Effect of the Trimethoxy Group

Young Soo Choi

University of Rhode Island

Follow this and additional works at: https://digitalcommons.uri.edu/theses

Recommended Citation
https://digitalcommons.uri.edu/theses/188

This Thesis is brought to you for free and open access by DigitalCommons@URI. It has been accepted for inclusion in Open Access Master's Theses by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons@etal.uri.edu.
EFFECT OF THE
TERMINAL GROUP
BY
YOUNG SOO CHOI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
PHARMACOLOGY

UNIVERSITY OF RHODE ISLAND
1963
ACKNOWLEDGEMENT

The author is greatly indebted to Dr. John J. De Fee, Associate Professor and the Chairman of the Department of Pharmacology, who acted as research adviser, for his benevolent guidance and help.

Thanks are extended to Dr. David R. De Yunci, Assistant Professor of Pharmacology, Dr. Pierre F. Smith, Professor and the Chairman of the Department of Pharmaceutical Chemistry, and Dr. Leonard R. Worthen, Associate Professor of Pharmacognosy, for their help and advice.

Appreciation is also expressed to Mr. David W. Costes, Instructor of Pharmacology, for his help and encouragement.

This investigation was supported by the United States Public Health Service, Grant No. MY-4132.
ABSTRACT

The significance of the 3,4,5-trimethoxyphenyl group in possible psychotropic compounds was investigated by pharmacological screening of the 3,4,5-trimethoxyphenyl analogs of diphenhydramine and tripelestone, and comparative studies of these trimethoxy derivatives with their parent compounds were conducted.

The gross activity studies using the Actophotometer indicated enhancement of the depressant effect of tripelestone and the depressant as well as stimulatory effect of diphenhydramine.

The results of the sleeping time test (potentiation of pentobarbital sleeping time) showed that the presence of the trimethoxy group decreased the potentiation effect of the parent compounds.

LD₅₀ studies indicated that the trimethoxy derivatives are less toxic than the parent compounds.

The preliminary behavioral studies (rolling, reeling and inclined plane tests) indicated that the C₅₂₈₅₆ activity of these compounds is other than on the cerebro-cortical areas of the brain.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II.</td>
<td>REVIEW OF LITERATURE</td>
<td>2</td>
</tr>
<tr>
<td>III.</td>
<td>EXPERIMENTAL PROCEDURE</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Gross Activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sleeping Time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LD50 Studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preliminary Behavioral Studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rolling roller</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inclined Plane</td>
<td></td>
</tr>
<tr>
<td>IV.</td>
<td>RESULTS</td>
<td>15</td>
</tr>
<tr>
<td>V.</td>
<td>DISCUSSION</td>
<td>23</td>
</tr>
<tr>
<td>VI.</td>
<td>SUMMARY AND CONCLUSIONS</td>
<td>26</td>
</tr>
<tr>
<td>VII.</td>
<td>REFERENCES</td>
<td>28</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table | Description                                                                 | Page |
------|------------------------------------------------------------------------------|------|
1.    | Comparative Effects of Experimental Compounds on Normal Cross Activity in Mice by the Actophotometer | 16   |
2.    | Comparative Effects of Experimental Compounds on Cross Activity in Mice Influenced by Amphetamine Hydrochloride by the Actophotometer | 17   |
3.    | Comparative Effects of Experimental Compounds on Sodium Pentobarbital Sleeping Time in Mice | 18   |
4.    | Comparative Effects of Experimental Compounds on Sodium Pentobarbital Sleeping Time in Mice with All Compounds Tested during Same Time Period | 19   |
5.    | Comparison of LD₅₀ of Experimental and Parent Compounds in Mice | 20   |
6.    | The Effects of the Experimental Compounds in Mice Subjected to the Rotating Roller | 21   |
7.    | The Effects of the Experimental Compounds in Mice Subjected to the Inclined Plane | 22   |
INTRODUCTION

Diphenhydramine and tripelennamine are well known and widely used antihistamines. Their physiological role and pharmacological actions such as Central Nervous System (C.N.S.) stimulation and depression have been defined by several investigators, but the exact mechanisms of their action on the C.N.S. is still obscure.

The fact that reserpine, mesaline and colchicine which have a trimethoxy group in their structures, produce some C.N.S. activity suggested that it might be possible to synthesize various derivatives by the addition of the trimethoxy group to known antihistaminic compounds which would exert some effect on the C.N.S.

