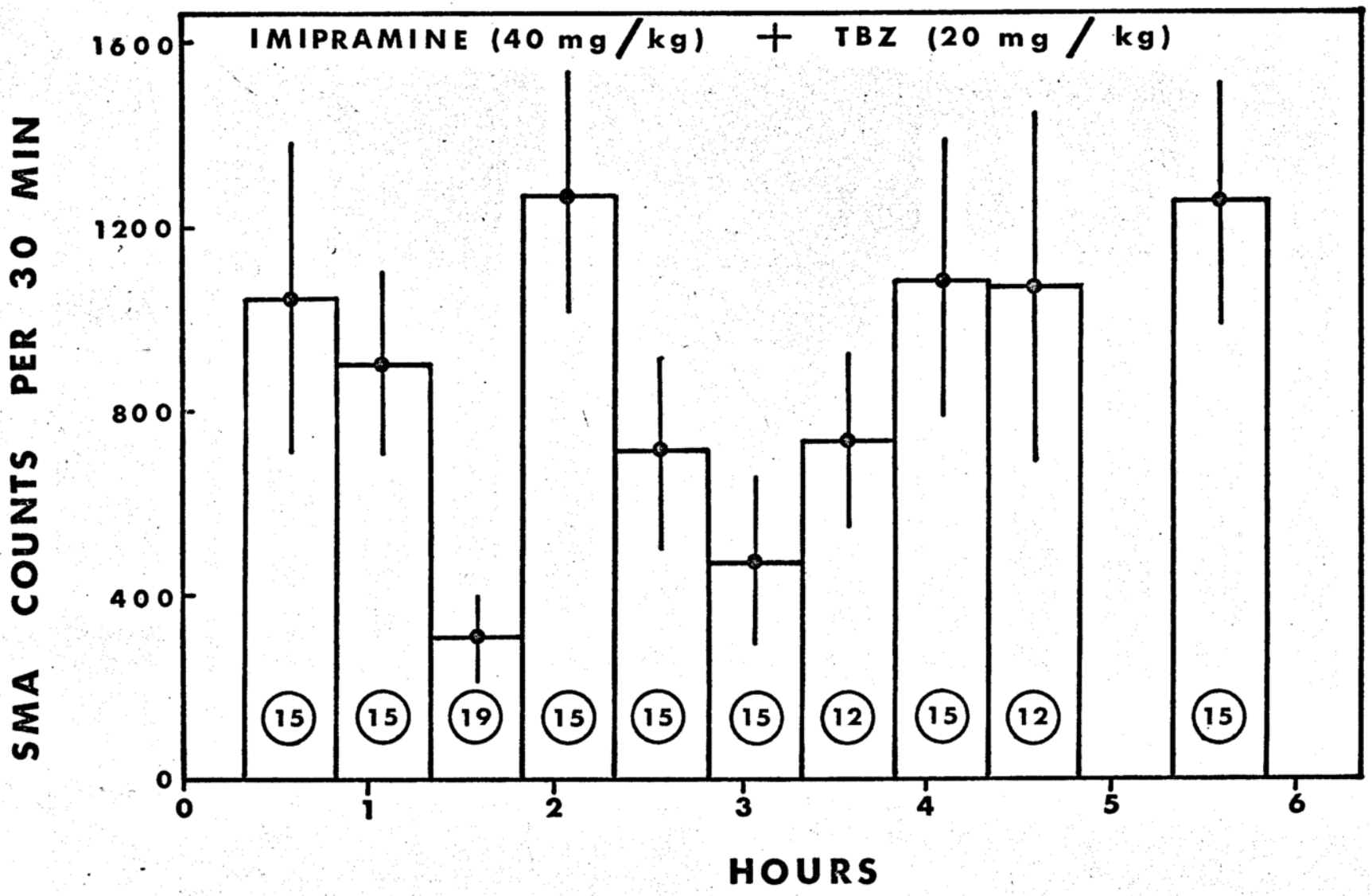
The reduction of SMA by imipramine is illustrated in Figure 16. This reduction of SMA (hypoactivity) can be reversed by hyperactivity by the concurrent or


( DESMETHYLIMIPRAMINE )
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
18
8
4
7
8
12
SALINE
1 ml
.75
2
5
10
40
DOSE ( mg / kg — ip )

subsequent administration of tetrabenazine (70,71). The following study was conducted to quantitate the degree of SMA reversal (hyperactivity) exhibited by rats when TBZ is administered at various times after imipramine treatment.

Imipramine, 40.0 mg/kg was administered intraperitoneally to rats, and at succeeding time points (0 to 5 hours) the animals were challenged with TBZ, 20 mg/kg. The SMA counts recorded in the 20-50 minute interval following TBZ injection were summated. A SMA counting lag-time was necessary for reasons previously discussed, and because Sulser has reported (96) that SMA reversal of imipramine or DMI by tetrabenazine is dependent upon the rapid release of central amine stores (primarily norepinephrine). This neurohumoral release in rats requires 15 to 30 minutes after administration of TBZ intraperitoneally. Both the rate of central norepinephrine release and the percentage of animals exhibiting SMA reversal are a function of the TBZ dose (96). The administration of a 20 mg/kg dose of TBZ to imipramine pretreated rats resulted in some degree of hyperactivity in about 75 percent of the animals within 20 minutes after TBZ injection. The results of TBZ administration on imipramine-induced SMA are shown in Figure 18.

It is immediately apparent that the degree of SMA reversal elicited by TBZ after imipramine pretreatment


IMIPRAMINE (40 mg / kg) + TBZ (20 mg / kg)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
0
1
2
3
4
5
6
HOURS
15
15
19
15
15
15
12
15
12
15

is not equal at every time interval. The SMA counts per 30 minutes at approximately 1.5 and 3.0 hours are significantly lower than the 0.5, 2.0, 4.0, and 5.5 hour SMA levels. The 1.5 and 3.0 hour SMA values are significantly greater than those obtained when imipramine or TBZ are administered alone. However, they are not significantly different from control values (Figure 15). The SMA values at the remaining time points are significantly greater than imipramine, TBZ, or saline (control) SMA levels. A previous report has indicated that the onset of reversal of imipramine reduced SMA by TBZ requires from 1.0 to 3.0 hours (42). The present studies show the time of initial onset of SMA reversal to be less than 30 minutes. It is quite probable that the other investigators chose different TBZ administration time points and thus did not detect the extreme hyperactivity noted at approximately 0.5 and 2.0 hours. The use of an insufficient number of animals at each counting period could also mask the hyperactivity noted early after TBZ administration to imipramine pretreated animals.