The presence of the trimethoxy group in certain C.N.S. acting drugs has led to the use of this group in the synthesis of new drugs. Trimethoxy diphenhydramine and trimethoxy tripelennamine resulted.

The purpose of this study was to determine what effect the trimethoxy group in diphenhydramine or tripelennamine has on the pharmacological action of the parent compounds. This was approached by administering the new drugs and the parent drugs to the experimental animals.
REVIEW OF LITERATURE

The antihistaminic drugs are capable of antagonizing to a varying degree many, but not all, of the pharmacologic actions of histamine. Moreover, they can modify allergic and anaphylactic reactions, this being the basis of the major therapeutic application of the antihistaminic drugs.

Antihistaminic activity was first proposed by Bover et al. (1923) who demonstrated this action by the use of certain phenolic amine.

Edelmeier (1927) demonstrated that although certain amino acids, histidine, oxyazine and arginine possess histamine inhibiting activity, this action is too weak to be of therapeutic use.

Since Bover, many investigators have tried to synthesize new antihistaminics. As a result, pyrimidines (Halsen, 1942), pyrethamine (Bover & Halsen, 1943), diphenhydramine (Low et al., 1945) and triphenylamine (Rover et al., 1943) have been produced.

Diphenhydramine was first reported as an antihistaminic agent by Low et al., (1945). This group was testing seventeen synthesized compounds of the type Ph₂C₆H₅N, and assessing the degree of protection capacity of these compounds against the bronchoconstriction induced in guinea pigs by exposure to a histamine aerosol. They found that diphenhydramine was the most powerful of this series, and was about 33 times
more potent than anaphylamines.

Many workers such as Wells et al. (1945), Lew et al. (1946), Winter et al. (1946), and Thomas (1946) showed that diphenhydramine acts as an antihistamine in allergic reactions, anaphylactic shock, smooth muscle spasm, vasomotor rhinitis, seasonal hay fever, serum reaction, vasodepressor effect of blood vessels, urticaria, and pruriginous dermatitis.

Mayer et al. (1948) were testing eleven compounds of the general formula \( R_1 R_2 NCH_2CH_2NH(C_2H_5)_2 \) on the isolated strips of guinea pig intestine and found that the compound of \( R_1 = p\)-pyridyl, \( R_2 = benzyl \), and \( R_3 = ethyl \) (i.e., tripelennamine) was the most active one. This compound actively sensitized the guinea pig strip to horse serum and protected them from anaphylactic shock in doses of 0.1 mg/kg. A close relationship existed between the antihistaminic activity in vitro and the antianaphylactic property in vivo.

Antihistaminic effects including antiallergic and anti-anaphylactic actions of diphenhydramine and tripelennamine have been studied by numerous workers, Lew et al. (1945), Mayer (1946) and Winter (1947) on bronchoconstriction, Sherrod et al. (1947) and Lew (1947) on smooth muscle spasm, Lew et al. (1946), Kennedy et al. (1946), Harsh et al. (1947), and Sherrod et al. (1947) on vasodepressor effects, and Arbman (1946), Friedlander et al. (1946) and Heck (1947) on visceral formation and anaphylactic reaction.

Harumi-Uberti et al. (1961) reported that histamine induced gastric ulcer was inhibited 32.2% by previous intramuscular injection of tripelennamine, and proposed that their method appeared to be accurate.
enough for screening anti-snor agents.

Diphenhydramine and tripeleamine have been extremely useful in antihistamine therapy but they induce a variety of undesirable side-effects. The most common side-effect of the antihistaminic drugs is sedation, which is manifested by dizziness, lassitude, drowsiness, sleepiness, and motor incoordination.

Curtis et al. (1945) calculated the LD50 of diphenhydramine, as 167 mg/kg (oral) for mice and 545 mg/kg for rats. They also mentioned the violent excitement, convulsions, respiratory failure and death which resulted in from a few minutes to several hours after the administration of lethal doses. Nonlethal doses produced excitement and asomnia with recovery in one to two hours.

Low et al. (1946) observed no evidence of drowsiness in dogs which received diphenhydramine (10 mg/kg) subcutaneously and Winter et al. (1940) obtained similar results with similar doses in mice, rats, dogs, guinea pigs, and monkeys.

Bayer et al. (1946) observed no drowsiness resulting from low doses of tripeleamine, but did report excitability and convulsions with toxic doses given by intravenous injection. Similar results were obtained by Winter (1940) in rats and mice as well as dogs by intraperitoneal, oral and subcutaneous administration of tripeleamine.