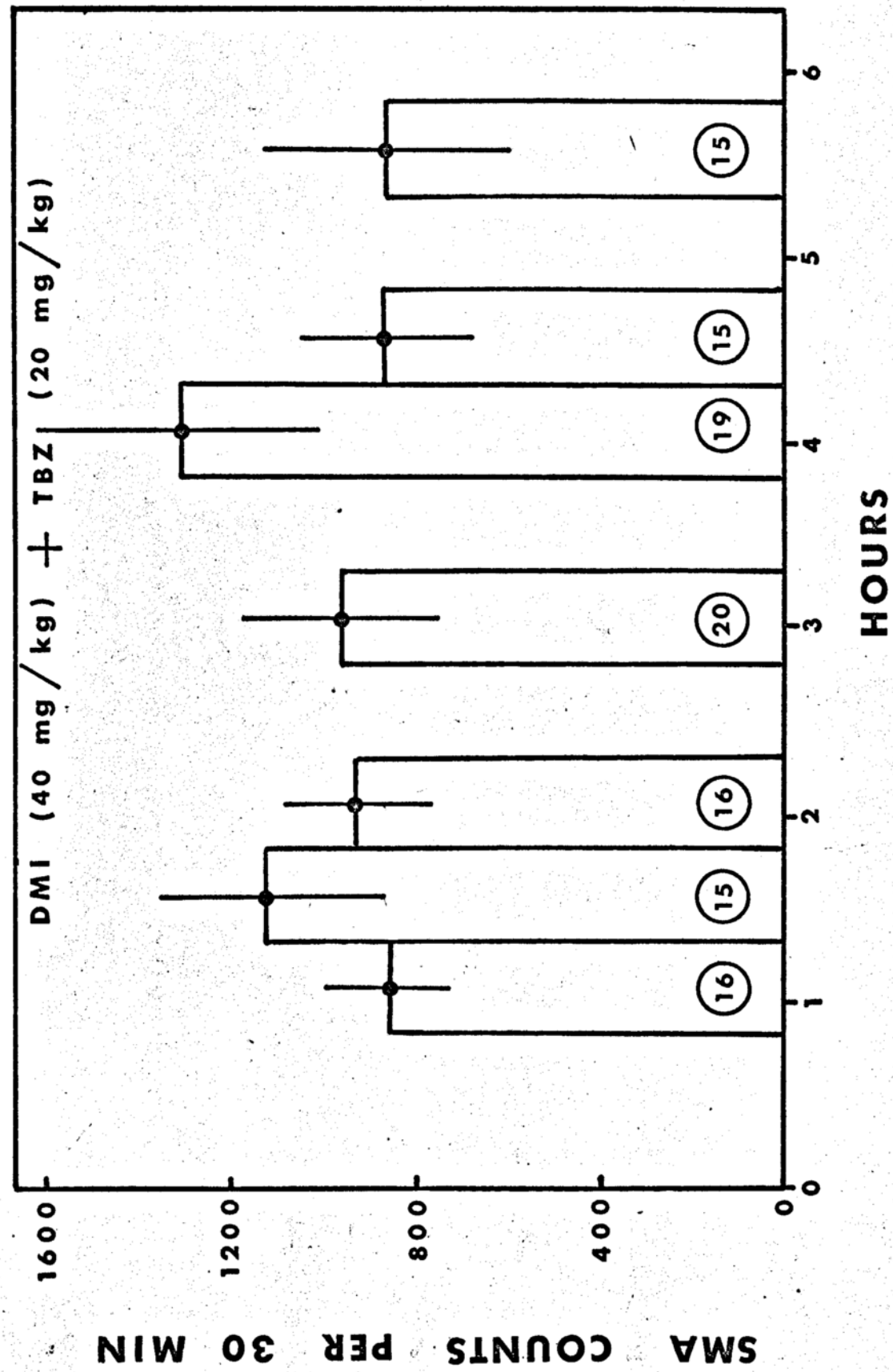
### H. Effect of Tetrabenazine Administration on Desmethylimipramine-induced Spontaneous Motor Activity in Rats

The degree of SMA reversal varied with the time of TBZ administration after imipramine. Thus, it was of

interest to investigate whether this variable SMA pattern would be reproduced when DMI was substituted for imipramine. These studies were carried out in order to elucidate the possible roles of imipramine and DMI in the TBZ reversal phenomenon.

Desmethylimipramine, 40 mg/kg, was administered intraperitoneally to rats, and at succeeding time points the animals were challenged with TBZ, 20 mg/kg. The SMA results of this study may be seen in Figure 19. The degree of TBZ reversal noted at all SMA counting intervals was not statistically different. However, at all counting intervals the levels of SMA reversal were significantly greater than the levels observed when DMI, TBZ, or saline were administered alone. The extreme variability in SMA exhibited when TBZ was administered at various times after imipramine was not apparent when TBZ was administered after DMI. The onset of SMA reversal, and the maximum degree of reversal for imipramine or DMI followed by TBZ did not appear to differ.

It was noted, however, that the concentrations of DMI in the blood and brain (Figures 8 and 9) after administration of DMI, 40 mg/kg, far exceeded the DMI concentrations attained after imipramine administration, 40 mg/kg (Figures 5 and 6). Since the variability of SMA reversal might be inhibited or prevented by higher

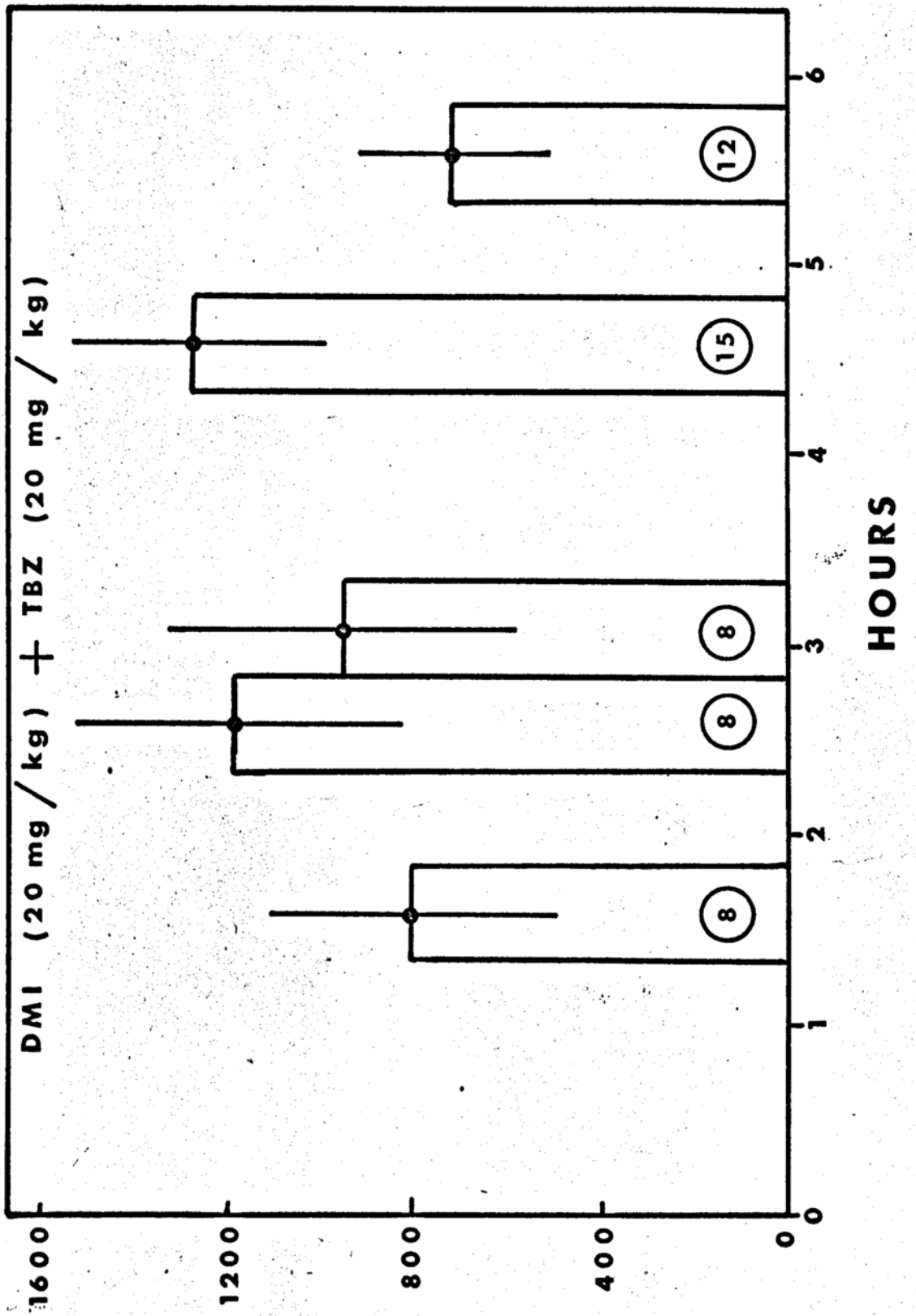

DMI (40 mg/kg) + TBZ (20 mg/kg)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
0
1
2
3
4
5
6
HOURS
16
15
16
20
19
15
15