Graham (1947) also observed the powerful central excitement and incoordination in white mice with intraperitoneal injections of sublethal doses of diphenhydramine.

The sedative effect of diphenhydramine and tripeleamine is also reported by Beck (1947). He mentioned many untoward side reactions
that occurred in 20 to 25% of cases treated with tripelemamine and in 50 to 65% of cases treated with diphenhydramine. The common reactions are depression of the C.N.S., resulting in drowsiness, lassitude, inability to concentrate, sleepiness, and in rare cases, narcolepsy, stupor or mental confusion and irritation of C.N.S. or peripheral nervous system resulting in insomnia, irritability, headache, nervous tension, chills and blurring of vision, and rarely causing dysuria, polyuria, frequency and olfactory hallucinations. These reactions are much more common and more severe with diphenhydramine than with tripelemamine.

McCraeck et al. (1947) also mentioned about the side effects of diphenhydramine involving the C.N.S., which are apparently unrelated to its antihistamine potency. These effects in men are drowsiness, which often follows administration of therapeutic doses, and convulsions after toxic doses. Therefore, diphenhydramine appears to exert a depressant effect at low dosage, and a stimulant effect at toxic dosage.

Clarke (1955) showed that diphenhydramine was effective against seasickness. The hyoscine-like property of the antihistamine determined its essential action on motion sickness.

Beard et al. (1954) found that the administration of diphenhydramine in doses up to 25 mg/kg of body weight had no effect upon measurement of complex, coordinated, reflex activity in albino rats. Large doses of diphenhydramine augmented the measured reflex activity and oral doses of 300 mg/kg produced convulsions and a mortality of 80%.

The excitation of the C.N.S. by tripelemamine and diphenhydramine in clinical studies has been reported by Feinberg et al.
(1947), Henderson et al. (1947) and Churchill et al. (1949).

Zuker (1957) determined the subcutaneous LD₅₀ in mice of
diphenhydramine (147.6 mg/kg) and tripelennamine (97.7 mg/kg), and
observed excitement, paralysis effect and antagonistic action against
acetylcholine.

The effects of antihistamines on the C.N.S. consist of both
stimulation and depression, with large doses producing the stimulation.
In animals, restlessness, excitement and convulsions occur when toxic
doses are administered and death results from respiratory depression.
In the therapeutic dose range, antihistamines may occasionally cause
restlessness, nervousness and insomnia, but are frequently they exert
a sedative action, and they produce somnolence presumably due to
cortical depression.

The most important actions of diphenhydramine and tripelenn-
amine are on the C.N.S. These consist in both stimulation and depression.
The mechanisms of both actions on the C.N.S. are obscure, but it is
supposed that they do possess some anticholinergic properties and
atropine like actions. Since these compounds were synthesized, numerous
workers studied the C.N.S. actions of diphenhydramine and tripelennamine.

Winter (1943) found that the potentiating effect of diphen-
hydramine upon the sedative action of the barbiturates was much greater
than that of tripelennamine and both diphenhydramine and tripelennamine
pupling the sleep-producing effects of hexobarbital in mice with 10 or
20 mg/kg subcutaneous injection, and that the mean waking time was
prolonged about 10% by tripelennamine and about 49% by diphenhydramine.
He concluded that whatever potentially sedative effect antihistaminic
Tomlin (1932) studied the effects of electroencephalographic stimulation on the brain of the rabbit. He found that the effects were not the same in all cases, and that the results differed in different areas of the brain. The general behavior of the animals was also different in different cases. The authors suggested that the effects were due to the different areas of the brain being stimulated in the two cases. They also suggested that the effects of electroencephalographic stimulation were due to the different areas of the brain being stimulated in the two cases. The authors also suggested that the effects of electroencephalographic stimulation were due to the different areas of the brain being stimulated in the two cases. The authors also suggested that the effects of electroencephalographic stimulation were due to the different areas of the brain being stimulated in the two cases. The authors also suggested that the effects of electroencephalographic stimulation were due to the different areas of the brain being stimulated in the two cases. The authors also suggested that the effects of electroencephalographic stimulation were due to the different areas of the brain being stimulated in the two cases.
other hand, in his experiments, diphenhydramine given intraventricularly in the rabbit, acted as a central depressant and produced marked changes in the E.E.G., characterized by high voltage slow activity.

Way et al. (1951) reported that sodium pentobarbital increased the intraperitoneal LD₉₀ of tripelennamine and diphenhydramine in rats and acted similarly with both tripelennamine and diphenhydramine. It did not affect the death rate significantly in animals overdosed with diphenhydramine and tripelennamine, although convulsions were shortened in part and survival time possibly increased. They also reported that some protection was evident when animals were antidoted with a low dose of sodium pentobarbital (30 mg/kg), but they died following convulsions, whereas with a higher dose (60 mg/kg) the animals died of respiratory depression shortly after the barbiturate was injected. The difference in the manner in which death was produced might be explained by the fact that certain antihistamines appeared to depress as well as stimulate the C.N.S.

Belouzov (1953) reported that diphenhydramine was quite active in potentiating the effect of pentobarbital in rats.

Nair et al. (1954) reported that tripelennamine had a statistically insignificant effect upon locomotor activity with oral and subcutaneous administrations in the amount of from 1 mg/kg body weight up to lethal doses in albino rats.

Solovëv (1956) summarized through his experiments that the latent period of defensive reflexes was usually prolonged by subcutaneous diphenhydramine (12 - 40 mg/kg) in mice and rats, and diphenhydramine had an antispasmodic effect in strychnine poisoning due to
depression of the C.N.S.

Shumaw's studies (1956) with diphenhydramine demonstrated that, at a single dosage level, alone pharmacologically inactive, it could enhance the C.N.S. activity of two compounds (pentobarbital and strychnine) long recognized as being pharmacological antagonists. In an attempt to account for diphenhydramine activity, two modes of action might be considered. The first would involve an inhibition of the biotransformation of the barbiturates and strychnine. The second possible mode of action would postulate a more direct effect on the elements of the C.N.S., resulting in alterations in levels of neuronal activity that, in turn, could quantitatively affect the response to strychnine.

Tocher and his co-workers (1957) reported that some hypnoactivity was observed with effective doses of diphenhydramine (25 - 60 mg/kg) and that the ED₅₀ was demonstrated to increase pentobarbital sleeping time in mice by 200%. The effect of pentobarbital was due to generalized cortical depression or interruption of spinal polysynaptic pathways leading to stunned.

Chen (1959) studied the kinds of C.N.S. stimulants and concluded that diphenhydramine belonged to excitants because it did not produce tonic extensor seizures in mice when administered intravenously. In his experiments it was observed that animals receiving diphenhydramine would run or jump intermittently with slight attacks of clonic seizures but without tremors.

Onisuka (1958) reported that intravenous injection of diphenhydramine caused weakness and convulsions in rabbits and anesthetic
action by intraspinal injection, and that excitation by subcutaneous injection of diphenhydramine in mice was antagonized by pentobarbital, chloral hydrate, and urethane. It was concluded that the excitation by diphenhydramine was thought to be due to its unbalanced inhibition of central nerves and since this drug is essentially a central inhibitor, its action resembled those of antihistamines, chlorpromazine, and procaine.

Kawali et al. (1960) observed some C.N.S. actions with tripelennamine and suggested that there might be a component in central nervous function which was mediated through an adrenergic mechanism.

Kartali (1961) reported that tripelennamine inhibited the catalysis of pentobarbital in vivo or in vitro.

Gilmour et al. (1960) and Shaé (1962) summarized the actions of antihistamines on the C.N.S. as follows: High doses of antihistamines elicit a marked cerebral stimulation and convulsions, which are followed eventually by severe depression. Seizures are readily controlled by barbiturates. The depressant and stimulant effects of the antihistamines are probably due to the diallylaminoethyl structure; the same responses are elicited by conduction anesthetics and antiparkinsonian agents.
III

EXPERIMENTAL PROCEDURE

Gross Activity

The Actophotometer* consists of a circular chamber with adjustable-height screen mesh floor for retaining the animal. The lower portion of the chamber is cross hatched at the animal's level by six light beams activating six photocell units. Each circuit break is recorded on a six digit counter by means of an amplifying system built into the bottom section of the cage.

Two male and two female albino mice were selected for each test group. Each group was placed in an Actophotometer cage for a period of 30 minutes, and activity was recorded as counts per five minute periods for a total of 30 minutes. Then they were removed and the experimental groups were injected intraperitoneally with the experimental drug. The controls received an equal volume of 0.9% saline or drug vehicle. The animals were then returned to their respective cages for 30 minutes, and the activity was recorded for 60 minutes as before. Another similar series of tests was conducted employing various doses of amphetamine-hydrochloride. The activity was recorded in the same manner as previously described.