concentrations of DMI, a lower DMI dose for TBZ reversal studies was chosen. A DMI dose of 20 mg/kg was found to produce blood and brain DMI levels comparable to those seen after the administration of imipramine, 40 mg/kg.

Desmethylimipramine, 20 mg/kg, was administered to rats, and at succeeding time points the animals were challenged with TBZ, 20 mg/kg. The SMA data resulting from this study are shown in Figure 20.

Again, the SMA reversal levels did not appear to be significantly different at any counting interval despite the fact that DMI levels in the blood and brain approximated those attained after imipramine in a dose of 40 mg/kg. At all time points, the levels of SMA reversal were significantly greater than the levels attained when DMI, TBZ, or saline were administered alone.

These findings suggest that imipramine <u>per se</u> modifies the degree of SMA reversal elicited when TBZ is administered to rats pretreated with imipramine. Previous studies on the disposition of DMI in the blood and brain after imipramine administration have shown a double peaked disappearance curve of metabolically formed DMI (Figures 5 and 6), and it was proposed that imipramine by substrate inhibition might be responsible for this biphasic disposition pattern. This modifying effect of imipramine on DMI disposition might also account for the extreme variations in SMA reversal noted


DMI (20 mg / kg) + TBZ (20 mg / kg)
SMA COUNTS PER 30 MIN
HOURS
0
400
800
1200
1600
1
2
3
4
5
6
8
8
8
15
12

when TBZ is administered after imipramine treatment. A modification of SMA reversal and DMI disposition by imipramine would indicate a major role for DMI in the tetrabenazine reversal phenomenon. These findings appear to substantiate, with respect to "tetrabenazine reversal," the role of imipramine as a "pro-drug," and the responsibility of DMI in the reversal phenomenon.

## I. Effect of SKF-525A Pretreatment on the Reversal of Imipramine-induced Spontaneous Motor Activity by Tetrabenazine in Rats

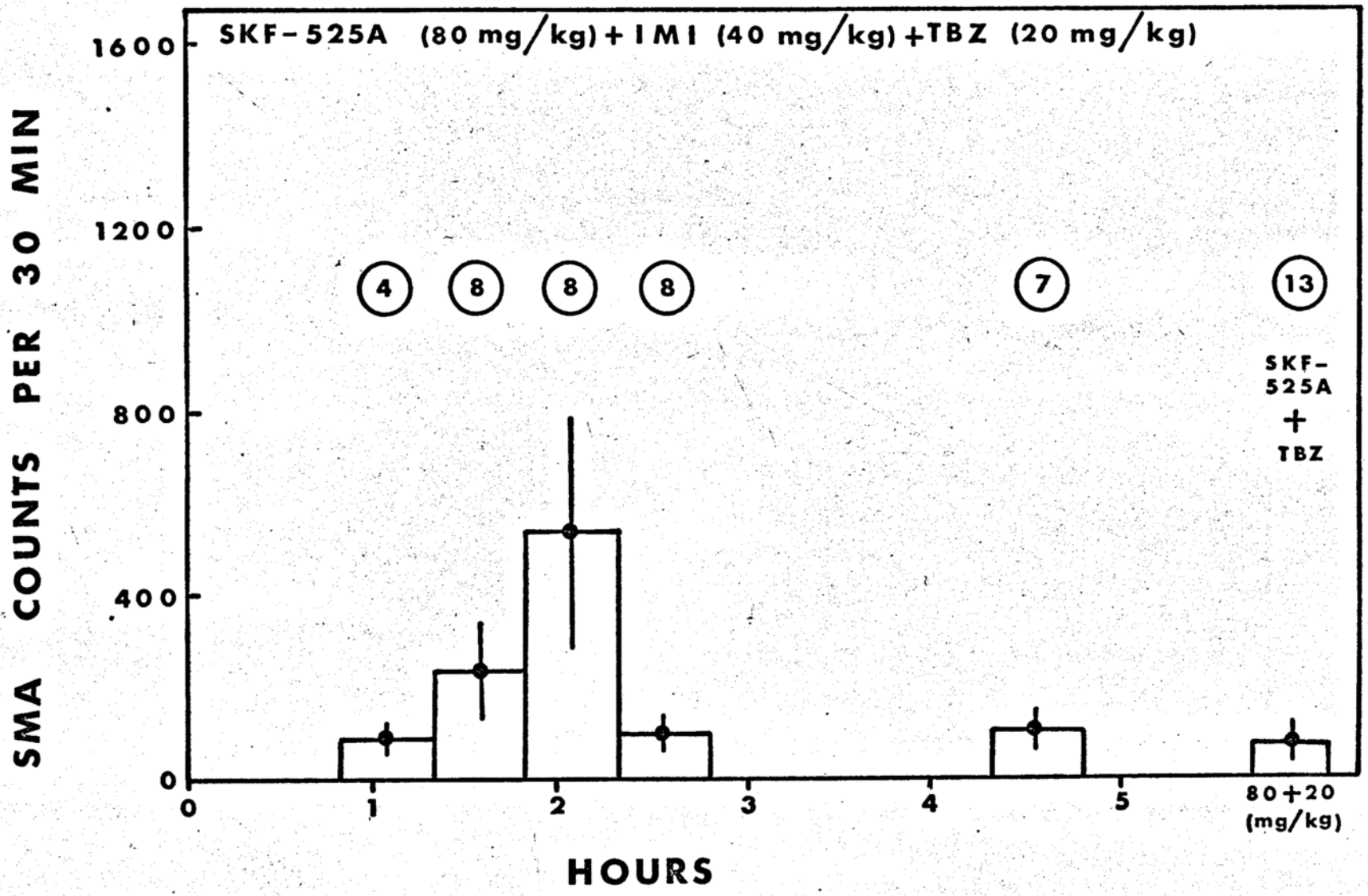
Other investigators have shown that SKF-525A effectively inhibits the metabolic transformation of imipramine to DMI in rats (8). However, the effect of SKF-525A pretreatment on the degree of SMA reversal exhibited when TBZ is administered after imipramine treatment has not been studied thoroughly. An inhibition or lack of inhibition of SMA reversal with SKF-525A pretreatment could further verify the indispensibility of DMI for induction of the TBZ reversal phenomenon in rats.