* Metro Industries, Niagara, L. I., N. Y.
It should be noted that the doses of drugs were calculated on an equimolar ratio between the parent compounds and its derivatives throughout the experiments.

The % change in activity was determined as follows: Each group of four mice were tested for control values. Then they were again tested for drug effects and saline effect on one group. The counts for 5 minute periods were totaled and a mean 5 minute count was determined. For each group the drug or saline mean was then compared to the control mean and a % change was calculated. Then the saline % change was compared to each drug % change and the final % change in activity was determined.

**Sleeping Time**

Five male and five female albino mice were selected for each group. They were deprived of food for a period of 24 hours prior to the experiments. The experimental groups received various doses of the drugs intraperitoneally and the control groups received equivalent volumes of 0.9% saline by the same route. After 30 minutes sodium pentobarbital (80 mg/kg) was injected intraperitoneally into both groups. The sleeping time was measured from the moment of the loss of the righting reflex until the return of this reflex, as indicated by the animal righting itself.

**LD50 Studies**

Five male and five female albino mice were selected for each dose group. They were deprived of food for a period of 24 hours prior to the experiments. The different doses of drugs for each group were injected intraperitoneally and deaths were recorded during a 24 hour period.
Rolling Roller

The rolling roller used in this experiment was a modified apparatus from Dunham and Hicks' rolling roller. The rolling roller apparatus employs a 115 v, 60 cycle, a.c., Bird Electric Rexograph, as a power source for turning the roller. The end of the motor shaft is connected by means of a belt to one end of a wooden roller, 15 inches long and 3/8 inch diameter. Metal rods protruding from both ends of the roller are inserted into holes in the vertical metal supports.

The speed was set such that the roller made 25 revolutions per minute. In order to perform multiple tests simultaneously, 6 circular aluminum disks, 6.5 inches in diameter, were placed on the roller at suitable space intervals so as to divide the roller into 5 equal compartments. The roller was held stationary by suitable clamps at a height of 5 inches above the table top.

Albino mice were trained to stay on the rolling roller at least 10 minutes for the experiments. They were deprived of food for a period of 24 hours prior to the experiments. Two male and two female mice were selected for each group, and were first placed on the rolling roller for 5 minutes maximum as a control. They were then removed and injected intraperitoneally with the experimental drugs. The mice were then returned to the cages for 30 minutes and then were placed on the rolling roller again. They were observed for a maximum of 5 minutes to see whether or not they would fall off the rolling roller.
Inclined Plane

Albino mice were trained to descend from the top of an inclined plane; 16.5 inches in length of the inclined plane and 45° angle with the horizontal plane. They were deprived of food for a period of 24 hours prior to the experiments. Two male and two female mice were selected for each group. The mice were placed at the top of the inclined plane and were given three trials each to determine control activity. They were then injected intraperitoneally with the experimental drugs, put back on the top of the inclined plane, and observed at 5, 30, and 60 minutes intervals to see if they would descend the inclined plane in a manner similar to that of the controls.
IV

RESULTS

The results of the gross activity of mice are recorded in Tables 1 and 2.

The results of the sleeping time tests are tabulated in Tables 3 and 4, and the significant values in the statistical analysis of these results were made at a F value of 0.05.

Determinations of the LD₅₀ for the experimental compounds were carried out along with the parent compounds and the results are listed in Table 5.

Tables 6 and 7 are lists of the results from the preliminary behavioral studies by the rolling roller and the inclined plane.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg</th>
<th>% Change from normal</th>
<th>Compound</th>
<th>Dose mg/kg</th>
<th>% Change from normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-NCI**</td>
<td>12.16</td>
<td></td>
<td>**</td>
<td>16.0</td>
<td>98 ↓</td>
</tr>
<tr>
<td></td>
<td>19 ↓</td>
<td></td>
<td></td>
<td>54</td>
<td>92 ↓</td>
</tr>
<tr>
<td>24,32</td>
<td>10 ↑</td>
<td></td>
<td>32,0</td>
<td>82 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>143 ↑</td>
<td></td>
<td></td>
<td>80 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99 ↓</td>
<td></td>
<td></td>
<td>91 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 ↑</td>
<td></td>
<td></td>
<td>93 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 ↓</td>
<td></td>
<td></td>
<td>46 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ↑</td>
<td></td>
<td></td>
<td>30 ↓</td>
<td></td>
</tr>
<tr>
<td>PHE-NCI**</td>
<td></td>
<td></td>
<td>THPE-NCI**</td>
<td>17.6</td>
<td>51 ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64 ↓</td>
<td></td>
</tr>
<tr>
<td>22.2</td>
<td>63 ↓</td>
<td></td>
<td>26.4</td>
<td>93 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 ↓</td>
<td></td>
<td></td>
<td>93 ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72 ↓</td>
<td></td>
<td></td>
<td>89 ↓</td>
<td></td>
</tr>
</tbody>
</table>

* Metro Industries, Mumbai, India.
** Diphenhydramine hydrochloride
*** Trimethoxy diphenhydramine hydrochloride
**** Tripalocamamine hydrochloride
***** Trimethoxy tripalocamamine hydrochloride
<table>
<thead>
<tr>
<th>Comp.</th>
<th>Dose mg/kg</th>
<th>% Change from normal</th>
<th>Comp.</th>
<th>Dose mg/kg</th>
<th>% Change from normal</th>
<th>Comp.</th>
<th>Dose mg/kg</th>
<th>% Change from normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphet. HCl*</td>
<td>5.0 ↑</td>
<td>120 ↑</td>
<td>Be*</td>
<td>12.16 ↑</td>
<td>62 ↑</td>
<td>THPM*</td>
<td>16.0 ↑</td>
<td>189 ↑</td>
</tr>
<tr>
<td>&amp;</td>
<td>1.56 ↑</td>
<td>HCl*</td>
<td>148 ↑</td>
<td>HCl*</td>
<td>136 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp;</td>
<td>184 ↑</td>
<td>&amp;</td>
<td>42 ↑</td>
<td>&amp;</td>
<td>108 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp;</td>
<td>113 ↑</td>
<td>AmpH HCl*</td>
<td>96 ↑</td>
<td>AmpH HCl*</td>
<td>236 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>62 ↑</td>
<td>24.32 ↑</td>
<td>78 ↑</td>
<td>35.0</td>
<td>200 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>256 ↑</td>
<td>74 ↑</td>
<td>57 ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82 ↑</td>
<td>107 ↑</td>
<td>122 ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>61 ↑</td>
<td>24.32 ↑</td>
<td>183 ↑</td>
<td>35.0</td>
<td>60 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119 ↑</td>
<td>127 ↑</td>
<td>130 ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89 ↑</td>
<td>78 ↑</td>
<td>107 ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphet. HCl*</td>
<td>5.0 ↑</td>
<td>362 ↑</td>
<td>FES*</td>
<td>14.8 ↑</td>
<td>83 ↑</td>
<td>THPM*</td>
<td>17.6</td>
<td>79 ↑</td>
</tr>
<tr>
<td>&amp;</td>
<td>176 ↑</td>
<td>HCl*</td>
<td>192 ↑</td>
<td>HCl*</td>
<td>119 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp;</td>
<td>125 ↑</td>
<td>&amp;</td>
<td>91 ↑</td>
<td>&amp;</td>
<td>78 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp;</td>
<td>103 ↑</td>
<td>AmpH HCl*</td>
<td>68 ↑</td>
<td>AmpH HCl*</td>
<td>135 ↑</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Metro Industries, Mimeo, L. E. R. H. Y.
* Amphetamine hydrochloride
* Dibenzhydramine hydrochloride
*** Triethoxy dibenzhydramine hydrochloride
** Tripelaminine hydrochloride
*** Triethoxy tripelaminine hydrochloride
## TABLE 3