SKF-525A, 80 mg/kg, was administered orally to rats one hour prior to intraperitoneal injection of imipramine, 40 mg/kg. At succeeding time points, tetrabenazine (20 mg/kg) was administered, and SMA was quantitated during the subsequent 20 to 50 minute interval. The

results of this study appear in Figure 21.

It is evident that SKF-525A pretreatment produced a pronounced decrease in the degree of SMA reversal noted when TBZ was administered to rats previously treated with imipramine only (Figure 21). With the exception of the 2.0 hour SMA interval, the degree of reversal was significantly less than reversal levels attained without pretreatment with SKF-525A. In addition, these reduced SMA levels were not statistically different from SMA levels observed when SKF-525A and TBZ were administered in conjunction. With regard to the 2.0 hour SMA interval, the level attained was not statistically different from the saline (control) SMA level. However, this level was significantly greater than SMA levels observed when imipramine, DMI, TBZ, SKF-525A or SKF-525A plus TBZ were administered (Figure 15).

To insure that SKF-525A inhibition of SMA reversal was due to an inhibition of metabolically formed DMI, and not to some intrinsic pharmacological action of SKF-525A, DMI was substituted for imipramine, and the previous experiment was repeated. SKF-525A (80 mg/kg) was administered orally to rats one hour prior to intraperitoneal injection of DMI, 20 mg/kg. After 240 minutes, TBZ (20 mg/kg) was administered, and SMA was


SKF-525A (80 mg/kg) + IMI (40 mg/kg) +TBZ (20 mg/kg)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
4
8
8
8
7
13
SKF-525A + TBZ
0
1
2
3
4
5
80+20 (mg/kg)
HOURS

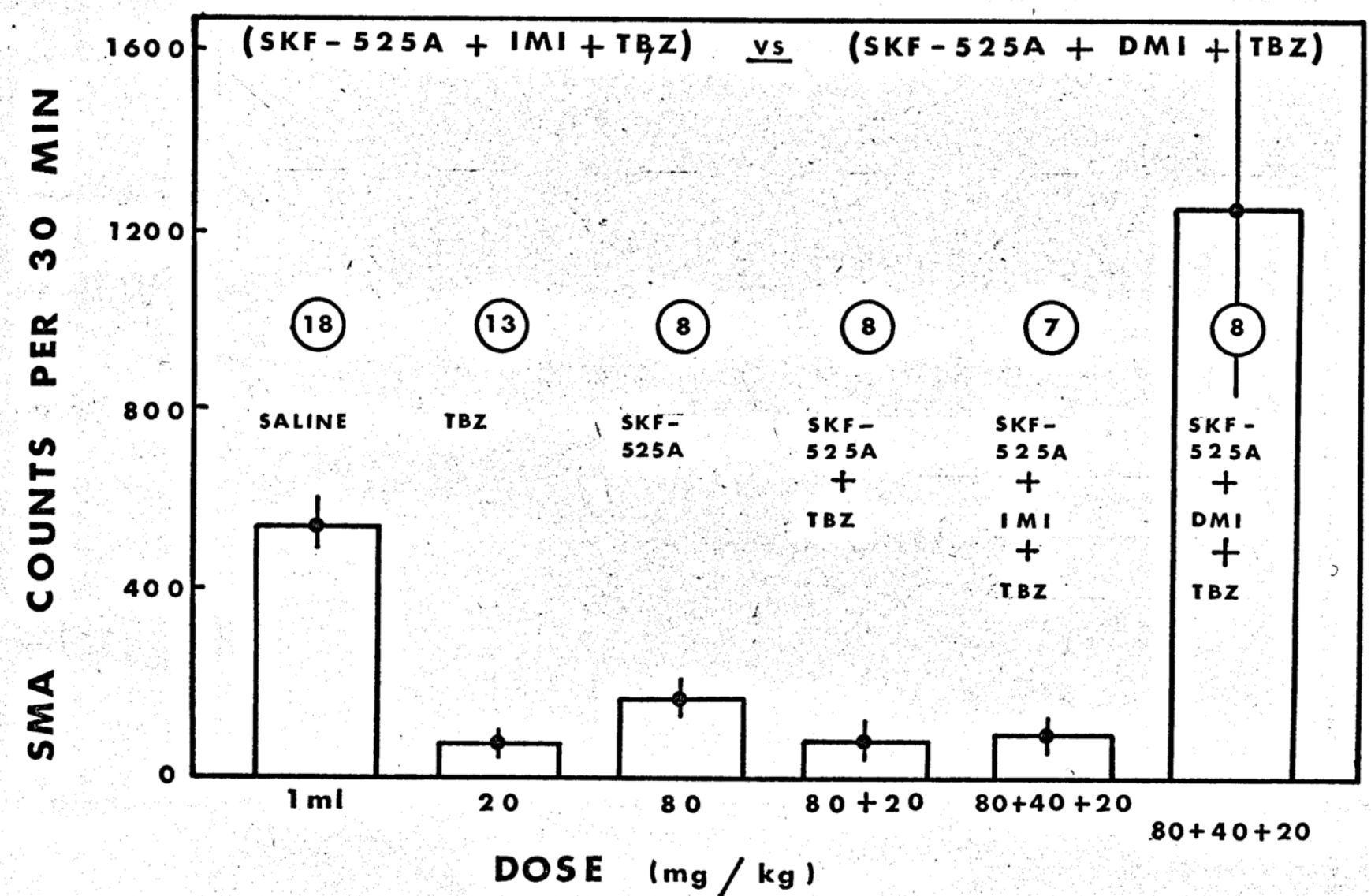
quantitated during the following 20 to 50 minute interval. Figure 22 illustrates the results of this experiment.

It is apparent that SKF-525A does not prevent the reversal phenomenon elicited when TBZ is administered after DMI. The SMA level attained was significantly greater than the saline (control) level, and SMA levels elicited by TBZ, SKF-525A, SKF-525A plus TBZ, or SKF-525A, imipramine, and TBZ in combination.

These data strongly suggest a requirement for DMI in the TBZ reversal phenomenon. The SMA reversal noted when TBZ was administered after imipramine may be due to the action of metabolically formed DMI, and not to the action of imipramine. These findings further substantiate the proposed "pro-drug" role for imipramine in the tetrabenazine reversal phenomenon and verify the necessity for DMI.

### J. Effect of Varying the Dose of Desmethylimipramine on the Degree of Reversal by Tetrabenazine of Desmethylimipramine-induced Spontaneous Motor Activity

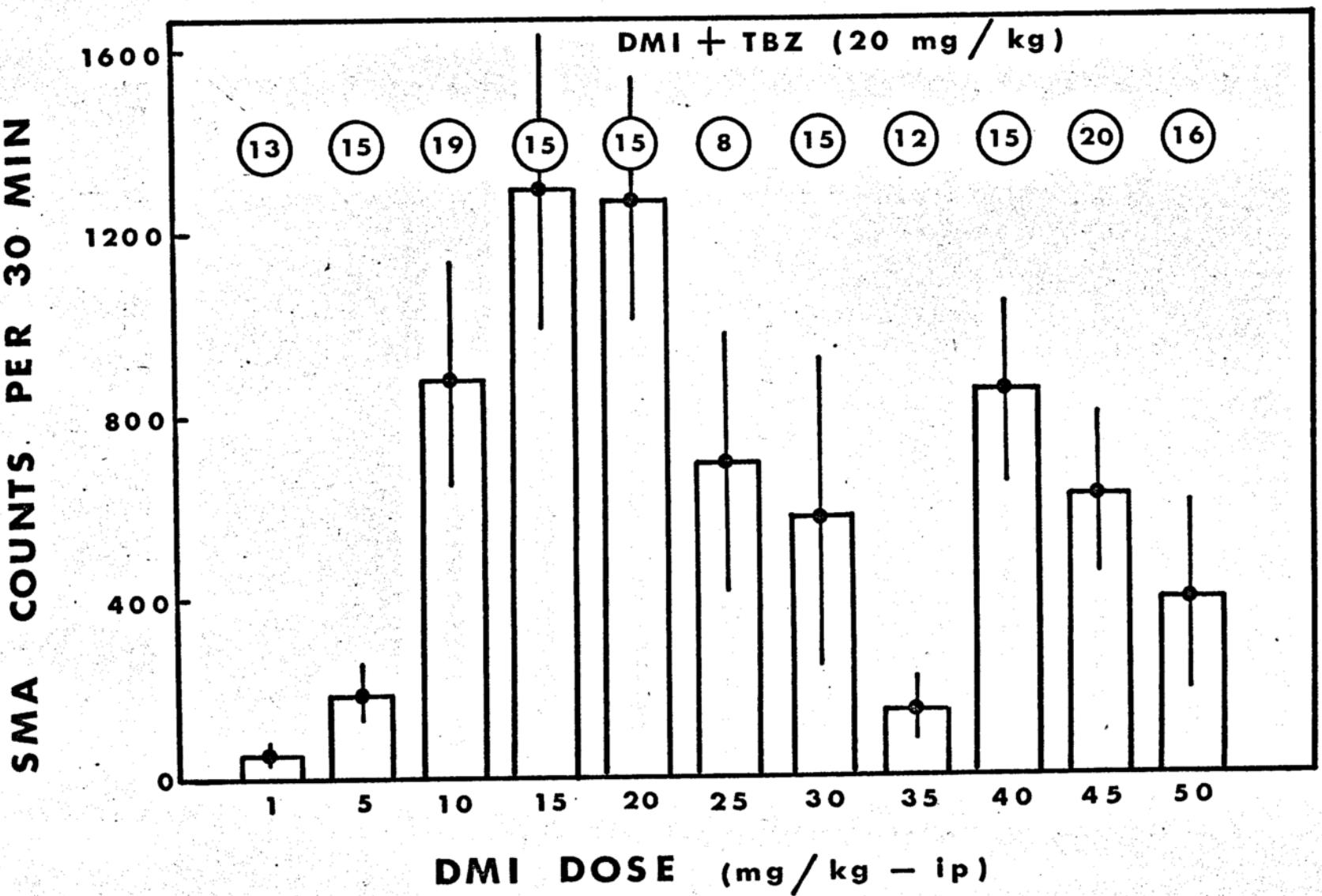
The role of DMI in the reversal of imipramine-induced SMA by TBZ promoted speculation as to whether variations in the amount of DMI administered would alter the degree of SMA elicited when TBZ is administered to DMI treated rats. The following study attempts to establish a dose-response relationship between DMI concentration and the degree of SMA reversal.


(SKF-525A + IMI + TBZ) vs (SKF-525A + DMI + TBZ)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
18
13
8
8
7
8
SALINE
TBZ
SKF-525A
SKF-525A + TBZ
SKF-525A + IMI + TBZ
SKF-525A + DMI + TBZ
1 ml
20
80
80+20
80+40+20
80+40+20
DOSE (mg / kg)

Desmethylimipramine concentrations ranging from 1.0 to 50.0 mg/kg were administered intraperitoneally to rats. The animals were challenged 240 minutes later with TBZ (20 mg/kg), intraperitoneally. Immediately after TBZ injection, the animals were placed in photocell cages, and SMA was quantitated during the following 20 to 50 minute interval. The results of this study appear in Figure 23.

A DMI dose of 10.0 mg/kg was shown to initiate TBZ reversal to a significantly greater extent than control SMA levels, or SMA levels observed after administration of TBZ and DMI separately. The degree of SMA reversal was greatest with 15.0 and 20.0 mg/kg DMI doses, but declined with DMI doses of 25.0, 30.0, and 35.0 mg/kg. A dose of 35.0 mg/kg followed by TBZ did not elicit significant SMA reversal. However, larger DMI doses (40.0 and 45.0 mg/kg) produced SMA reversal levels significantly greater than control levels. Thus, it appears that the dose of administered DMI alters the degree of SMA reversal noted when TBZ is administered after DMI. However, the dose-response relationship is atypical insofar as low or high doses of DMI trigger TBZ reversal, while intermediate doses are ineffective in producing this response.

This observation parallels the anomalous dose-response data reported on the interaction of imipramine


DMI + TBZ (20 mg / kg)
SMA COUNTS PER 30 MIN
1600
1200
800
400
0
13
15
19
15
15
8
15
12
15
20
16
1
5
10
15
20
25
30
35
40
45
50
DMI DOSE (mg / kg – ip)

with barbiturates and amphetamine (41,53,54). Low and high doses of imipramine potentiate or depress the behavioral effects of barbiturates and amphetamines, while intermediate doses do not modify the central nervous system effects of these agents. The data from the present studies suggest the possibility that the interactions of imipramine with barbiturates and amphetamines might also be attributable to metabolically formed DMI.

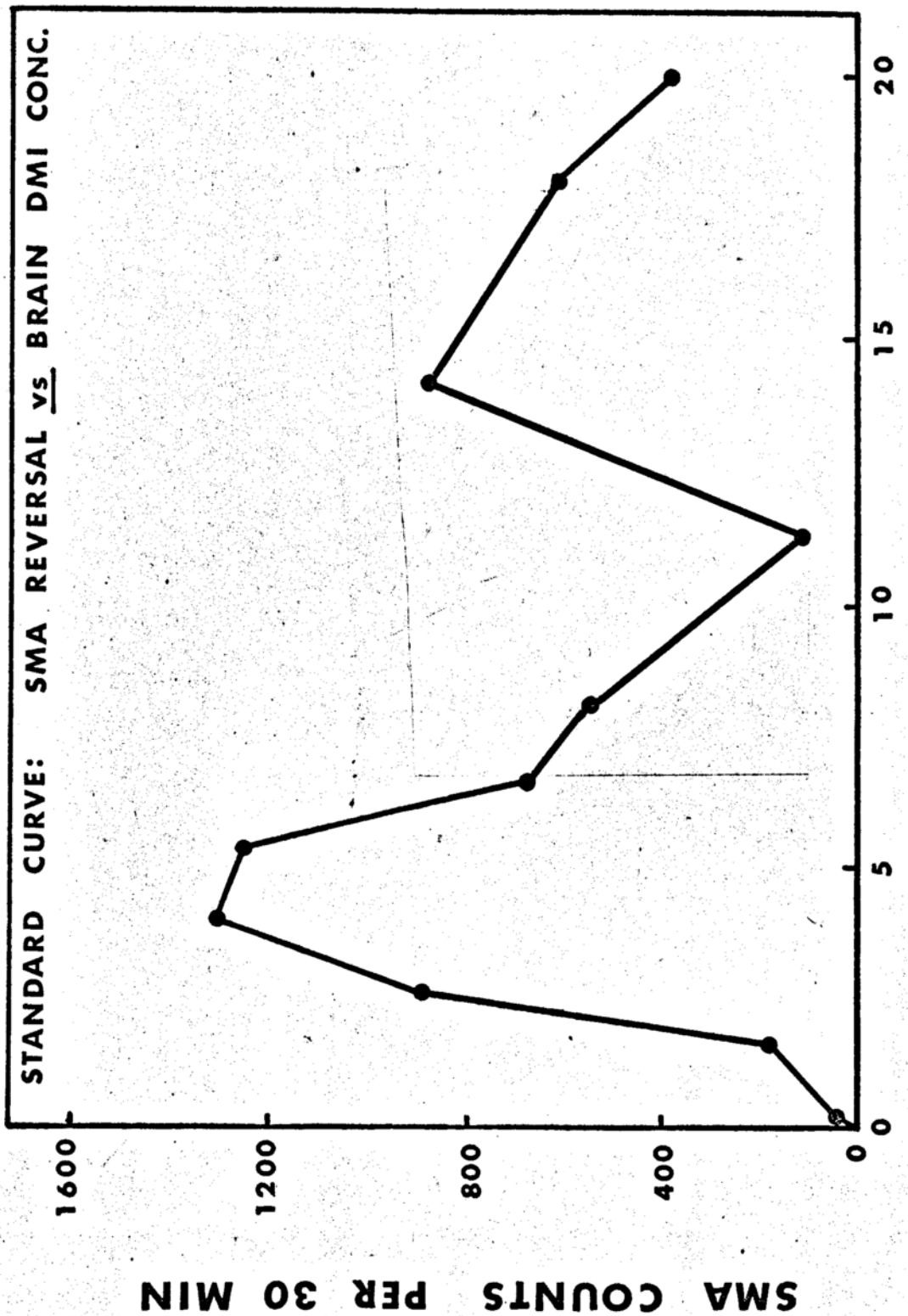
### K. The Correlation Between Brain Desmethylimipramine Levels and Reversal of Imipramine-induced Spontaneous Motor Activity by Tetrabenazine in Rats