**COMPARATIVE EFFECTS OF EXPERIMENTAL COMPOUNDS ON MORPHINE PENTOBARBITAL* SLEEPING TIME IN MICE**

<table>
<thead>
<tr>
<th>No.</th>
<th>Rooms</th>
<th>Sleeping Time (min.) Mean ± S.D.</th>
<th>No.</th>
<th>Rooms</th>
<th>Sleeping Time (min.) Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>13.7 ± 4.12</td>
<td>5</td>
<td>10</td>
<td>53.0 ± 17.0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>23.0 ± 13.7</td>
<td>6</td>
<td>7</td>
<td>50.8 ± 5.0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>20.3 ± 15.8</td>
<td>7</td>
<td>10</td>
<td>53.6 ± 16.3</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>12.5 ± 3.4</td>
<td>8</td>
<td>10</td>
<td>66.2 ± 23.9</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>14.1 ± 3.0</td>
<td>9</td>
<td>8</td>
<td>28.3 ± 16.0</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>13.3 ± 5.0</td>
<td>11</td>
<td>9</td>
<td>19.9 ± 6.9</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>39.0 ± 10.4</td>
<td>12</td>
<td>10</td>
<td>28.0 ± 19.8</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>22.5 ± 16.3</td>
<td>13</td>
<td>10</td>
<td>42.3 ± 12.0</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>43.0 ± 13.0</td>
<td>14</td>
<td>6</td>
<td>40.6 ± 30.8</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>56.6 ± 19.9</td>
<td>15</td>
<td>6</td>
<td>51.3 ± 14.2</td>
</tr>
</tbody>
</table>

* : Sodium pentobarbital  
** : Diphenhydramine hydrochloride  
*** : Triphenyl diphenhydramine hydrochloride  
**** : Triphenyl triphenylamine hydrochloride

* : Significant at 0.05 level  
** : Not significant
### Table 4

**Comparative Effects of Experimental Compounds on Sodium Pentobarbital** Sleeping Time in Mice

*With all compounds tested during same time period*

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Control</th>
<th>B-HCl</th>
<th>B-HCl***</th>
<th>PHE-HCl</th>
<th>B-PHE-HCl***</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>1</td>
<td>52.0 ± 8.7</td>
<td>100 ± 17.5</td>
<td>88.0 ± 24.6</td>
<td>70.2 ± 22.3</td>
<td>60.3 ± 13.0</td>
</tr>
<tr>
<td>2</td>
<td>29.0 ± 10.7</td>
<td>51 ± 17.0</td>
<td>43.0 ± 19.3</td>
<td>40.0 ± 9.8</td>
<td>30.0 ± 3.8</td>
</tr>
</tbody>
</table>

*; Sodium pentobarbital
++; Diphenhydramine hydrochloride
+++; Triazolopyridazine hydrochloride
**; Trimipramine hydrochloride
***; Triazolopyridazine hydrochloride

*6 animals for each determination*
<table>
<thead>
<tr>
<th>Compound</th>
<th>LD₅₀</th>
<th>Compound</th>
<th>LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-HCl++</td>
<td>73 mg/kg</td>
<td>FSH-HCl+++</td>
<td>76 mg/kg</td>
</tr>
<tr>
<td></td>
<td>70 - 85</td>
<td></td>
<td>64 - 78</td>
</tr>
<tr>
<td>THD-HCl+++</td>
<td>142 mg/kg</td>
<td>DMPAZ-HCl+++</td>
<td>155 mg/kg</td>
</tr>
<tr>
<td></td>
<td>130 - 149</td>
<td></td>
<td>146 - 164</td>
</tr>
</tbody>
</table>

++ : Diphenhydramine hydrochloride  
+++ : Trimethoxy diphenhydramine hydrochloride  
+++ : Triphenylmethyl diphenhydramine hydrochloride  
+++ : Triphenylmethyl diphenhydramine hydrochloride  

* From literature: Ref. 19, 31, & 37

* Determined by Litchfield & Wilcoxon method - Ref. 21

Lower figures are confidence limits at 19/20 level
### TABLE 6

THE EFFECTS OF THE EXPERIMENTAL COMPOUNDS
IN MICE SUBJECTED TO THE ROLLING ROLLER

| Compound   | Dose  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>D-HCl**</td>
<td>12.16</td>
</tr>
<tr>
<td>D-HCl</td>
<td>24.32</td>
</tr>
<tr>
<td>TRS-HCl***</td>
<td>16.0</td>
</tr>
<tr>
<td>TRS-HCl</td>
<td>32.0</td>
</tr>
<tr>
<td>TRS-HCl**</td>
<td>14.8</td>
</tr>
<tr>
<td>TRS-HCl***</td>
<td>29.6</td>
</tr>
<tr>
<td>TRS-HCl**</td>
<td>17.6</td>
</tr>
<tr>
<td>TRS-HCl***</td>
<td>35.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>No. of Animals falling within 3 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

**; Diphenhydramine hydrochloride
***; Triamcinolone diphenhydramine hydrochloride
**; Tripelemonine hydrochloride
***; Triamcinolone tripelemonine hydrochloride
<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>5 min.</th>
<th>30 min.</th>
<th>60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-MeI††</td>
<td>12.16</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>24.32</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>THD-MeI†††</td>
<td>16.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>32.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PhH-MeI‡‡‡</td>
<td>14.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>29.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NPhMeH-MeI§</td>
<td>17.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>33.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