The previous investigations were conducted in an attempt to correlate reversal of imipramine-induced SMA by TBZ and the concentration of DMI attained in rat brain after administration of imipramine. It is obvious that the double peaked disappearance curve of DMI in rat brain, observed after imipramine administration (Figure 6), does not correlate visibly with the variable SMA reversal pattern observed when TBZ is administered at succeeding time points after imipramine treatment (Figure 18). Thus, if a true correlation does exist between brain DMI concentration and SMA reversal in rats treated with tetrabenazine after imipramine administration, it is a more subtle one.

Prior studies have indicated that the dose-response relationship between intraperitoneally administered DMI and the degree of SMA reversal elicited upon subsequent TBZ administration is atypical. Low and high doses of DMI trigger TBZ reversal, while intermediate doses are ineffective in producing this response. These findings suggested the possibility that low, intermediate, and high concentrations of DMI in the rat brain might account for the atypical dose-response relationship observed when TBZ is administered to rats pretreated with various doses of DMI. For this reason, the degree of SMA reversal elicited after various intraperitoneal doses of DMI followed by TBZ was plotted versus brain concentrations of DMI determined after various intraperitoneal doses of DMI (Figure 24).

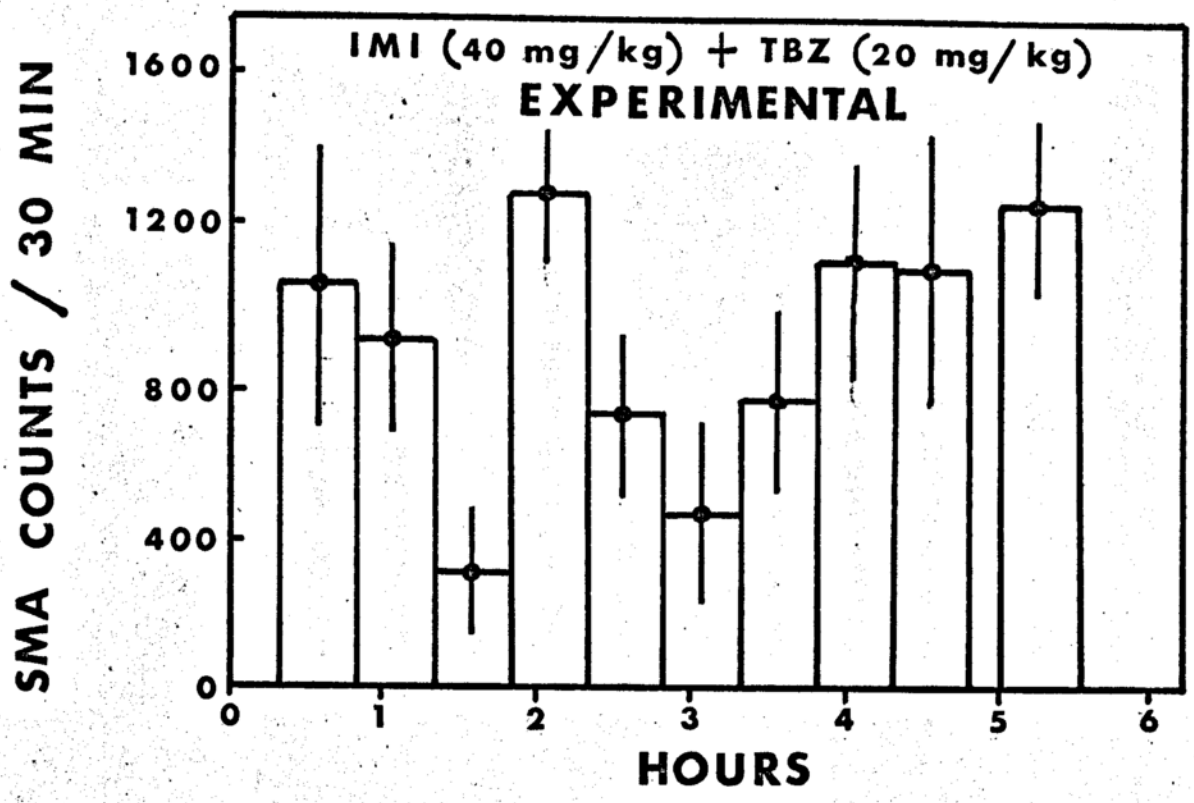
As in Figure 23, which illustrates SMA reversal versus intraperitoneal DMI dose, SMA reversal is apparent with low and high brain DMI concentrations, but not at intermediate concentrations. The degree of SMA reversal was greatest at brain concentrations of approximately 4 and 14 micrograms per gram brain, while an intermediate brain DMI concentration of 10.5 micrograms per gram brain did not elicit SMA reversal.

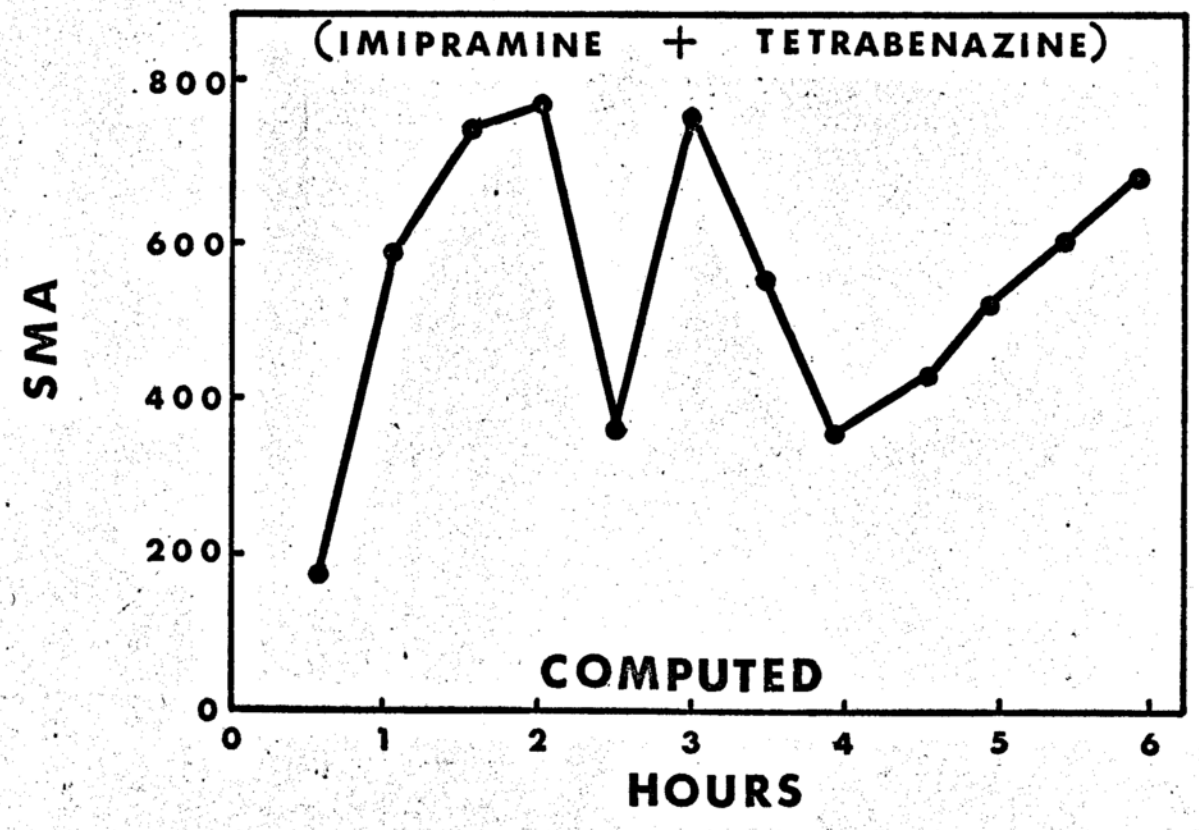
Having established an SMA reversal versus brain DMI concentration standard curve (Figure 24), and with the knowledge that variable brain DMI concentrations after


STANDARD CURVE: SMA REVERSAL vs BRAIN DMI CONC.
SMA COUNTS PER 30 MIN
0
400
800
1200
1600
0
5
10
15
20
µG DMI / GM BRAIN

imipramine administration would alter the degree of SMA reversal noted when TBZ is given after imipramine, another attempt was made to correlate brain DMI levels with the SMA reversal levels observed when TBZ is administered after imipramine. Using DMI concentrations at various times during the double peaked DMI concentration curve after imipramine administration (Figure 6), and extrapolating the degree of SMA reversal expected from each concentration of DMI from the standard curve (Figure 24), an SMA pattern was computed. Figure 25 presents the experimentally determined SMA pattern observed when TBZ is administered at various times after imipramine, and the SMA pattern computed from brain levels using the SMA reversal versus brain DMI concentration standard curve.

It is immediately apparent that a visible correlation exists between the experimental SMA pattern and the SMA pattern computed from brain DMI concentrations (97). Thus, indirectly, the levels of DMI in the brain can be shown to correlate with the degree of TBZ reversal noted when TBZ is administered to rats pretreated with imipramine. These data provide additional credence to the hypothesis that the SMA reversal phenomenon observed when TBZ is given to rats pretreated with imipramine is dependent upon the metabolic formation of DMI, and not upon the presence of parent drug.


IMI (40 mg/kg) + TBZ (20 mg/kg)
EXPERIMENTAL
SMA COUNTS / 30 MIN
1600
1200
800
400
0
0
1
2
3
4
5
6
HOURS


(IMIPRAMINE + TETRABENAZINE)
SMA
800
600
400
200
0
COMPUTED
0
1
2
3
4
5
6
HOURS

## CONCLUSIONS

Investigation of the biological disposition of imipramine and its major metabolite, desmethylimipramine (DMI), in blood and brain after injection of imipramine disclosed a double peaked disappearance curve for DMI (Figures 5 and 6). However, the parent drug imipramine exhibited a typical first order disappearance curve in this series of studies. Spontaneous motor activity (SMA) experiments carried out under these conditions at various time points showed that the amplitude of tetrabenazine (TBZ) reversal of imipramine-induced SMA did not correlate with blood or brain levels of imipramine (Figures 5 and 6). However, the amplitude of reversal with time was suggestive of a possible correlation with DMI levels in blood and brain.

In contrast, the injection of the metabolite, DMI, resulted in the production of a typical first order decay curve in blood and brain (Figures 8 and 9). Furthermore, the magnitude of SMA reversal was relatively constant, and correlated with blood and brain DMI levels. These data suggested that imipramine might modify the disposition of metabolically formed DMI, and that DMI is involved in the reversal of imipramine-induced SMA by TBZ.

Studies utilizing SKF-525A further substantiated the role of DMI in the TBZ reversal phenomenon. Pretreatment with SKF-525A was shown to inhibit the metabolic conversion of imipramine to DMI (Figures 11 and 12) and to prevent the reversal of imipramine-induced SMA by TBZ (Figure 21). In contrast, SKF-525A pretreatment prior to injection of DMI did not prevent the reversal of DMI-induced SMA by TBZ (Figure 22).

DMI dose versus brain DMI concentration studies revealed a biphasic increase in brain DMI concentration as the dose was increased (Figure 14). This suggests that more than one uptake mechanism might be involved in the entry of DMI into the brain. The particular mode of uptake might affect the degree of SMA reversal noted when TBZ is given after DMI.

Additional studies with TBZ showed that variable doses of administered DMI dramatically altered SMA reversal (Figure 23). However, the dose-response relationship was atypical in that low or high doses of DMI initiated TBZ reversal while intermediate doses were ineffective in producing this response. This observation suggests that more than one mechanism of action for DMI is involved in the reversal phenomenon. Hypothetically, at low DMI doses (10-20 mg/kg), TBZ reversal could be attributed to an indirect adrenergic effect dependent upon the demonstrated central blockade

of norepinephrine uptake by DMI (26). As intermediate doses of DMI are approached (25-35 mg/kg), the adrenolytic effects of DMI which are dependent upon the affinity of the drug for adrenergic receptor sites (33, 72) could account for the reduction of TBZ reversal. The appearance of TBZ reversal at high DMI doses (40-50 mg/kg) might be attributed to the central cholinolytic action of DMI (83). Inhibition of central cholinergic activity might enhance adrenergic effects which, in turn, might facilitate TBZ reversal in the manner suggested for low DMI doses. Thus, both central adrenergic and cholinergic mechanisms, dependent upon the relative brain concentration of DMI, might be involved in the reversal of DMI-induced SMA by TBZ.

Integration of the data from brain DMI concentration studies (Figure 6) and dose-response investigations of DMI versus SMA reversal (Figure 23) revealed that DMI levels in the brain indirectly correlate with the degree of SMA noted when TBZ is administered to rats pretreated with imipramine (Figure 25). This correlation provides additional evidence to substantiate the influential function of DMI in the reversal of imipramine-induced SMA by TBZ.