†† 3-MeI: Diphenhydramine hydrochloride
††† THD-MeI: Trimethoxy diphenhydramine hydrochloride
‡‡‡ PhH-MeI: Tripelensine hydrochloride
§ NPhMeH-MeI: Trimethoxy tripelensine hydrochloride
ND: Normal descent
DISCUSSION

In attempting to evaluate the results of the gross activity of mice it was difficult to apply statistical methods due to the inconsistency of the results. However, some comparisons may be made which should be of value. As the dose for diphenhydramine was increased (Table 1) the effects on the gross activity varied from almost complete inactivity to an increase in activity of 143%. On the other hand, the trimethoxy derivatives produced a more or less consistent depressant effect. All the results for triphenylaniline and its trimethoxy derivative indicated a depressant effect with those of trimethoxy derivatives once again appearing to produce the greater effect. When tested against the various doses of amphetamine hydrochloride (Table 2) the results once again were very variable. In some cases there was an increase of activity and in others a very definite decrease was noted.

One interesting fact was demonstrated by control mice which following administration of 0.9% saline showed a definite depressant effect in some cases. This could be explained on the basis of a stress reaction following the injection. Following the tests of normal gross activity those mice exhibiting the greater decrease in activity were examined and found to be awake, when prodded or pricked they appeared to respond normally, however, when left alone they just sat in an
apparently normal position.

The statistical evaluation of the combined results of the sleeping time studies (Table 3 & 4) indicated that the sedative effects of sodium pentobarbital were definitely potentiated by diphenhydramine and trihexyphenidyl, by tripelennamine to some degree, but not to any apparent degree by trimethoxy tripelennamine. In both series it appears that the parent compound is the most potent with the presence of the 3,4,5-trimethoxy group decreasing the potentiating effect.

By the results of the LD₅₀ studies for the experimental compounds with the parent compounds (Table 5) it was obvious that the trimethoxy group reduces the toxicity of the parent compounds.

In all cases most of the animals that died did so within two hours. Prior to death all the animals went through a period of classic convulsions, with those receiving diphenhydramine exhibiting the most severe activity. Those mice receiving diphenhydramine or trimethoxy diphenhydramine became excited about 3 minutes after injections and soon went into convulsions and died. Those receiving tripelennamine exhibited milder excitement than those receiving diphenhydramine or its trimethoxy derivative. Trimethoxy tripelennamine elicited depression at first then convulsions and death. After the excitatory period trimethoxy diphenhydramine elicited depression. The animals stayed together in one place and exhibited postis similar to that observed during reserpine sedation.

Results from the preliminary behavioral studies by the
rolling milling and the inclined plane (Tables 6 & 7) were inconclusive and yielded no significant results at the dose levels employed which produced an effect on the gross activity. The lack of results from these preliminary behavioral studies, measuring coordination of the animals, gives an indication that the C.N.S. activity of these compounds is other than on the cerebral cortical areas of the brain.

It appears that these compounds are producing some effects on the C.N.S., which at the moment are not clearly elucidated. Further studies employing various behavioral testing methods are needed to help clarify the problem.
VI

SUMMARY AND CONCLUSIONS

1. The results from gross activity were quite variable. However, it was found that the trimethoxy compounds showed some depressant effects on gross activity.
   a. Diphenhydramine with dose response showed very inconsistent effect.
   b. Trimethoxy diphenhydramine showed more or less consistent depressant effect.
   c. Both tripeleamine and trimethoxy tripeleamine showed depressant effect with the trimethoxy tripeleamine showing the greater effect.

2. At times, control mice displayed depression during the gross activity tests.

3. The sleeping time tests showed consistent results, and the statistical analysis indicated that diphenhydramine, trimethoxy diphenhydramine and tripeleamine potentiated the effect of sodium pentobarbital sleeping time whereas trimethoxy tripeleamine produced no apparent effect.

4. The LD₅₀ studies indicated that trimethoxy compounds are less toxic than the parent compounds.

All of the animals that died went through a period of clonic
convulsions before death.

3. At the dose levels employed which produced an effect on the gross activity, no observable effects on the coordination of the mice was seen as measured by the preliminary behavioral studies with the rolling roller and the inclined plane.
REFERENCES