The observation that low or high doses of DMI initiate TBZ reversal while intermediate doses are ineffective parallels the anomalous dose-response data

reported on the interaction of imipramine with several biological systems, and various chemical agents. The physiological responses to sympathetic nerve (33-35) and vagal (37) stimulation are enhanced by low doses of imipramine, but antagonized with higher doses. In addition, barbiturate and amphetamine interactions with imipramine do not exhibit a typical dose-response relationship. Intermediate doses of imipramine prolong barbiturate sleeping time while lower or higher doses shorten hypnosis (41,54). In contrast, amphetamine hyperactivity is enhanced by low doses of imipramine, unaffected by intermediate doses, and inhibited by high doses (53). The dosage similarities between the reversal of DMI-induced SMA by TBZ and the biological and drug interactions with imipramine suggest that DMI might contribute in whole or in part to the diverse pharmacological interactions observed with imipramine. However, additional research would be required to substantiate this hypothesis.

There exists an extensive body of evidence to suggest that depression as a disease state might be related to the levels of endogenous biogenic amines in the brain (22,79,80,98). In fact, Schildkraut (79) has proposed that some, if not all, depressions are associated with an absolute or relative decrease in catecholamines, particularly norepinephrine, available

at central adrenergic receptor sites. Elation, conversely, may be associated with an excess of such amines. The depletion of norepinephrine by TBZ is associated with a decrease in motor activity, and has been labeled as "pseudodepression" insofar as this state resembles human inactive or regressive depression (80). Pharmacological studies have suggested that imipramine exerts its antidepressant action by potentiating the physiological effect of released norepinephrine (75,77). Thus, after imipramine treatment, the adrenergic activity of norepinephrine released by TBZ would be enhanced and might exert an "antidepressant" effect. Clinically, administration of imipramine and imipramine-like antidepressants has been shown to antagonize the pharmacological effects of TBZ in normal and depressed patients (99,100). Thus, the use of TBZ to induce a model experimental depression would appear to be a valid and useful test system for evaluation of antidepressant activity in lower species.

Although it has been proposed that the antidepressant activity of imipramine might actually be due to DMI (86,87), a body of evidence also exists to suggest that this activity might be due to the parent drug. Specifically, the onset of therapeutic action and the degree of antidepressant activity have been shown to be essentially identical following therapy with either

imipramine or DMI (22). The present rat studies have demonstrated a sedative action of imipramine in that SMA was markedly reduced after administration of imipramine alone (Figure 16). This sedation induced by imipramine might account for the apparent antidepressant activity associated with imipramine insofar as this pharmacological effect might prove beneficial in alleviation of the anxiety component of depressive anxiety states. Thus, the antidepressant activity of DMI might be enhanced, but masked by the sedative action of imipramine, and thus might account for the similar onset times for imipramine and DMI in antidepressive therapy. In addition, clinical studies with DMI (101) have shown individual differences in steady state plasma concentrations (8-290 mμg of DMI/ml of plasma). In subjects with the highest steady state plasma levels, undesirable side effects were reported. This observation is in accord with clinical observations which indicate that antidepressant activity is greatest in patients exhibiting lower plasma levels of DMI (102). These dose related toxicity and efficacy findings might be similar to the atypical dose-response data observed when TBZ was administered to rats pretreated with various doses of DMI (Figure 23). Low doses of DMI might promote antidepressant activity, with little or no toxicity, while with higher doses both toxicity and anti-adrenergic

effects of DMI might contribute to a decline in antidepressant activity. Thus, the onset of the antidepressant activity for imipramine might depend upon the metabolic conversion of this compound to DMI, and the degree of therapeutic efficacy for both imipramine and DMI might require the maintenance of low steady state plasma levels of DMI.

In conclusion, the present studies verify the necessity for conversion of imipramine to DMI prior to the reversal of TBZ-induced depression in rats, thereby implicating imipramine as a "pro-drug," DMI, however, would appear to be the active agent with respect to TBZ reversal. These findings substantiate the hypothesis that although imipramine exerts a sedative action in animals and man the antidepressant activity is due mainly to the metabolite, DMI (86,87).

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## SUMMARY

In 1961, a new theory regarding the mechanism of antidepressant activity of imipramine was proposed. It was suggested that despite the mild sedative action of imipramine in animals and man, the antidepressant activity is due to the metabolite desmethylimipramine (DMI). Numerous investigations were conducted to support or disprove this theory during the 1960's. However, significant findings were few and inconclusive.

The present studies were conducted to elucidate the relative importance of imipramine and/or DMI in the reversal of imipramine-induced spontaneous motor activity (SMA) by tetrabenazine (TBZ) in rats. The disposition of imipramine and DMI in whole blood and brain were monitored over 24 hours, and a detailed time-SMA response study of the interaction between imipramine or DMI and TBZ was undertaken. It was anticipated that these studies would clarify the present "imipramine or DMI" controversy, and possibly provide new insight concerning the pharmacological action of antidepressants.

Male Holtzman rats were given imipramine or DMI intraperitoneally, and sacrificed at various times. Whole blood samples were collected and whole brains homogenized with water. Both blood and brain samples

were extracted and assayed fluorimetrically for imipramine and DMI content by the technique of Dingell, Sulser, and Gillette (12).

SMA was measured in circular six-beam photoelectric activity cages. Each rat was given imipramine or DMI and challenged with TBZ at a succeeding time point. A single rat was placed in each activity cage immediately after TBZ administration, and SMA was recorded periodically.

Investigation of the biological disposition of imipramine and DMI in blood and brain after imipramine administration disclosed a typical first-order disappearance curve for imipramine, and a double peaked disappearance curve for DMI. SMA determinations at various time points showed that the amplitude of TBZ reversal of imipramine-induced SMA did not correlate with blood and brain levels of imipramine. However, the amplitude of reversal with time was suggestive of a possible correlation with DMI levels in blood and brain.

In contrast, injection of DMI resulted in the production of a typical first-order decay curve in blood and brain. Furthermore, the magnitude of SMA reversal was relatively constant, and correlated with blood and brain DMI levels.

SKF-525A pretreatment was shown to inhibit the metabolic conversion of imipramine to DMI, and to

prevent the reversal of imipramine-induced SMA by TBZ. In contrast, SKF-525A treatment prior to the administration of DMI did not prevent the reversal of DMI-induced SMA by TBZ.

DMI dose versus blood and brain DMI concentration studies revealed a linear increase in blood DMI concentration, and a biphasic linear increase in brain DMI concentration as the dose was increased. TBZ reversal studies showed that variable doses of administered DMI dramatically altered SMA. However, the dose-response relationship was atypical in that low or high doses of DMI initiated TBZ reversal, while intermediate doses were ineffective in producing this response. Integration of data from the brain DMI concentration studies and the dose-response investigation of DMI versus TBZ reversal revealed that DMI levels in the brain indirectly correlate with the degree of SMA noted when TBZ is administered to rats pretreated with imipramine.

The present studies verify the necessity for conversion of imipramine to DMI prior to the reversal of imipramine-induced SMA by TBZ, thereby implicating imipramine as a "pro-drug." DMI, however, would appear to be the active agent with respect to TBZ reversal. These findings provide additional credence to the hypothesis that the antidepressant action of imipramine is due mainly to the metabolite, DMI, and not the parent drug.